



Societas Europaea Herpetologica

HERPETOLOGIA BONNENSIS II



edited by
Miguel Vences, Jörn Köhler, Thomas Ziegler
& Wolfgang Böhme



13th CONGRESS
OF THE
SOCIETAS EUROPAEA
HERPETOLOGICA (SEH)



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Proceedings of the
13th Congress of the
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27 September – 2 October 2005
Bonn, Germany

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Bonn, December 2006



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FOREWORD

From September 27 to October 2, 2005, SEH returned for the 2nd time to its birth place. Founded in 1979 at the Zoologisches Forschungsmuseum A. Koenig (ZFMK) in Bonn, the society returned to its founding place for the first time in August 1995. The proceedings volume of that meeting was named “Herpetologia Bonnensis” and published in 1997.

It was our original intention to invite SEH again in 2009, not only because of the 30th anniversary of its foundation in our museum but also because one of the editors (WB) will have to face his retirement at the end of this year and he thought that a second SEH congress at ZFMK would be a very appropriate concluding event for his nearly 40 years of herpetological work in this institution. However, in spring 2005, we were surprised by the information that the 13th O.G.M. which originally was scheduled for an Italian site, could not take place there, and a new location was desperately searched for by the SEH council. So we advanced our invitation without hesitation for four years but were of course aware of the limited preparation time for this congress. But things went fairly well, more than 200 herpetologists registered, and the O.G.M. was mostly considered successful and generally appreciated by the participants. Again, as in 1995, Museum Koenig and its lecture hall proved to be too small to house all participants, but in the mean-time a new hotel (DERAG Hotel “Kanzler”) had opened just in the neighbouring building so that both locations could be linked for the purpose of this congress.

14 months later, we can now present the proceedings volume of this 2nd O.G.M. that was held in Bonn, and we consequently name it “Herpetologia Bonnensis II”.

Of nearly 80 oral presentations and roughly the same number of poster presentations, 56 papers were submitted to the editors for the present volume. They cover a particularly wide array of topics, wherefore we decided to arrange them in the alphabetical order of the respective (first) authors. The two workshops on ophidian sensory biology and on herpetodiversity of Vietnam follow separately, with equally alphabetically listed participants resp. authors.

A third workshop integrated into the congress was the IUCN Global Assessment Regional Workshop on non-Mediterranean Reptiles of the Western Palearctic, coordinated by Neil Cox and Carlo Rondinini. Its results will of course be published elsewhere, in the framework of IUCN’s publications.

The editors are aware of the fact that neither the congress nor this book could have been successfully completed if not numerous persons would have helped in a very effective manner. First of all, we should like to express our gratitude to those persons who - next to us - met our organisation committee (in alphabetical order): Wolfgang Bischoff, Ursula Bott, Viola Gossmann, Monika Hachtel, Peter Sound and Philipp Wagner. Secondly, we cordially thank our student crew: Alexander Burmann, Albia Consul, Anke Frank, Alexandra Großerichter, Astrid Heidrich, Ralf Hendrix, Monique Hölting, Claudia Koch, Tobias Kohl, Melanie Madscher, Lisa Meier, Daniel Ortmann, Birgit Rach, Jürgen Roder, Sarah Schellberg, Peter Schmidt and Klaus Weddeling, for being always present, assisting with the media during the lectures, and being always available for the participants, for answering questions and solving problems of any kind that may come up during such an international event.

Thirdly, we wish to deeply acknowledge the help and support of the following organisations:

- the Deutsche Forschungsgemeinschaft (DFG) for making the participation of our Russian and Ukrainian colleagues possible;

- the Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT) for generously sponsoring the joint evening dinner at the boat tour on the Rhine;
- The Alexander-Koenig-Gesellschaft (AKG) and the Cologne Zoo for providing the funds necessary for the participation of two colleagues from Vietnam.

But also the ontology of this book required many helpful persons and we were lucky enough to experience much support. Each of the articles contained in the present volume was peer-reviewed, and the colleagues who were willing to serve as reviewers, next to ourselves, were (again in alphabetical order): Raoul Bain (New York), Patrick David (Paris), Michael Franzen (München), Frank Glaw (München), Monika Hachtel (Bonn), Julian Glos (Würzburg), Ulrich Joger (Braunschweig), Franz Krapp (Bonn), Axel Kwet (Stuttgart), Alexander Kupfer (London), Mark-Oliver Rödel (Würzburg), Ulrich Sinsch (Koblenz), Andreas Schmitz (Genève), Sebastian Steinfartz (Bielefeld), Bryan Stuart (Chicago), Frank Tillack (Berlin), Klaus Weddeling (Bonn), David R. Vieites (Berkeley), Klaus Weddeling (Bonn), Katharina Wollenberg (Mainz). Mrs. Lieselotte Schulz, Cologne Zoo, was kind enough to prepare the table of contents of this book, and Uwe Vaartjes (Bonn) designed the logo of the congress and the title page of this volume.

Finally, special thanks are due to Edoardo Razzetti, webmaster of SEH: for the first time, the articles of an SEH Proceedings volume are being made available as open-access-PDF files from the SEH website, and we thank Edoardo for volunteering for this work.

The whole process of generating this volume was accompanied and reliably influenced by the indispensable help of Ursula Bott (ZFMK Bonn). Without her, the project would have come hardly to a positive end.

Bonn, 16 December 2006

For the editors: Wolfgang Böhme



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Phylogeny and microendemism of the New Caledonian lizard fauna

Aaron M. Bauer, Todd Jackman

Abstract. The lizard fauna of New Caledonia is both diverse and highly endemic. Molecular phylogenetic analyses of the diplodactylid geckos and lygosomine skinks reveal that the island supports a minimum of 106 endemic lizard species. New Caledonian diplodactylids are monophyletic, but recognized genera are not, whereas New Caledonian skinks are paraphyletic with respect to New Zealand skinks, although all but one genus is monophyletic. Geological events in the Eocene and Oligocene are likely to have been responsible for initial cladogenesis within both geckos and skinks in New Caledonia, although the lineages themselves may be of different ages. Microendemism is the result of geologically and climatically-mediated fragmentation of habitats throughout the second half of the Tertiary and poses significant problems for conservation management in New Caledonia today.

Introduction

The biota of New Caledonia is noteworthy both for its phyletic and ecological diversity and for its high level of endemism (Holloway, 1979) and the New Caledonian region has recently been identified as one of the world's hotspots of tropical biodiversity (Myers, 1988, 1990; Mittermeier et al., 1996; Myers et al., 2000; Lowry et al., 2004). Although the botanical significance of the island has long been recognized (Morat, 1983; Morat et al., 1986; Jaffré et al., 1998), the uniqueness of the terrestrial and freshwater fauna has only recently been emphasized (Chazeau, 1993; Platnick, 1993; Séret, 1997). Among vertebrates, lizards constitute the most diverse and highly endemic component of the fauna (Bauer, 1989, 1999; Bauer and Sadlier, 2000). A diversity of habitat types within New Caledonia, including humid forest, sclerophyll forest, and both low and high elevation maquis, certainly contributes to the maintenance of high biodiversity, but the ultimate source of the observed patterns of diversity among the reptiles of New Caledonia is the island's long and complex geological and climatic history. The Grande Terre, the main island of New Caledonia, has a land area of 16,648 km² and is dominated by chains of mountains (to 1600 m elevation) that parallel the long axis of the island. Parts of the Grande Terre have been emergent for at least 100 Ma and were originally adjacent to Australia. The opening of the Coral and Tasman Seas isolated New Caledonia by about 65 Ma, although sporadic connections to New Zealand and other, smaller land masses may have existed (Kroenke, 1996).

Perhaps the most important events in the biotic history of New Caledonia occurred in association with the Eocene ophiolitic obduction (39-36 Ma; Lowry, 1998; Lee et al., 2001), which resulted in the overthrusting of peridotite sheets, which today dominate the southern one third of the Grande Terre as well as a series of isolated massifs extending to the north and west as far as the Belep Islands. This was followed by Oligocene marine transgressions, which reduced neighboring New Zealand to an area of about 18% of its current aerial land mass (Cooper and Millener, 1993) and may have submerged the majority of the Grande Terre, and by Miocene marine regression and mountain building, ultimately resulting in the modern, highly-dissected topography of the island.

An intensive series of field trips by the authors and their colleagues during the period 2001-2004 provided material from numerous areas of New Caledonia that had not been previously sampled for lizards, including the northwest ultramafic peaks and numerous northern offshore islands. Combined with more than 20 years of accumulated specimens and tissue samples, the new material provided an unprecedented opportunity to reevaluate the systematics of the New Caledonian herpetofauna and to erect hypotheses of relationship for both of the major lizard groups occurring on the Grande Terre: diplodactylid geckos and lygosomine skinks of the *Eugongylus* group. We here summarize the broader results of molecular phylogenetic studies on the New Caledonian herpetofauna, although both new taxon descriptions and details of phylogenetic hypotheses have been or will be presented elsewhere (e.g., Sadlier, Smith, Bauer and Whitaker, 2004; Sadlier, Bauer, Whitaker and Smith, 2004; Bauer et al., 2006, submitted).

Materials and methods

Molecular methods

Nucleotide sequences from the mitochondrial ND2 and ND4 genes and five tRNAs, and from nuclear Rag-1 and c-mos genes were obtained from representatives of most genera and species of New Caledonian geckos and skinks, including numerous putatively new species. In total 2286 bp of sequence were generated for 405 diplodactylid gecko samples including 14 outgroup taxa and all 21 recognized ingroup taxa. 1950 bp of sequence were generated for 382 skinks, including 92 taxa, 39 of which were outgroups. Genomic DNA was extracted using the Qiagen QIAmp tissue kit and PCR amplification was conducted under a variety of thermocycler parameters using a diversity of primers (see Sadlier, Smith, Bauer and Whitaker, 2004; Bauer et al., 2006, submitted). Products were visualized via 1.5% agarose gel electrophoresis. Amplified products were purified either using AmPure magnetic bead PCR purification kit or reamplified products were purified on 2.5% acrylamide gels (Maniatis et al., 1982) after being reamplified from 2.5% low melt agarose plugs. DNA from acrylamide gels was eluted from the acrylamide passively over two days with Maniatis elution buffer (Maniatis et al., 1982). Cycle-sequencing reactions were performed using the Applied Biosystems Big-Dye™ primer cycle sequencing ready reaction kit. The resulting products were purified using SeqClean magnetic bead purification kit. Purified sequencing reactions were analyzed on an ABI 373A stretch gel sequencer or an ABI 3700 automated sequencer. To insure accuracy, negative controls were included in every reaction, complementary strands were sequenced, and sequences were manually aligned by eye using the original chromatograph data in the program SeqMan II. All ingroup sequences are being deposited in GenBank as primary research papers are published.

Phylogenetic methods

Phylogenetic trees were estimated using parsimony, likelihood and Bayesian analysis. PAUP* 4.0b10a (Swofford, 2002) was used to estimate parsimony and likelihood trees. Parsimony searches were conducted with 100 heuristic searches using random addition of sequences. Non-parametric bootstrap resampling was used to assess support for individual nodes using 1000 bootstrap replicates with ten random addition searches. For maximum likelihood analyses, ModelTest version 3.5 (Posada and Crandall, 1998) was used to compare different models of sequence evolution with respect to the data. The chosen model was used to estimate parameters on the most parsimonious tree. These likelihood parameters were fixed and the most parsimonious trees were used as starting trees for branch swapping in 25 heuristic searches with random addition of taxa to find the overall best likelihood topology. To estimate a phylogenetic tree with a Bayesian framework MrBayes 3.0 (Huelsenbeck and Ronquist, 2001) was used with the model chosen using ModelTest 3.5. The Bayesian analyses were initiated from random starting trees and run for 2,000,000 generations with four incrementally heated Markov chains. Likelihood parameter values were estimated from the data and initiated using flat priors. Trees were sampled every 100 generations, resulting in 20,000 saved trees. To ensure that Bayesian analyses reach stationarity, the first 5000 saved trees were discarded as 'burn-in' samples.

Results

Diplodactylid geckos

The diplodactylid geckos of New Caledonia form a monophyletic group that has as its sister group the viviparous geckos of New Zealand. This result is strongly supported by Bayesian analysis, although under maximum parsimony, the Australian Pseudotochecadactylus is weakly supported as the immediate sister group of the New Caledonian clade. Outgroup relationships and basal ingroup relationships were chiefly supported by Rag-1 sequence data. Although relationships among basal groups was equivocal, all analyses retrieved the same series of strongly supported New Caledonian clades, each deeply divergent from all other such clades. Groupings did not correspond to the three diplodactylid genera currently recognized in New Caledonia. Indeed, only the highly autapomorphic *Eurydactyloides* was unambiguously monophyletic. The monophyly of the giant geckos, *Rhacodactylus*, was falsified, as was that of the morphologically plesiomorphic genus *Bavayia*. Although most described species of *Bavayia* are members of a single clade, other taxa previously assigned to this genus appear in two other basal clades. In addition, a newly discovered species with superficial resemblances to *Bavayia* was found to be the sister group of all other New Caledonian diplodactylids (Bauer et al., 2006). Molecular data, supplemented by morphological traits (discussed elsewhere) also revealed many undescribed species among New Caledonian diplodactylids. These include cryptic taxa, as well as easily recognized novelties. New taxa identified include one new *Eurydactyloides*, two new "*Rhacodactylus*" (as well as one resurrected from synonymy), and 32 new *Bavayia*, chiefly in the *B. cyclura*, *B. sauvagii*, and *B. validiclavis* clades.

Lygosomine skinks

The bulk of the New Caledonian skink radiation is part of a single clade withing the Eugongylus group, with only *Cryptoblepharus novocaledonicus* and *Emoia* spp. (limited to the Loyalty Islands within the New Caledonian region) falling outside this clade. The New Caledonian clade also subsumes the New Zealand skinks, which appear to be monophyletic. All of the recognized New Caledonian endemic genera are monophyletic except *Lioscincus*, which is polyphyletic. Most generic level taxa are, however, well supported and have long branch lengths. A new genus and species, *Kanakysaurus viviparous*, has recently been identified and described as one such distinctive clade (Sad-

lier, Smith, Bauer and Whitaker, 2004). Relationships among skink genera are not as well supported as those among diplodactylids, but there is strong support, chiefly from mitochondrial data, for patterns of species relationships. In two of the most speciose genera, *Nanoscincus* and *Caledoniscincus*, molecular and morphological data are inconsistent with respect to species boundaries. In the former case, several morphological species appear to be paraphyletic and one pair of morphologically distinctive species are genetically indistinguishable. In the latter genus, molecular data reveals the existence of several cryptic species, but also suggest that not all species previously recognized on the basis of allozyme data (Sadlier et al., 1999) should be recognized. At a minimum, phylogenetic data indicate the existence of six more skink species than are currently recognized, despite the requirement for the synonymization of some nominal species.

Discussion

The monophyly of New Caledonian diplodactylids is consistent with earlier, morphologically based studies (e.g., Kluge, 1967; Bauer, 1990), but the non-monophyly of the constituent genera has not been previously proposed (Bauer, 1990; Vences et al., 2001; but see Good et al., 1997). Among skinks, the current system of generic divisions established initially by Sadlier (1986) has been supported. Although no previous studies have explicitly examined the higher order phylogenetics of New Caledonian skinks, the monophyly of the New Caledonian + New Zealand clade is at odds with at least some earlier conjectures of affinity (e.g., Böhme, 1976; Bauer and Sadlier, 1993).

Perhaps most surprising among our findings is that such a large proportion of New Caledonian lizard diversity remained hidden, despite two decades of intensive research on an island of only moderate size. Indeed, based on our current research, the Diplodactylidae is represented on New Caledonia by a minimum of 58 species, whereas there are at least 51 species of New Caledonian lygosomine skinks. Of these, all of the diplodactylids and all but three of the skinks are strictly endemic to New Caledonia and its islands. Thus there are at least 106 endemic lizard species in New Caledonia. This is an increase of 72 (212%) since 1980 and 46 (77%) since 2000 (Bauer and Sadlier, 2000).

Much of the increased diversity, especially among geckos, has been the result of recent explorations of the ultramafic massifs of northwestern New Caledonia (Whitaker et al., 2004). This has revealed that most iso-

lated peaks and plateaus support one or more endemic species. Likewise, increased sampling in central and southern New Caledonia has revealed species breaks that could not have been localized without fine scale sampling and which were not suspected until sample sizes permitted the distinction between minor regional or clinal variation and species-specific differentiation – sometimes a difficult task among morphologically conservative genera such as *Bavayia* and *Caledoniscincus*. This new picture of New Caledonian lizard diversity further emphasizes a previously signalled pattern of microendemism (Sadlier, 1986; Bauer and Vindum, 1990; Bauer and Sadlier, 1993, 2000). In addition to previously recognized areas of microendemism, such as the southern ultramafic block of the Grande Terre and the Panié Massif, our phylogenetic results and recognition of cryptic species suggests that virtually all montane blocks in New Caledonia (Bauer et al. submitted), as well as lowland limestones (Sadlier et al., 1999) and certain vegetation types at all elevations (Bauer et al., 2006) may be considered areas of intra-island endemism.

How has the extreme microendemism seen in New Caledonia evolved? Both diplodactylid geckos and lygosomine skinks are commonly associated with certain substrates or microhabitats. This connection has probably promoted speciation in both groups in association with the fragmentation of once continuous habitat/substrate types over geological time. The Eocene ophiolitic obduction and Oligocene marine transgressions that impacted New Caledonia are candidate historical events that may have played a role in at least basal cladogenesis within the lizard lineages. Indeed, a comparative analysis of the New Caledonian and New Zealand skink and gecko fauna suggest that basal within-island cladogenesis in both taxonomic groups occurred approximately 30 million years ago (Jackman, 2005; Bauer et al., submitted), at a time consistent with the “Oligocene bottleneck” that is credited with the reduction of genetic and phyletic diversity of the New Zealand fauna (e.g., Cooper and Cooper, 1995; Hickson et al., 2000; Chambers et al., 2001). Within the *Bavayia validiclavus* lineage, the most recent speciation events correspond to an age of 5–6 Ma (Bauer et al., submitted) suggesting that cladogeneic events throughout the Mid- to Late Tertiary may have played a role in the fragmentation and speciation of the New Caledonian lizard fauna. Climatic and vegetational changes in New Caledonia during this period were substantial (Lowry, 1998; Lee et al., 2001) and might

well be relevant to herpetofaunal diversification, although specific candidate cladogenetic events remain elusive.

Although there is no evidence for divergences compatible with Gondwanan cladogenesis within New Caledonian lizards, their Gondwanan origin is not excluded. These age estimates merely suggest that the modern radiations of lizards date from the Oligocene, but it is plausible to suppose that older lineages may have become extinct, perhaps during the period of Eocene overthrusting or subsequent drowning of much of the Grande Terre, leaving a single surviving lineage which subsequently diversified. Rough dating of the divergence between New Caledonian and New Zealand diplodactylids, as well as that between East Tasman and Australian diplodactylids, is consistent with Late Cretaceous to Early Tertiary geological events occurring along the eastern margin of Gondwanaland (Jackman, 2005). No such evidence exists for skinks and we think it likely that the founders of the New Caledonian/New Zealand skink lineage reached the Grande Terre via overwater dispersal in the mid-Tertiary (Bauer, 1999).

Microendemism poses particular problems for conservation and new data from New Caledonia will necessitate new priorities for conservation management. Based on our results, very few endemic New Caledonian lizards have island-wide distributions, and most are restricted to very localized areas. Many such areas are associated with geological features of economic importance and are subject to exploitation by mining, New Caledonia's most important industry. Small, localized populations are also at greater risk from introduced predators, which are widespread in New Caledonia (Gargominy et al., 1996), fire ant invasion (Jourdan et al., 2001), and agricultural activities. If most or all endemic lizards in New Caledonia are to receive protection, it will necessitate the establishment of a much more extensive system of protected areas, incorporating much of the remaining forested habitat on many of the Grande Terre's mountains, as well as a diversity of habitats at low and middle elevation.

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Ecology and conservation aspects of *Neurergus strauchii* (Amphibia: Salamandridae)

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Abstract. Characteristics of breeding streams and terrestrial habitats of 11 populations of *N. strauchii* are presented. Streams are fast running with rock pools. They are fed by melting snow and rain and are 0,5 to 2 meters wide. Bottom consists of rocks, big stones and stone chippers added with gritty sand. The terrestrial habitat is rocky with mostly only herbaceous vegetation and hardly any shrub or tree layer. Terrestrial habitat degradations are caused by overgrazing, sometimes the establishment of cultivated grounds. Conservation aspects and future research aspects are discussed. Conservation should be first focussed on *N. s. barani*.

Introduction

Few ecological data are available concerning the salamandrid genus *Neurergus*. The genus comprises four species of which two are found in Turkey: *Neurergus strauchii* (Steindachner, 1887) and *N. crocatus* Cope, 1862 (Baran and Öz, 1986). The nominate subspecies of *N. s. strauchii* (Steindachner, 1887) is known south and west of lake Van (Schmidtler and Schmidtler, 1970) up to south of Hazar Gölü (Pasmans et al., 2006). In 1994, the subspecies *N. s. barani* Öz, 1994 was described from the Kubbe mountains on the Malatya – Pütürge road (Öz, 1994) and seems to be restricted to these and surrounding mountains (Pasmans et al., 2006). Both subspecies are probably separated by the river Euphrates (Pasmans et al., 2006).

N. strauchii is strictly protected species by the Convention on the Conservation of European Wildlife and Natural Habitats (also known as Bern Convention) and listed on appendix II, ratified by Turkey on the 2nd of May 1984. Artikel 6 states for these species that each Contracting Party shall take appropriate and necessary legislative and administrative measures to ensure the special protection of the wild fauna species specified in Appendix II. The following (in short) will in particular be prohibited for these species: all forms of deliberate capture, keeping, killing, disturbance, insofar as disturbance would be significant in relation to the objectives of this Convention, deliberate destruction or taking of eggs from the wild and possession of and trade in these animals, alive or dead. In Resolution No. 6 (1998) of the Standing Committee, *N. strauchii* is listed

as a species requiring specific habitat conservation measures.

Aims of our study were to determine the presence of populations of *N. strauchii* outside the known areas, to collect data on their morphology and ecology, to assess the level of molecular and morphological differentiation among them and to determine possible threats. Here we present ecological data and aspects for conservation measures. Morphological and molecular data concerning biogeography are published elsewhere (Pasmans et al., 2006).

Materials and methods

Four field trips to Turkey were undertaken in the period between April –May 2000, 2001, 2003 and 2005. In 2001 and 2003 the area between Malatya and Bitlis was investigated. In 2005 special attention was paid to the mountain areas west and south of the Malatya mountains and the area between Malatya and Muş. The breeding streams and terrestrial habitats were characterized with methods used in previous studies (see Winden & Bogaerts, 1992). Roughly 35 streams were investigated by walking along and in the stream in search for newts. Mostly 15 to 30 minutes were spent per stream. Of streams in which newts were present width, type of substrate in the streams, presence of vegetation in the stream and the percentage of vegetation coverage within roughly 10 meters on both sides of the stream were estimated and presence of human activity was noted.

Results

In 11 streams *N. strauchii* was found present out of 35 streams investigated. Numbers of localities are presented in figure 1. *N. strauchii* could not be found west or south of the Malatya mountains area or in streams in mountain areas north of the Euphrates between Malatya and Muş. Adult newts were found in breeding condition in mountain brooks in all localities with the exception of two localities (4, 9) where also animals were found on land. At locality 4, 14 sub-adult and 4 adult individuals were found on land under in between crevices of rocks and under stones in the only rock formation available

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Figure 1. Distribution of *Neurergus strauchii* in Turkey. Numbers refer to the locations in Tables, Figures and the text.

in the valley about 10 meters away from the stream, and at locality 9 one adult female was found on land under a stone about 0,5 m from the stream having just left the water after laying eggs. In all streams animals were found active at day time. Location 1 was also visited at night where approximately two to three times as many animals were observed. Cyprinid fish (species undetermined) were present at location 11; newts were remarkably shy at this location. We found fresh spawn at several locations (1, 9, 10) up to 93 eggs (10) on the underside of rocks. Mostly large groups of eggs (> 20) were found with some exceptions (2-14). At location 9 we found eggs attached to the rock bottom and branches exposing the eggs to direct sunlight.

In table 1 habitat characteristics of 11 localities are presented. There are no obvious differences between the habitats of the two subspecies. Breeding streams are 0,5 to 2 meters wide, only one stream was 2-4 meters wide.

Half of them are 0,5 to 1 meter wide, 25 % is 1 to 1,5 meters wide and 25% is up to two meters or more wide. Bottom coverage consists of solely rocks, big stones and stone chippers (36 %) added with gritty sand (45 %) or gritty and fine sand (18 %). Parts with loam were only found at isolated spots in the streambed, but never over several meters. Only in three streams vegetation was present.

The terrestrial habitat of *N. strauchii* is only sparsely covered with vegetation. A layer of herbs is always present. Only in two localities this layer was dense (81-100%). In all other cases herb coverage varied between 11 – 40% (n = 4) or 41 – 80% (n = 5). Shrubs were present at only 7 localities and coverage varied between 0 – 11% (n = 5) to 11- 40% (n = 2). Trees were only scarcely present or absent. They consisted of planted willows or poplars (n = 3) or of single natural trees (n = 3). Location 3 had the richest vegetation structure along the stream.

Human activities consisted mainly of grazing by goats and sheep (or even cows at location 1). In most cases the area's outside the roughly 10 meter zone surrounding the

Table 1. Characteristics of the breeding stream and land habitat of *Neurergus strauchii*. Breeding stream characteristics determined on the area of approximately 50 m of the stream where adults were present. Coding of the bottom coverage. 1 = rock – big stones, 2 = stone chippers, 3 = gritty sand, 4 = fine sand and 5 = loam or clay.

Land habitat characteristics determined on the area of approximately 10 m wide on both sides of the stream. Herb = vegetation lower than 0,5 meter, shrub is vegetation 0,5 to 3 meter and tree is vegetation higher than 3 m. Coding of the vegetation coverage. 1 = 0 – 10 %, 2 = 11 – 40 %, 3 = 41 – 80 % and 4 = 81 – 100 %.

Locality	Date	Width (m)	Bottom coverage	Aquatic vegetation	Herb	Shrub	Tree	Type of tree	Human activities
38°15'N;38°37'E(1)	30-4-2001	0.5 - 1	1, 2	Algae and grass	3	0	0		grazing with goats and cows
38°15'N;38°39'E(6)	30-4-2001	0.5 - 1	1, 2	None	3	1	1	willow	cultivated grounds
38°15'N;38°38'E(7)	30-4-2001	0.5 - 1	1, 2, 3	None	2	0	1	poplar	none observed
38°21'N;42°15'E(2)	4-5-2001	0.5 - 1	1, 2, 3	None	3	1	0		grazing with goats
38°24'N;42°05'E(8)	4-5-2001	2 - 4	1, 2, 3	None	4	0	1	div	grazing with goats, cultivated grounds, houses
38°34'N;39°44'E(3)	25-4-2003	1 - 2	1, 2	Grass	3	2	2	div	grazing with goats, cultivated grounds
38°44'N;40°32'E(4)	26-4-2003	0.5 - 1	1, 2, 3, 4	None	4	1	1	div	grazing with goats
38°40'N;40°27'E(5)	26-4-2003	1 - 1.5	1, 2, 3, 4	None	3	1	1	div	grazing with goats
38°17'N;38°35'E(9)	14-5-2005	0.5 - 1	1, 2	None	2	2	0	oaks	none observed
38°36'N;40°01'E(10)	15-5-2005	1 - 1.5	1, 2, 3	None	2	0	1	willow	none observed
38°41'N;41°11'E(11)	16-5-2005	1 - 2	1, 2, 3	Algae	2	1	1	div	none observed

stream were used as meadows (n = 6) and/or cultivated grounds (n = 3). At location (8) houses were very close to the stream. Stream 3 was used by local people of a small village nearby for drinking water. Near all streams roads were present, parallel along the stream or crossing it.

The following species of amphibians and reptiles were found along the streams in which *N. strauchii* was found: *Rana macrocnemis*, *Rana ridibunda* complex, *Bufo viridis*, *Hyla savigny*, *Testudo graeca*, *Ophisops elegans*, *Lacerta cappadocica*, and *Lacerta media*. Both *Rana macrocnemis* and *Lacerta media* were found very frequently along side the streams. *B. viridis* was found breeding in the same stream as *N. strauchii* (1).

Discussion

The finding of five new populations of *N. s. strauchii* (locations 3, 4, 5, 10, 11) has expanded the distribution range approximately 300 km to the west (Pasmans et al., 2006). The presence of *N. s. barani* seems restricted to the Kubbe mountains where only one new location (9) was found just outside the Kubbe valley (Pasmans et al., 2006). The aquatic and land habitats of *N. strauchii* are for the first time characterised.

The terrestrial habitat is always situated in rocky surroundings with a scarce shrub layer and hardly any trees present. On only two occasions we could find animals on land, despite intensive searching at all localities. Slopes providing deep crevices through

compilation of rubble might be very important for the survival of newts on land. Schmidler and Schmidler (1970) found *N. strauchii* hibernating at 25 meters away from the stream and about 5 meters higher in a heap of stones. There is more information needed on how far newts migrate from the streams. Most areas are grazed by sheep and goats. Overgrazing can cause erosion which could turn out negative for the populations of *N. strauchii*. Newts were not found in optical suitable habitats where stream bottoms are covered with loam or clay. In wide streams (wider than 2 meters) it is difficult to detect newts and it is possible that in those streams newts are present but not detected.

Papenfuss et al. (2004) lists *N. strauchii* as “vulnerable” because its area of occupancy is less than 2,000 km², its distribution is severely fragmented, and there is continuing decline in the extent and quality of its habitat in Turkey. We estimate the distribution area of *N. s. strauchii* to be around 7,500 km² and that of *N. s. barani* to be 1,000 km². It seems that different populations are isolated from each other. We could notice disturbance of *N. strauchii* terrestrial and breeding habitat on several occasions: road construction works, tapping of sources, household sewage and overgrazing. These threats are also noted for *N. microspilotus* (Sharifi and Assadian, 2004) and to a lesser extent for *N. kaiseri* in Iran (Sharifi et al., in press). Collecting of adult animals during breeding season by animal traders has occurred as *Neurergus strauchii barani* were offered for sale in

Figure 2. Breeding habitat of *Neurergus strauchii* near Bitlis (nr. 8).



2002 and 2003 in Germany (personal observations). All these single threats combined might lead to local extinction of *N. strauchii* throughout its known range. The Bern Convention is not implemented in Turkish national law yet. The new Nature Conservation Law, is in preparation and it will include the concept of “protected species” (Güven Eken, pers. comm.). However, strict protection of habitats is required to conserve the current status and prevent local extinction. We propose to concentrate conservation first on *N. s. barani*. Only four populations are known. Threats are current like road reconstructions close to the breeding streams and the construction of a dam is planned on the river catchments in the Kubbe Dağı (Wagener, 2003). The area is also of great importance for the butterfly *Polyommatus dama dama* of which the only population worldwide is found (Wagener, 2003). Data on population numbers, size and range and population dynamics are urgently needed when conservation of this species is taken seriously (see also Papenfuss et al., 2004). More research is needed to determine the exact distribution of *N. strauchii* south, south-east and east of Lake Van and in the area’s between the known populations.

We propose education to the people living in these remote areas that treating the streams and surroundings with more care is essential for the survival of newts and people both using these streams as primary water source.

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The adductor mandibulae in *Elaphe* and related genera (Serpentes: Colubridae)

Bartosz Borczyk

Abstract. In this paper I describe the jaw adductor musculature in colubrid snakes that formerly belonged to the genus *Elaphe* Fitzinger. The group studied shows a high level of homoplasy, and particular lineages exhibit a mixture of advanced and primitive characters. The presence of the levator anguli oris in this group is questioned.

Introduction

The head anatomy of snakes has been a subject of numerous studies and the trigeminal musculature has attracted significant attention (e.g. Haas, 1973; Rieppel, 1980; Zaher, 1994). However, most of these studies have been purely descriptive and the comparisons were on a family rather than generic level. There are only a few studies dedicated to comparisons of closely related species, of *Thamnophis* (Cowan and Hick, 1951; Varkey, 1979), *Heterodon* (Weaver, 1965), *Entechinus*, *Opheodrys* and *Symphimus* (Cundall, 1986).

In this paper I present preliminary results of my studies of the jaw adductor musculature of *Elaphe* and its allies. I describe the jaw adductors and present their evolution based on a recently published reconstruction of their phylogeny. Also I discuss the 'levator anguli oris' problem.

Materials and methods

I studied the following species: *Coronella austriaca* (IZK 400-403), *Elaphe dione* (ZMB 31427), *E. quatuorlineata* (ZMB 63769), *E. quadrivirgata* (ZMB 66114), *E. schrenckii* (IZK 362-363), *Gonyosoma oxycephala* (IZK 331-333, MNHUr unnumbered specimen), *Lampropeltis getula* (BB 008, IZK 385-386), *L. mexicana* (IZK 394) *L. triangulum* (IZK 358, MNHUr unnumbered specimen), *Oreocryptophis porphyraceus* (ZMB 48053), *Orthriophis taeniurus friesi* (BB 042-043, IZK 365-366), *Pantherophis guttatus* (BB 015-016, 044), *Zamenis longissimus* (IZK 338, 364), *Z. situla* (IZK 384, MNHUr 2 unnumbered specimens). The institution abbreviations are as follows: ZMB – Museum für Naturkunde, Humboldt-Universität, Berlin, IZK – Laboratory of Vertebrate Zoology Collection, University of

Wrocław, MNHUr – Natural History Museum, University of Wrocław, BB – author's collection.

The homologies of muscles were established on the basis of their aponeuroses and topography. I follow the terminology proposed by Zaher (1994). The phylogeny I used in this studies is based on the recent papers by Rodriguez-Roblez and De Jesus-Escobar (1999), Helfenberger (2001), Lenk et al. (2001) Utiger et al. (2002, 2005) (Fig. 1). Character states were analyzed using McClade 4.03 software (Maddison and Maddison, 2001).

Results

The studied taxa show a typical colubrid pattern of the adductor mandibulae (fig. 2 a, b), as described by Albright and Nelson (1959). The main differences are the sites of origins, insertions and the aponeurotic pattern. The only two muscles that do not show variation in the studied group are the superficialis and profundus parts of the adductor posterior.

The main variation of the musculus adductor mandibulae externus superficialis proper involves its aponeurotic pattern and insertion sites (fig. 2 c). This muscle passes in postero-ventral direction and curves around the mouth corner behind the Harderian gland. In this area this muscle is tightly covered by tissue in the mouth corner, although there are no fibers inserting there. The externus adductor superficialis inserts either via its aponeurosis only, or via aponeurosis and directly to the compound bone. I haven't found any divisions of this muscle in the studied specimens. Musculus adductor mandibulae externus medialis shows variation in the pattern of its subdivision by the quadrate aponeurosis. In some cases this muscle is undivided, divided in two, or in three slips by the quadrate aponeurosis. These slips are clearly distinguished near the muscle origin, but in the ventral part they become indistinguishable. Musculus adductor mandibulae externus profundus shows interspecific variation in the origin. The

bodenaponeurosis is reduced, but present. Musculus pseudotemporalis shows variation in the pattern of origin, which can be on the parietal, parietal and occipital or parietal and postorbital.

The externus adductor medialis evolved from undivided or divided (2 subdivisions) conditions, and both scenarios are equivocal. All North American forms I studied have this muscle subdivided in two parts, and some of the Euro-Asiatic species, too. Taking into account the early divergence of Euro-Asiatic and North American lineages (e.g. Utiger et al. 2002), I suggest the primitive character state for this group is the subdivision of this muscle in two parts. The Nearctic species retain the primitive condition, and some Palearctic species have evolved independently the undivided condition or subdivided on three parts several times. The broad aponeurotic insertion of superficial externus adductor is the primitive condition. The North American forms, except *L. triangulum*, show a tendency towards a narrower insertion on the compound bone only (type II and III). Such reduction is also seen in not closely related *C. radiatus*, *E. dione*, *E. schrenckii*

and *Z. longissimus*. The hypothesis of the primitive condition of the insertion of superficial externus adductor only via its aponeurosis or the insertion of superficial externus adductor via aponeurosis and directly to the compound bone requires the same number of steps, but the first condition is more common among the studied taxa.

I cannot say which lineages of the studied group are more morphologically conservative. All studied species show a mixture of primitive and advanced characters, both myological and osteological (Borczyk, unpublished data). To resolve this problem more species have to be studied and more characters used.

Discussion

The adductor mandibulae of the studied colubrid genera is highly variable, which may reflect the adaptive plasticity of this group. However, most of the variations involve changes in relative position and shape of origins and insertions of this muscles, but it does not produce any major changes in muscle arrangement. It is possible that the observed

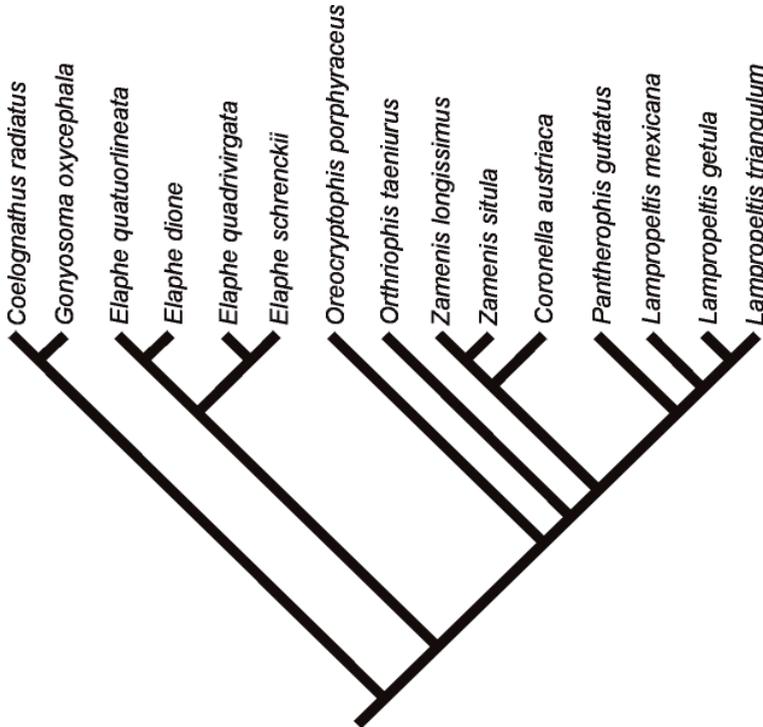
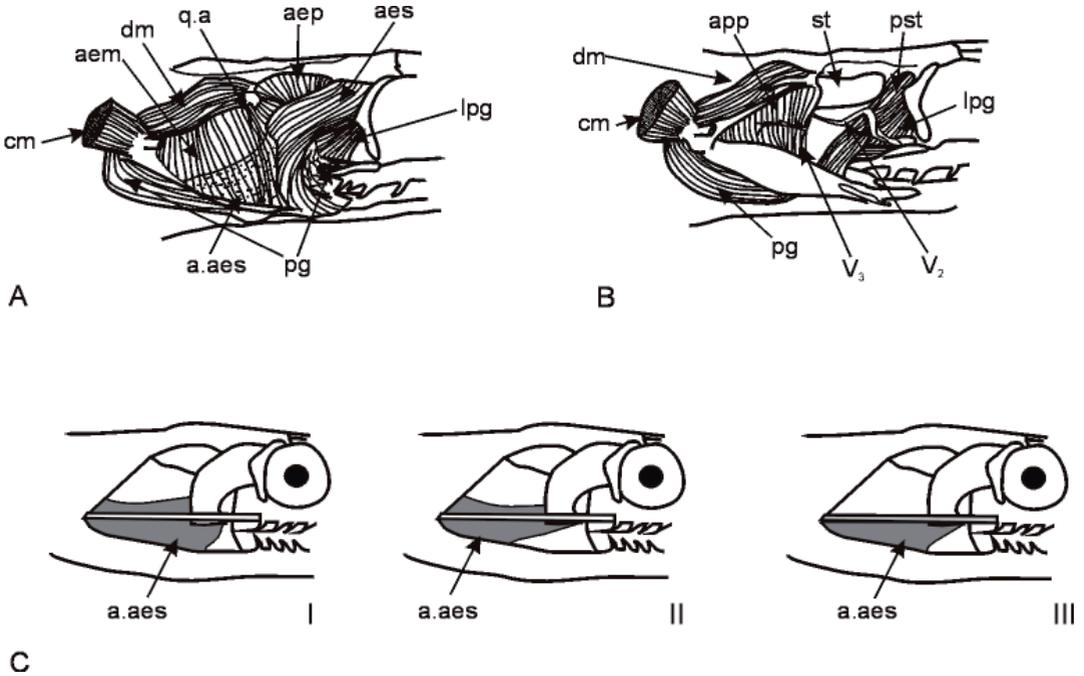


Figure 1. The phylogenetic relationships of studied species. The phylogeny used here is based on the recent papers by Rodriguez-Roblez and De Jesus-Escobar (1999), Helfenberger (2001), Lenk et al. (2001) and Utiger et al. (2002, 2005).

Figure 2. The jaw adductors of *Elaphe schrenckii* (IZK 362). A) The skin, Harderian and labial glands and quadrato-maxillary ligament removed. B) External adductors and adductor mandibulae posterior superficialis removed. C) Schematic representation of the three basic types of aponeurotic insertions of the adductor externus superficialis. Abbreviations: a.aes – aponeurosis of superficial external adductor; aes – m. adductor mandibulae externus superficialis proper; aem – m. adductor mandibulae externus medialis; aep – m. adductor mandibulae externus profundus; app – m. adductor mandibulae posterior profundus; cm – m. cervicomandibularis; lpg – m. levator pterygoidei; pg – m. pterygoideus; pst – musculus pseudotemporalis; q.a – quadrato aponeurosis; V₂ – maxillary branch of the trigeminal nerve; V₃ – mandibular branch of the trigeminal nerve.



differences in the origins, insertions and aponeurotic pattern are of little functional significance, and thus are easily accumulated during evolution. The direction of the fibers is similar in all studied species, and factors favouring the parallel fibers arrangements can limit greater variability (Cundall, 1986).

The posterior adductors are constant in their arrangement. I suggest that the reason is space constraint. They originate on the antero-ventral part of the quadrato, and insert in the mandibular fossa (the superficial posterior adductor) and the profundus posterior adductor inserts on the medial part of the compound bone. Anteriorly, these muscles are constrained by the mandibular branch of the trigeminal nerve and profundus external adductor. The space constraints are believed to limit the arrangement of muscles near the mandibular articulation (e.g. Elzanowski, 1993).

The levator anguli oris (LAO) is a problematic muscle in terms of its homology with the lacertilian

LAO as well as its homology among snake taxa (Zaher, 1994). The lacertilian LAO originates on the edge of the lateral temporal fenestra and inserts on to the rictal plate. The snake LAO originates on the parietal/postorbital and inserts to the rictal plate (Rieppel, 1980 McDowell, 1986). Underwood (1967) reported the superficialis inserting on the lower jaw and lower lip or rictal plate in *Coronella austriaca*, *E. quatuorlineata*, *Z. longissimus* but I have not found any insertions on the rictal plate and only in the smooth snake (*Coronella austriaca*) I found the insertion on the lower jaw. Also in *E. quatuorlineata* studied, there was no insertion of superficialis on the compound bone. This suggests a polymorphism in the attachment of this muscle. I have not found any fibers inserting on the rictal plate in the studied species, as the muscle inserts via its aponeurosis. In some cases the adductor superficialis inserts directly on the compound bone and via its aponeurosis. In addition, this muscle

does not form any slip distinct from the rest of the muscle. The distribution of character states of insertion of the superficial external adductor shows either the multiple origins of this condition or a loss in closely related species.

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Terrestrial habitat use of the common spadefoot (*Pelobates fuscus*) in an agricultural environment and an old sanddune landscape

W. Bosman¹, P. van den Munckhof²

Abstract. The terrestrial habitat use of the common spadefoot was studied in an agricultural area and an old riverdune landscape. In an agricultural area potatofields were the most important terrestrial habitat, in the old sanddune landscape half open sanddunes, sandy paths between a deciduous wood and a pinewood and sandy paths in a deciduous wood.

Introduction

In the Netherlands the endangered common spadefoot (*Pelobates fuscus*) can be found in different habitat areas. In two of these areas we studied the terrestrial use during the summer period. One important condition of the terrestrial habitat for the common spadefoot is the availability of a soil to dig in, in which to spend their inactive period. Loss of suitable terrestrial habitat can lead to extinction of populations.

In order to conserve the common spadefoot for the Netherlands, the government developed a protection programme especially for this species. In this programme the LIFE Nature "AMBITION" proposal was written and funded (including four other endangered species) in order to be able to finance measures to improve the biotopes of this species (Bosman et al., 2004).

In general little is known about terrestrial habitat use of the common spadefoot. Eggert (2002) studied the migration of the common spadefoot in a floodplain. A preliminary investigation was carried out in the Netherlands in a semi natural nature reserve with old sanddunes in 1987 (Bosman et al., 1988). The main aim of this study is to describe the terrestrial habitat types the common spadefoot use in order to be able to improve the management of the terrestrial area of this species.

Material and methods

The study area 'Groot Soerel' is situated on the edge of the valley of the river IJssel in the east of the Netherlands. It is an agricultural area dominated by meadows of pasture land. Other parts are maisefields, small meadowlands, some shrubs and a seed refinement company. Especially for the common spadefoot a nature management organization planted different products on

four pieces of land. Two were planted with biological potatoes, one with rye and another with barley. These field crops were new in the area. Within 500 meters from each other there are two reproduction sites in the area (Bosman, 2005).

The "Overasseltse en Hatertse vennen" is a nature reserve along the river Meuse with dunes either covered with spruce or pine trees, oak and birch or (half) open dunes. Beside that there is also some agricultural activity in the area. There are four reproduction sites in this study area (Dijk and Struijk, 2005).

In both areas an investigation route was established to include all the present habitat types in the area. If a road or path was part of the investigation route, it is named after the adjoining habitat types. The route in "Groot Soerel" has a length of approximately 3000 metres, the route in the "Overasseltse en Hatertse vennen" is 1375 metres. In "Groot Soerel" data was collected in 2003 and 2004 (Bosman, 2005). For the Overasseltse en Hatertse vennen" data are used that was collected between 1988 and 1992 (Bosman and van den Munckhof, 1993).

Both studies lasted each year from the beginning of May till the end of September, the period the common spadefoot is in its summer habitat. As often as possible, but at least once every two weeks, half an hour after sunset the routes were searched for amphibians and especially the common spadefoot. Amphibians were located visually by using a torch and acoustically. For every specimen data was collected, carefully recording the place where it was found and at what time it was found. A detailed description of each location is given. Pictures were taken of the back of the common spadefoot for individual recognition, sex was determined and length was measured.

During every visit more or less the same amount of time was spent in all different habitat types to search for toads in the agricultural area "Groot Soerel". From the total number of common spadefoots found, per habitat type the percentage of spadefoots found was calculated. The area was visited 23 times in 2003 and 2004. For the "Overasseltse en Haterste vennen" the results were corrected for length of the different habitat types as a part of the total length of the route. The number of visits, 82, was equal for all habitat types.

Results

Figure 1 shows the habitat types the common spadefoot used in the agricultural area "Groot Soerel" in 2003 and 2004. Eight specimen of the common spadefoot were found in two habitat types. In potatofields 62.5%

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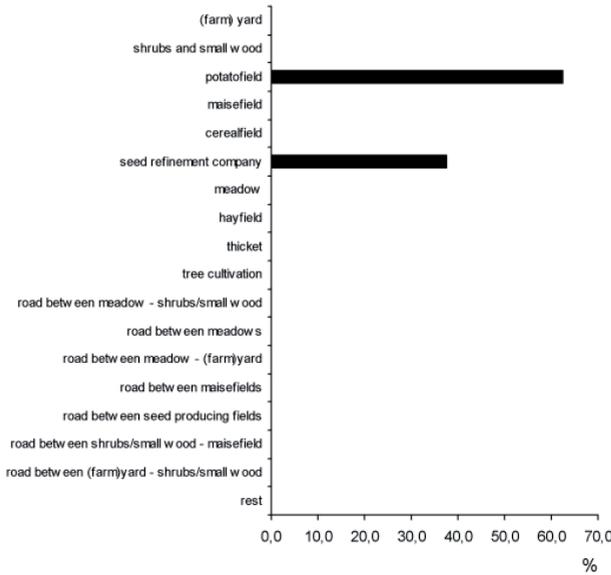


Figure 1. Terrestrial habitat use (%) of the common spadefoot (n = 8) in an agricultural landscape “Groot Soerel”, 2003-2004.

of the common spadefoots were found. The other specimen, 38.5 % were found at the property of a seed refinement company.

The results of the old seminatural dune landscape “Overasseltse en Hatertse vennen” in the period 1988 – 1992 are shown in figure 2. Data was collected from 279 specimens. 42,5 % was located on the half open sanddune. On a sandy path between a deciduous wood and a pinewood 20 % of the common spadefoots were found. 13 % of the common spadefoots were found on a sandy path in a deciduous wood. Eight of the habitats scored less then 5 % (Figure 2). No common spadefoots were found on a sandy path between meadows and none were found on a sandy path between meadow and

farm(yard) nor on a sandy path between the maize field and (farm) yard.

Discussion

In the agricultural area the common spadefoot used potatofields and the fields of a seed refinement company as terrestrial habitat. Half open sanddunes, sandy paths in a deciduous wood and between a deciduous wood and a pinewood were most used as terrestrial habitat in the old seminatural dune landscape.

A low number of common spadefoots was found in the agricultural area “Groot Soerel”. Unfortunately it is unclear why numbers are low. From another study in the same area we learned that at least 88 specimens

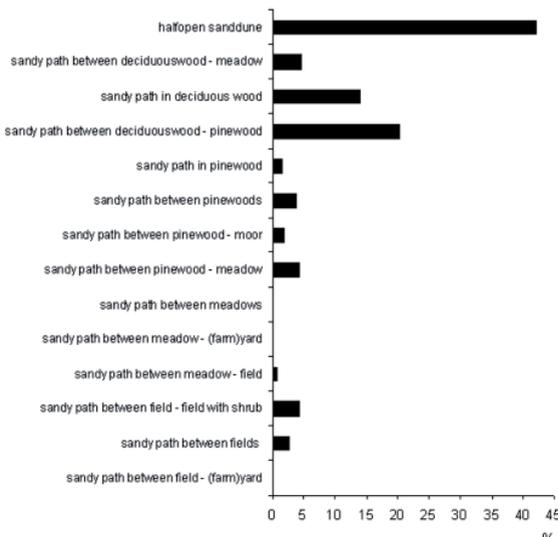


Figure 2. Terrestrial habitat use (%) of the common spadefoot (n = 279) in an old seminatural dune landscape “Overasseltse en Hatertse vennen”, 1988-1992.



Figure 3. A half open old riverdune - an important habitat of the common spadefoot.

reproduced at the two spawning sites in 2004 (Bosman, 2005). Including subadults the population normally should be at least twice as big. Visits to this study area were primarily planned on rainy nights with high temperatures but later on also on dry(er) evenings with high temperatures. Unfortunately we were not able to collect more data. On the contrary visits to the nature reserve “Overasseltse & Hatertse vennen” were hardly ever really planned according to the weatherconditions and nearly always common spadefoots were found (Bosman and van den Munckhof, 1993).

Two habitat types in the agricultural area Groot Soerel could not be investigated optimally. (Farm)yards are private property. This means that nearly always people also live there. Apart from a few exceptions most yards, for privacy reasons were not searched for common spadefoots. Another habitat type that could not be searched well, were the cereal fields. The two fields were sowed too densely with cereal. This made it impossible to find any common spadefoots after the cereal grew higher than 40 cm. It also seemed that the vegetation was so dense that there was hardly any space left for a common spadefoot to dig in. For those reasons these habitat types will be underestimated as terrestrial habitat type of the common spadefoot. When suitable conditions exist, common spadefoots can be found in a private gardens (own observations) and also not too densely sowed cereal fields are known to be used by the common spadefoot (Tobias, 2000).

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Reproductive cycles of the European amphibians: A brief history of studies on the role of exogenous and endogenous factors

Rossana Brizzi, Claudia Corti

Abstract. The European amphibians show seasonal reproductive cycles typical for each species. This behaviour allows that mating, egg-laying and larval development occurs when environmental conditions are most favourable. In addition to exogenous factors (mainly temperature), also endogenous mechanisms (hormonal in nature) play a crucial role in the periodicity of the sexual activity. In this context, we wish to recall the researches of Mario Galgano in clarifying the dynamics of the reproductive cycles on the basis of both external and internal constraints. Galgano described the whole process of gametogenesis in several anurans and urodeles, both under natural and experimental conditions. In addition, from the observations of various species Galgano recognized two different types of gametogenesis (really discontinuous and potentially continuous) according to the prevalence of endogenous rhythms or environmental conditions, respectively.

Mode and time of reproduction in amphibians depends strictly on physiological and morphological responses of these vertebrates to the environment, on the basis of endogenous and exogenous mechanisms. The result is an extraordinary variety of breeding patterns reflecting a compromise among many selective pressures (Duellman and Trueb, 1986).

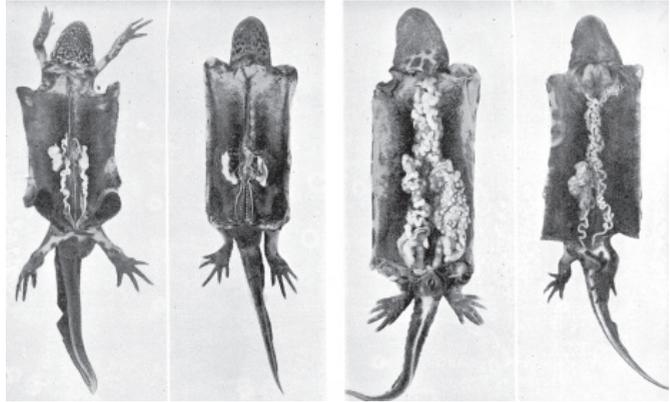
All amphibians of temperate areas, therefore including Europe, show a seasonal reproductive activity that ensures that mating, egg-laying and larval development can take place at those times of the year when environmental conditions are most favourable (Halliday, 1990; Griffiths, 1996). As a rule, the reproductive season is initiated by rising temperatures and spring rains and in some cases extends into the summer. The autumn, usually characterized by an equally suitable climate (at least in the Mediterranean regions) is less used by the amphibians for reproduction. Possibly, this depends on selective and adaptive processes related to the short duration of appropriate conditions, followed by a rapid decrease of temperatures and considerable difficulties for the larvae to complete their development before the winter.

However, the tight correlation between reproductive pattern and climatic parameters is underlined by the comparison of populations of the same species distributed in different areas, for example populations on mainland and islands, or on plains and mountains. Accordingly, the sexual activity is

early or late. The only European amphibians which do not display a clear cyclic pattern of breeding are those that live permanently in caves (*Proteus anguinus*; Durand & Bouillon, 1964), as these environments fluctuate much less in temperature and other physical parameters than surface habitats. In amphibians, reproduction uses up a lot of energy resources (mainly stored in the form of fat bodies) and both sexes must be ready as soon as environmental conditions become favourable. For this reason, all amphibians of temperate regions go through an annual cycle of physiological changes which produces growth and regression of gonads and hormonal changes related to the reproductive activity. At present, it is well known that the mechanisms of hormonal controls are subject to some genetic limitations and their integration produces certain reproductive patterns as a reaction to environmental variables and further constraints imposed by the organism's microhabitat and its species-specific characteristics (Duellman and Trueb, 1986; Houck and Woodley, 1995).

In this respect, we wish to recall the researches of Mario Galgano (1907-1985; Fig. 1) in clarifying the role of exogenous (climatic) and endogenous (mainly hormonal) factors on the reproductive cycles of the European amphibians, both anurans and urodeles. M. Galgano has been one of the most active researchers of the 20th century in the field of amphibian reproduction. On the basis of his wide knowledge of the international literature on this subject, he produced about 80 papers devoted to amphibian caryology, sexual determination

Figure 4. Male (A-B) and female sexual apparatus (C-D) of *T. carnifex* collected in different seasons. A and C: animals in spring reproductive phase, B and D animals in reproductive stasis and gonadic regression in late summer (from the original paper of Galgano, 1944, Tab.II).



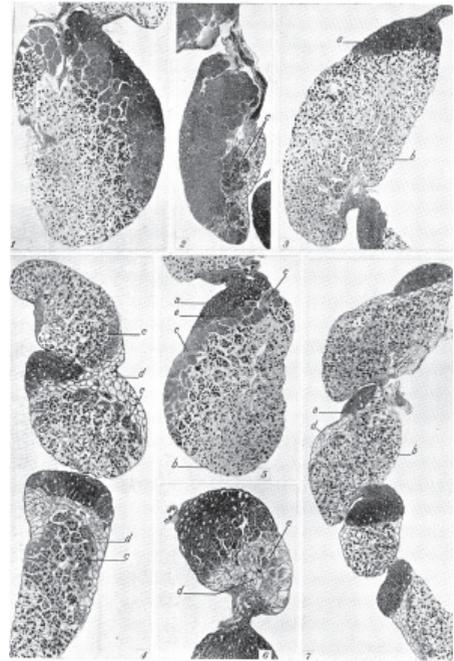
blocked by inappropriate environmental conditions, but can start again when the climatic conditions become better. In these cases we have a potentially continuous gametogenesis (Galgano, 1952 a,b).

The dynamics of reproductive cycles in Amphibia is also under endocrine control, as pointed out by further Galgano studies. From 1940 on, the author focused his attention on the effects of hormonal applications to amphibians at different phases of their reproductive cycles and maintained in experimental conditions (Galgano, 1942a, Galgano and Lanza, 1951). Besides the tight correlation between hypophysis and gonadic activity, the author noticed hormonal control also on secondary sexual characters (Galgano, 1940, 1942b), for example the mating livery (fig. 7). In particular, he demonstrated that the gametogenetic stasis and the regression of the secondary sexual characters depend upon a scarce release of gonadotrophins by the hypophysis. Concerning the production of the latter, this may depend on external factors -as in the case of the potentially continuous cycles- or on strictly endogenous mechanisms, as typical in the really discontinuous cycles. The identification of these two different types of mechanisms is one of the most important results of Galgano's research, and still today these criteria are fundamental for all studies on the reproductive biology of the amphibians (for a review of Galgano's results and a wide discussion on the sexual cycles of the European amphibians see Lanza, 1951).

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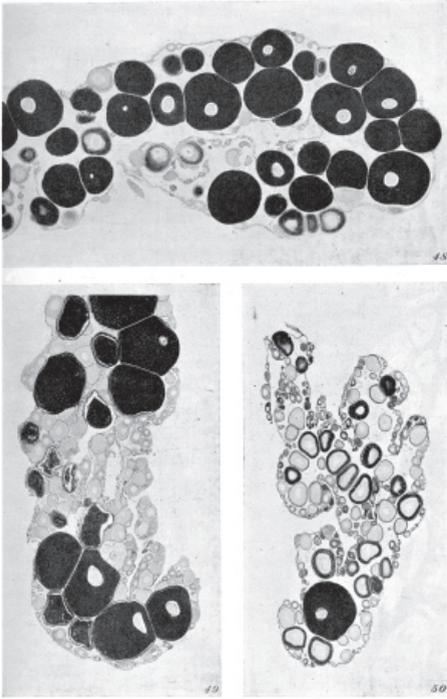
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Figure 5. Sections of testes of *T. carnifex* collected in different seasons (explanations in the original paper of Galgano, 1947, Tab. I).



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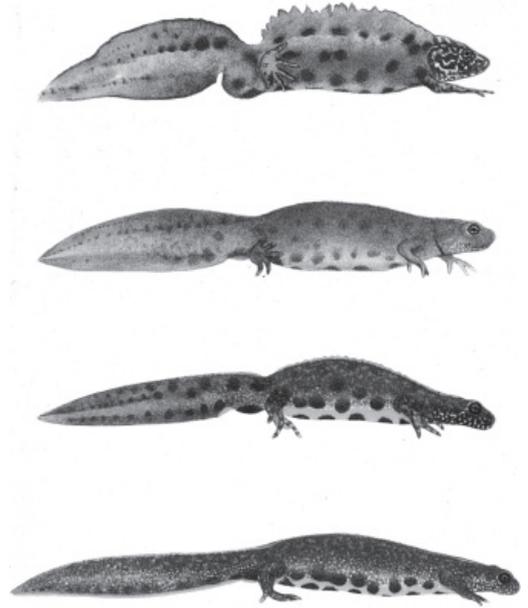
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Establishing an amphibian monitoring program in two protected areas of Romania

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Abstract. Aquatic habitats and amphibian species were inventoried during 2004-2005 in two nearby protected areas in Romania. The Retezat National Park and the nearby Hateg Geopark cover together an area of 1400 km² along an altitudinal range between 400-2500 m. We inventoried over 200 aquatic habitats and identified 11 amphibian species. We selected non-randomly 14 sites for monitoring based on several criteria as accessibility, likely permanence on medium term, representative coverage throughout the protected areas, and high amphibian species richness. We propose a low and a medium cost amphibian monitoring programs.

Introduction

The widespread decline of amphibian populations and the multitude of factors causing this (Green, 2003; Kiesecker et al., 2001; Stuart et al., 2004) suggest a need to monitor amphibian populations (Dodd, 2003). Since factors affecting population dynamic may have both natural and anthropogenic origins, monitoring programs are required to track changes in populations, communities, and habitat quality to better identify the causes of their changing in time (Pechmann, 2003).

Two elements are crucial to ensure the success of a monitoring program: well-defined objectives and science-based data from which reliable inference can be made (Pellet and Schmidt, 2005). Information and data obtained from regular monitoring programs can be enhanced, if data from pristine or low impact areas are available and can be used as a reference.

Romania still has large areas of relatively pristine habitats that can offer useful information for a continental scale amphibian monitoring program. Baseline information on the status and trends of Romanian amphibians is sparse. To fill this gap we surveyed the aquatic habitats from two nearby protected areas from Romania. We focused on two objectives: (1) gather baseline data through the inventory of habitat availability and use by amphibian communities, and (2) select aquatic habitats and identify species for the establishment of a medium-term (5-10 years) monitoring program.

Materials and methods

Study area. - Retezat Mountains National Park (RNP) and the nearby Hateg Geopark (HG) together cover an area of 1400 km² ranging in elevation between 400-2500 m a.s.l. Several arguments point in favor of establishing a monitoring program in these areas. RNP is one of the least human-affected protected areas in Central Europe, with dense forests including old-growth stands. It is the oldest national park in Romania, established by law in 1935. Glacial and cryonival relief are extremely widespread allowing lakes to form in the deeper parts of moraines. Fifty-eight permanent glacial lakes were recorded at elevations between 1700-2300 m. The Retezat Mountains have the highest rainfall and runoff in the Romanian Carpathians. Their glacial lakes are some of the least impacted freshwaters in Europe (Curtis et al., 2005). HG borders RNP to the north, and was established as a protected area in 2004. It is located at a lower elevation and has a highly diverse rural landscape (e.g., hayfields, forests, orchards, agroecosystems, sparsely disseminated rural areas). Together the two parks contain a variety of aquatic habitats, ranging from low elevation reservoirs, irrigation canals, fens and marshes to high elevation alpine lakes and temporary ponds. Of the 19 species of amphibians inhabiting Romania, 11 species occur in the two parks (Cogălniceanu et al., 2001).

Habitat and species inventory. - We began an inventory of aquatic habitats in 2000 in RNP and in 2004 in HG. A prior identification of permanent aquatic habitats was based on detailed 1:25.000 or 1:5.000 topographic maps. We measured geographical coordinates, elevation, area and average depth, water pH, conductivity, temperature, and transparency for each aquatic habitat. We also recorded the presence/absence of aquatic predators such as insects and insect larvae, leeches, and fish. We characterized the surrounding terrestrial habitat in terms of substrate, vegetation cover and human activities. - Species inventory in the two parks was based on repeated visits and the combination of a variety of census techniques (see Dodd et al., in press) in order to maximize the detection of species, as recommended by Ryan et al. (2002).

Species accumulation curves are good estimates of the amount of sampling required for a complete species inventory within a certain area. We computed species accumulation curves using

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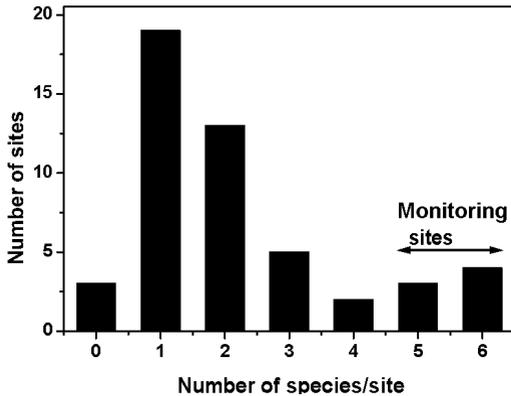


Figure 1. Species richness of the aquatic habitats inventoried in Hațeg Geopark.

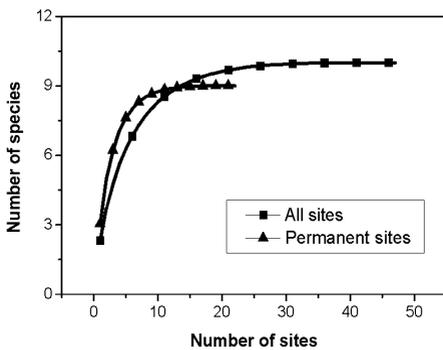


Figure 2. Cumulative species accumulation curves for amphibians inventoried at Hațeg Geopark: for all of the sites visited (where each site was considered as a single data point despite the multiple visits made), and for the six permanent sites (where each visit to a particular site was considered as an independent event).

EstimateS 5.0 (Colwell, 1997). By randomizing sample order (100 randomizations) and computing the mean species richness estimate for each sample accumulation level, EstimateS removes the effect of sample order and generates a smooth species accumulation curve.

We assembled baseline data for the species identified for monitoring, including estimates of population size and reproductive effort and success. A database was begun with digital images for individual identification based on the dorsal (e.g. in *Rana temporaria*) or ventral pattern (e.g. in *Bombina variegata*) (Plăiașu et al., 2005), for mark-recapture population estimates.

Results

Aquatic habitat inventory

We inventoried aquatic habitats throughout the major parts of the two parks: 46 in HG and 156 in RNP. We selected 14 sites for monitoring of which

six were located at low elevations (HG), and eight sites above the tree-line (RNP). The selection was non-random, based on several criteria: accessibility, likely permanence over the course of the study (i.e. no immediate threat of destruction), representative coverage throughout the protected areas, origin and types of habitats, and high amphibian species diversity (table 1; fig. 1).

Apart from the permanent sites selected for monitoring, we selected an alpine valley in the RNP (Judele Valley, elevation range 2000-2170 m a.s.l., lat. 45.35 N, long. 22.8 E) for a detailed analysis of habitat availability and use by amphibians. We inventoried 23 aquatic sites (including clusters of ponds), in which three species of amphibians were found: *Rana temporaria* (present at 52% of the sites), *Triturus alpestris* (18.5%) and *B. bufo* (9.2%).

Amphibian species inventory

Ten amphibian species and a species complex occur in the two protected areas: *Triturus vulgaris*, *T. alpestris*, *T. cristatus*, *Salamandra salamandra*, *Bombina variegata*, *Hyla arborea*, *Bufo viridis*, *B. bufo*, *Rana dalmatina*, *R. temporaria* and *R. esculenta* complex.

Species detectability at the six sites selected for permanent monitoring in HG varied according to species, among sites, and time of year from between 0 (i.e., no species recorded) and 100% (i.e., all known species recorded during one visit) with an overall mean value of 45%.

The species accumulation curves computed for HG showed that species richness reached an asymptote (i.e. the inventory was completed) more rapidly when based on repeated visits to sites selected for permanent monitoring, when compared to all of the aquatic habitats sampled (fig. 2). Monitoring the selected sites is thus more informative in terms of detecting shifts in amphibian community richness and composition.

Since multispecies surveys are usually inefficient for rare species (Pellet and Schmidt, 2005), we selected three species with wide range and high detectability for intensive monitoring at a population level (fig. 3). *Bombina variegata* is the most widespread species in the lowlands, but is also expanding into the alpine area. *Rana temporaria* is widespread between 600-2200 m, and the only species reaching high elevation lakes. *Triturus alpestris* is rather abundant but due to lower dispersal abilities, has rather isolated populations, especially in alpine

Table 1. Elevation and physico-chemical parameters of the aquatic sites investigated in Retezat Mountains National Park (RNP) and Hateg Geopark (HG). Only the results from 2004-2005 are presented below, and only high elevation site information is included for RNP. The values given are the mean \pm one standard deviation and, in parentheses, the minimum and maximum values.

Protected area	Elevation (m)	pH	Conductivity (μ S)	Water temperature ($^{\circ}$ C)	Species richness
RNP Alpine area, N=59	2080 \pm 80 (1920-2260)	7.04 (4.73-8.46)	12.8 \pm 5.4 (4.5-38)	13.9 \pm 4.2 (2.4-26)	Only three species present
HG Selected sites, N=6	490.6 \pm 86.9 (384-594)	7.43 (5.42-8.47)	185 \pm 156 (75 - 465)	20.5 \pm 1.5 (14.5 - 25)	5.66 \pm 0.5 (5-6)
HG Temporary ponds N=40	472 \pm 117 (337-813)	6.76 (6.08-7.54)	251 \pm 149 (86-678)	23.1 \pm 4.6 (12-34)	1.65 \pm 1.1 (0-5)

areas. We estimated population size for these three species in all permanent monitoring sites, as reference data.

Discussion

Our proposed monitoring program is facing two important sources of variation: selecting areas for surveying that will permit inference to the entire area of interest, and detection of the species selected for monitoring. Due to the inconspicuous behaviour of amphibians, the use of abundance and site occupancy estimators is highly recommended (MacKenzie et al., 2002).

One of the major issues is identifying the basic demographic unit for monitoring population trends, i.e. if it should be an individual pond or a cluster of local ponds. We agree with Petranka et al. (2004) that pond populations only several tens or hundred meters apart should be treated as subpopulations of the same monitoring unit. Thus, several of the sites selected for monitoring consist of clusters of up to six different ponds.

Large monitoring programs may involve high costs still the resulting data can often be biased. Our approach towards establishing a monitoring program is more realistic and practical, emphasizing feasibility. It is difficult to convince decision makers of the utility and importance of a long-term monitoring program. Recently, two long-term amphibian monitoring programs in Europe were stopped due to lack of funding (Hachtel et al., 2005, Jehle et al., 1997). One of our concerns was how to avoid a similar outcome. The solution seemed to be a low-cost program, with reasonable data output, that would justify its continuation. The difficulty was in deciding the minimal threshold of effort required for

generating statistically robust data. Finally, low-cost (with minimal coverage) and medium-cost (which would generate more high quality data) scenarios were proposed.

We suggest that the project should be evaluated after five years to assess its utility and reconsider its goals. An additional 15-45 sites each year (depending on funds available) randomly selected from previously inventoried sites will be monitored each year besides the permanent sites. Minimum sampling effort will involve one experienced investigator-hour for a permanent site, with at least three visits annually. The low-cost scenario will involve monitoring only permanent sites and an additional randomly chosen 15 sites (five at high elevations in RNP and 10 in HG). Our approach would include each temporary aquatic site in the inventory at least once every 4-5 years. The medium cost approach will involve sampling an additional 30-35 randomly chosen sites (with 10-15 in RNP). From the variety of inventory techniques available, we recommend time-constrained visual surveys and dip-netting. This means that each site will be sampled every 2-3 years. Since temporary

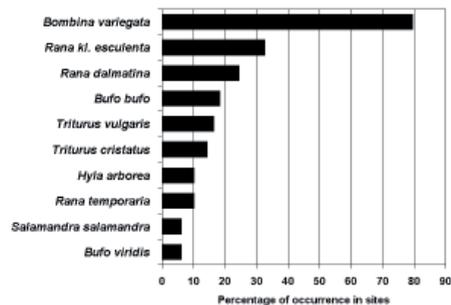


Figure 3. Frequency of occurrence of amphibians within the Hateg Geopark.

ponds are important but unpredictable habitats for amphibians (Griffiths, 1997; Cogălniceanu, 1999), regular monitoring might provide data too variable for reliable trend analyses.

Expected outcomes of the monitoring program

There are several important outcomes of the proposed monitoring program, including the opportunity to detect climate changes at high elevations in RNP due to global weather changes, and the resulting shifts in distribution of *Bufo bufo*, *Bombina variegata* and *Triturus alpestris* at higher elevations. We expect an increase in human pressure or changes in human activities at lower elevations in HG due to the EU Common Agriculture Policy that will affect rural communities. The amphibian monitoring program could provide a much needed and rapid assessment of the resulting environmental impact. It will also provide support for the protected areas management plans (Semlitsch, 2000), currently being prepared for HG and awaiting revision for RNP.

Monitoring activities usually yield a low publication output and are thus unattractive to the highly competitive scientific community. The baseline data gathered during the proposed monitoring program can identify and support the establishment of directed research projects and thus become more attractive. Finally, the approach outlined in our proposed monitoring program could be extended to three other large protected areas linking the Danube Valley with the Southern Carpathians (Iron Gates Natural Park, Cerna-Domogled National Park and Grădiște-Cioclovina Natural Park).

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The Snakes of Sulawesi

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Abstract. From the existing literature, museum specimens and field data an overview was produced of all currently known Sulawesi land snakes. The checklist of In den Bosch (1985) contained fifty-five species of Sulawesi land snakes. We consider forty-four of these to inhabit Sulawesi with certainty, and added eight species, bringing the total number of certain species to 52. Eleven species of In den Bosch's list and four added by us are of doubtful occurrence for Sulawesi, giving a total of 15 doubtful species. The taxonomy of species in several genera needs to be reviewed (e.g. *Enhydryis*, *Rhabdophis*, *Cylindrophis*). In order to determine which populations are threatened and in need of conservation, surveys should be carried out. This should be done with priority for the following species, since it is our impression that they are relatively rare or (potential) victim of human activities: *Candoia carinata carinata*, *Candoia paulsoni tasmai*, *Boiga tanahjampeana*, *Elaphe flavolineata*, *Gonyosoma janseni*, *Ophiophagus hannah*, *Python molurus bivittatus*, *Python reticulatus*, *Trimeresurus fasciatus* and the red and green colour morph of *Tropidolaemus wagleri*.

Introduction

Indonesia is a country in the tropics with over 200 million inhabitants. It consists of about 17,000 islands scattered around the equator between West-Malaysia and Australia. Indonesia is one of the two countries in the world, with ecosystems possessing the highest degree of biodiversity (Mittermeier et al., 1999). Its habitats and species are threatened by increasing demands from a growing population, resulting in habitat destruction and species overexploitation by hunting and collecting. In order to put into effect any form of nature conservation, we need to know which species are present and what their conservation requirements are.

Sulawesi (formerly Celebes) is one of the five largest Indonesian islands. It has a strangely contorted form with four peninsulas. This is the result of a number of collisions between parts of the ancient continent Gondwana, fifteen to three million years ago (Audley-Charles, 1987). Most of Sulawesi's entire surface is mountainous. Several volcanoes, eleven of which are still active, rise on the northern peninsula up to the Sangihe Islands. The landscape is covered with lowland forests, montane forests, forests on soils of ultrabasic rocks and of limestone, beach vegetation, swamp forests, and mangrove forests. In areas with a shortage of rain there are monsoon forests. Sulawesi is located in the wet tropical climatic zone. The temperature is relatively constant, 26 – 30 °C along the coast and about 5 °C lower in the mountains.

In the past several investigators observed a remarkable east-west differentiation in the fauna of the Indo-

Australian archipelago. Three biogeographical lines were defined: Wallace's Line, Weber's Line and Lydekker's Line (fig. 1). The Malay Peninsula and the Greater Sunda Islands Sumatra, Borneo, Java and Bali belonged to the former Sunda shelf, which is presently inundated in part. New Guinea and Australia were parts of the former Sahul shelf. Wallace's Line delimits the eastern boundary of the Asian fauna. Lydekker's Line delimits the western boundary of the Australian fauna. Both these lines effectively follow the 180-200 m depth contours of the Sunda and Sahul shelves. The area between the two lines, including Sulawesi, has been nominated as a separate region, called Wallacea. This area has always been isolated on a biogeographical basis. As a result a unique fauna developed, which is not a transition between the fauna of the two shelves, although a number of Papuan species reach their western limit in Sulawesi and a number of Asian species reach their eastern limit here (Whitten et al., 1987). How and Kitchener (1997) calculated the geographic similarities of all land snakes present on 36 Indonesian islands. They found that the major boundary in the snake fauna of Indonesia is not Wallace's Line, but Weber's Line.

Approximately 217 of the 2900 snake species worldwide (EMBL reptile database, January 2004, see www.embl-heidelberg.de/~uetz/LivingReptiles.html) occur on the Indonesian islands Sumatra, Borneo, Java and Sulawesi (Inger and Voris, 2001). In the last ten years several checklists and illustrated publications became available, describing the snakes of parts of Indonesia. The checklist of In den Bosch (1985) was a first attempt to provide an inventory of the snakes of Sulawesi. An illustrated field guide of the land snakes of Sulawesi did not exist however, and we therefore produced one (De Lang and Vogel, 2005).

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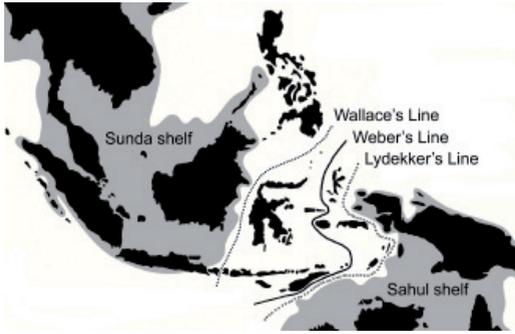


Figure 1. Biogeographical lines in the Indo-Australian archipelago.

Materials and methods

All records of Sulawesi land snake species in the scientific literature from 1837 up to and including 2003 and personal records of the authors and others were stored in a database and reviewed. A checklist of the snakes certainly inhabiting Sulawesi and a list of doubtful Sulawesi records were prepared. Distribution records of species on the checklist had to come from at least one, preferably two independent reliable sources. Sea snakes were not included. Species on the checklist were described in detail, using data from the existing literature, museum specimens and the field. Species on the doubtful list have in common that their records are old, that most of them have not been confirmed by other field workers in a period of over hundred years after publication and that their occurrence outside of Sulawesi is not rare.

The geographical area covered is Sulawesi's five administrative provinces: North-Sulawesi, including the Talaud and Sangihe Islands in the north; Gorontalo, in the central region of the northern peninsula; Central-Sulawesi, including the Togian Islands south of the northern peninsula and the Banggai and Bowokan Islands in the east; Southeast-Sulawesi, including the islands Wowoni, Buton, Muna, Kabaena, and the Tukangbesi Islands in the southeast; and South-Sulawesi, including the islands of Selayar and Tanahjampea, and the Bonerate Islands and Kalaotoa in the south.

Results

The checklist, containing fifty-two species, is presented in the Appendix.

In addition to the checklist, the following snakes have been discovered recently, but not yet formally described: *Enhydris* n. sp., a crescent-spotted snake from Mount Lompobatang, Southwest-Sulawesi, caught at an altitude of over 1200 m (D.T. Iskandar, pers. comm.); a paddle-tailed water snake from Lake Towuti (near Lake Matanna), Central-Sulawesi, which looks like an *Enhydris* species, but is probably a new homalopsine genus (D.T. Iskandar, pers. comm.); and

two *Calamaria* n. sp. from Buton Island, Southeast-Sulawesi (G. R. Gillespie, pers. comm.).

The list of doubtful records, contains fifteen species of which the presence in Sulawesi is unlikely but cannot be excluded: *Boiga multomaculata* (Boie, 1827); *Bungarus candidus* (Linnaeus, 1758); *Calliophis intestinalis* (Laurenti, 1768); *Enhydris enhydris* (Schneider, 1799); *Gonyosoma oxycephalum* (Boie, 1827); *Homalopsis buccata* (Linnaeus, 1758); *Naja sputatrix* Boie, 1827; *Oligodon octolineatus* (Schneider, 1801); *Pseudorabdion longiceps* (Cantor, 1847); *Rhabdophis chrysargos* (Schlegel, 1837); *Rhabdophis subminiatus subminiatus* (Schlegel, 1837); *Trimeresurus albolabris* (Gray, 1842) or *Trimeresurus insularis* Kramer, 1977; *Typhlops ruficaudus* (Gray, 1845); *Xenochrophis melanzostus* (Gravenhorst, 1807); *Xenochrophis vittatus* (Linnaeus, 1758).

Discussion

In his checklist, In den Bosch (1985) included fifty-five species of land snakes. We consider forty-four of them to be definitely present on Sulawesi. We added eight species, for the following reason: four species saw their geographic range expanded; one species was added because of a taxonomic change; one species which was discovered after 1985; one species because of a difference in opinion and one species which was overlooked. Therefore our checklist contains 52 certain species definitely occurring on Sulawesi. We found four species doubtful for Sulawesi in addition to the eleven species on In den Bosch's checklist, regarded by us as doubtful in retrospect. This brings the total number of doubtful records at 15.

The taxonomy of species in several genera is weak and needs to be reviewed. The differences between *Enhydris matannensis*, *E. plumbea* and *E. enhydris* for instance are small. One of the reasons is that the description of *Enhydris matannensis* is based on two specimens only. In the genus *Rhabdophis* it is not certain whether it is justified to distinguish *R. callistus* from *R. chrysargoides* only on the basis of a difference in colouration of the juveniles. The differences between the species *Cylindrophis isolepis*, *C. melanotus* and *C. ruffus ruffus* are also small and for *C. isolepis* data is taken from only three specimens.

That the island of Sulawesi has always been isolated can be seen from the low species richness and the high level of endemism in its fauna, relative to the Greater Sunda Islands (Whitten et al., 1987). This is the case for several taxa, including frogs and land snakes. In

Sumatra there are 127 species of land snakes, of which 16 % are endemic (David and Vogel, 1996). In Borneo there are 133 species (23 % is endemic) (Stuebing and Inger, 1999). For the land snakes of Sulawesi our data shows that 22 of the 52 species are endemic, which equals 42 %.

In order to determine which snake populations are threatened and in need of conservation, surveys should be carried out urgently. Thus far herpetofaunal inventories taken in Sulawesi have only been on a very limited scale. We believe that surveys should be made, with priority given to the following species, since it is our impression that they are relatively rare or (potential) victim of human activities: *Candoia carinata carinata*, *Candoia paulsoni tasmai*, *Boiga tanahjampeana*, *Elaphe flavolineata*, *Gonyosoma janseni*, *Ophiophagus hannah*, *Python molurus bivittatus*, *Python reticulatus*, *Trimeresurus fasciatus* and the red and green colour morph of *Tropidolaemus wagleri*.

During the production of the field guide “The Snakes of Sulawesi” we encountered some remarkable facts. It is generally known that Wagler’s Palm Viper (*Tropidolaemus wagleri*), a common snake in Sulawesi, is coloured green (fig. 2). We however point to the existence of a rare “red form”. Fig. 3 is the first colour photo of the “red form” ever published. This form is only found in North- and Central-Sulawesi. Boulenger (1897) gave the first description and a drawing of the “red form”. Heinrich (1932) showed a B/W photo of probably a “red form” specimen. Ahl (1933) was the last researcher mentioning the “red form”. So this form has been overlooked for a period of over 70 years.

Conclusions

1. We found fifty-two species of landsnakes certainly living in Sulawesi and fifteen species of doubtful occurrence.
2. Surveys should be carried out to determine which snake populations are threatened and in need of conservation. In our opinion priority should be given to the species *Candoia carinata carinata*, *Candoia paulsoni tasmai*, *Boiga tanahjampeana*, *Elaphe flavolineata*, *Gonyosoma janseni*, *Ophiophagus hannah*, *Python molurus bivittatus*, *Python reticulatus*, *Trimeresurus fasciatus* and the red and green colour morph of *Tropidolaemus wagleri*.

Acknowledgements. We are grateful to Mark Wootten, UK for the quick correction of the English language.



Figure 2. *Tropidolaemus wagleri*, common “green form” from Lambunu, North-Sulawesi.



Figure 3. *Tropidolaemus wagleri*, “red form” from Tangko-Batuangas Nature Reserve, Northeast-Sulawesi.

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Appendix. Checklist of the snakes of Sulawesi, containing the species of land snakes, known to occur with certainty in Sulawesi on January 1st 2004. Species or subspecies endemic for Sulawesi are marked with (E).

Family Acrochordidae

Acrochordus granulatus (Schneider, 1799)

Family Boidae - Subfamily Boinae

Candoia carinata carinata (Schneider, 1801)
Candoia paulsoni tasmai Smith & Tepedelen, 2001

Family Colubridae - Subfamily Calamariinae

Calamaria acutirostris Boulenger, 1896 (E)
Calamaria apraeocularis Smith, 1927 (E)
Calamaria boesemani Inger & Marx, 1965 (E)
Calamaria brongersmai Inger & Marx, 1965 (E)
Calamaria curta Boulenger, 1896 (E)
Calamaria muelleri Boulenger, 1896 (E)
Calamaria nuchalis Boulenger, 1896 (E)
Calamaria virgulata Boie, 1827
Calamohabdium acuticeps Ahl, 1933 (E)
Pseudorabdion sarasinorum (Müller, 1895) (E)
Rabdion forsteni Duméril, Bibron & Duméril, 1854 (E)

Family Colubridae - Subfamily Colubrinae

Ahaetulla prasina prasina (Boie, 1827)
Boiga dendrophila gemmicincta (Duméril, Bibron & Duméril, 1854) (E)
Boiga irregularis (Merrem, 1802)
Boiga tanahjampeana Orlov & Riabov, 2002 (E)
Chrysopelea paradisi celebensis Mertens, 1968 (E)
Chrysopelea rhodopleuron viridis Fischer, 1880 (E)
Dendrelaphis caudolineatus terrificus (Peters, 1872)
Dendrelaphis pictus pictus (Gmelin, 1789)
Elaphe erythrura celebensis (Jan, 1863) (E)

Elaphe flavolineata (Schlegel, 1837)
Gonyosoma janseni Bleeker, 1858 (E)
Lycodon capucinus Boie, 1827
Lycodon stormi Boettger, 1892 (E)
Oligodon waandersi (Bleeker, 1860) (E)
Psammodynastes pulverulentus pulverulentus (Boie, 1827)
Ptyas dipsas (Schlegel, 1837) (E)

Family Colubridae – Subfamily Homalopsinae

Cerberus rynchops rynchops (Schneider, 1799)
Enhydris matannensis (Boulenger, 1897) (E)
Enhydris plumbea (Boie, 1827)

Family Colubridae – Subfamily Natricinae

Amphiesma celebicum (Peters & Doria, 1878)
Amphiesma sarasinorum (Boulenger, 1896) (E)
Rhabdophis callistus (Günther, 1873) (E)
Rhabdophis chrysargoides (Günther, 1858)
Xenochrophis trianguligerus (Boie, 1827)

Family Cyliandrophiidae

Cylindrophis isolepis Boulenger, 1896 (E)
Cylindrophis melanotus Wagler, 1828
Cylindrophis ruffus ruffus (Laurenti, 1768)

Family Elapidae – Subfamily Bungarinae

Ophiophagus hannah (Cantor, 1836)

Family Pythonidae

Python molurus bivittatus Kuhl, 1820
Python reticulatus jampeanus Auliya et al., 2002 (E)
Python reticulatus reticulatus (Schneider, 1801)
Python reticulatus saputrai Auliya et al., 2002 (E)

Family Typhlopidae

Cyclotyphlops deharvengi In den Bosch & Ineich, 1994 (E)
Ramphotyphlops braminus (Daudin, 1803)
Ramphotyphlops olivaceus (Gray, 1845)
Typhlops ater Schlegel, 1839
Typhlops conradi Peters, 1874 (E)

Family Viperidae – Subfamily Crotalinae

Trimeresurus fasciatus (Boulenger, 1896) (E)
Tropidolaemus wagleri Wagler, 1830

Family Xenopeltidae

Xenopeltis unicolor Boie, 1827

The national amphibian monitoring program in the Netherlands and NATURA 2000

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Abstract. In the Netherlands, national flora and fauna monitoring programs are organised by NGOs in close cooperation with Statistics Netherlands. RAVON (Reptile, Amphibian and Fish Research Netherlands) is the NGO that coordinates the reptile and amphibian monitoring programs. For most species, volunteers collect the field data. Preferably, monitoring is carried out in all areas where a species is present. Providing data on the NATURA 2000 species *Triturus cristatus* and *Bombina variegata* has a high priority. In addition, the relatively widespread species in the Netherlands *Bufo calamita*, *Rana lessonae* and *R. arvalis* get special attention. Populations of the Annex IV species *Pelobates fuscus* and *Hyla arborea* are monitored within the scope of Species Protection Plans.

Introduction

In the Netherlands, national flora and fauna monitoring programs are organised by NGOs in close cooperation with Statistics Netherlands. The programs are carried out within the framework of the Network Ecological Monitoring (NEM). The NEM programs are funded by the Ministry of Agriculture, Nature and Food Quality. The main target of the NEM programs is to provide information for evaluation of Dutch nature policy. In the NEM there are programs for mycoflora, flora, birds, mammals, reptiles, amphibians, dragonflies, moths and butterflies.

RAVON (Reptile, Amphibian and Fish Research Netherlands) is the NGO that coordinates the reptile and amphibian monitoring programs (Zuiderwijk et al., 1999; Smit et al., 1999). The amphibian monitoring program developed into a network of, to date, more than 150 observers. New observers are recruited every year by promoting the program especially at local nature conservation and nature study groups, and by distributing biannual newsletters.

The main targets of the Amphibian Monitoring Program are:

- Detecting changes in populations of Habitats Directive species in the Netherlands and within NATURA 2000 areas.
- Detecting changes in populations of 'target' amphibian species of the Dutch Nature Policy Plan.

Target species for the Dutch nature policy are listed in the Nature Policy Plan of 1990. In 2004, the Dutch government proposed a list of Special Protection Areas, as part of NATURA 2000. This includes areas for *Bombina variegata* and *Triturus cristatus*, the two amphibian species in the Netherlands that are listed at Annex II, as well as Annex IV of the European Habitats Directive. In 2005, the NATURA 2000 target was added to the Amphibian Monitoring Program. In this article, we discuss the developments in the Program since its start and the consequences of the implementation of the NATURA 2000 target.

Target species

In the Netherlands, 16 native amphibian species are found. Nine species are considered as Red List species (Hom et al., 1996; Ministerie LNV, 2004). Seven species are listed at Annex IV, two species are also listed at Annex II of the Habitats Directive (table 1). As a result of the international reporting obligations, the focus of the Amphibian Monitoring Program recently switched from Red List species to Habitats Directive species. Providing data on the NATURA 2000 species *Triturus cristatus* and *Bombina variegata* has a high priority. In addition, the relatively widespread Annex IV species *Bufo calamita*, *Rana lessonae* and *R. arvalis* in the Netherlands get special attention. Populations of the Annex IV species *Pelobates fuscus* and *Hyla arborea* are monitored within the scope of Species Protection Plans.

Field methods

For most species, volunteers collect the field data. Preferably, monitoring is carried out in all areas where a species is present. For several species this

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requires a high investment of resources. In practise, coverage of the entire species' distribution area is only feasible for rare species and to some extent for species with a regionally restricted distribution.

On average, a monitoring unit is an area of a maximum one hundred hectares. Within this unit a number of potential breeding waters are sampled. This includes suitable reproduction sites as well as marginal reproduction sites. In areas with a large number of waters, where field surveys are relatively time consuming, we set a maximum of waters that can be handled within half a day of fieldwork. This implies that in most cases, volunteers invest half a day of fieldwork on each visit. Each year, all waters are visited approximately four times, including night-visits. For each water surveyed, all amphibian species are recorded. For each species the abundance is estimated and registered as an index-value (0-absent; 1-rare, some individuals are present; 2-common, approximately a dozen individual adults are present; 3-very common, several dozens or more are present). For rare species, the exact numbers of individuals are registered (Smit et al., 1999). The Amphibian Monitoring Program includes calling surveys of, for instance, *Hyla arborea* and *Bufo calamita*. Registering the numbers of calling males is an efficient way for surveying large areas (Pellet and Schmidt, 2005). For *Salamandra salamandra*, transect monitoring is applied by counting sightings along fixed routes. For every observer a handbook is available with detailed information about the methodology (Groenveld and Smit, 2001).

Index calculation

Indices and trends for the species are calculated using TRIM, a statistical program based on Poisson regression designed for fauna monitoring data with

missing values (Pannekoek and Van Strien, 1998, Van Strien et. al., 2001). Trends are presented with their slopes and standard errors. Annual indices represent the yearly numbers as a percentage of the numbers in the first year of monitoring. The index can be based on presence/absence and on the estimated abundance of a species. Assessing presence/absence is assumed to be more reliable than estimating numbers of animals. An index based on numbers of animals (abundance-index) is expected to be more sensitive for trend detection. We tested four options for calculating the indices:

1. the total presence per site as unit;
2. the total abundance per site as unit;
3. the total presence of all waters;
4. the total abundance of all waters.

The index based on the presence/absence can be interpreted to represent changes in local distribution (option 1). The index based on abundance represents the number of animals per sample site (option 2). The option for calculating the abundance differs for each species. In general, the procedure involves the following steps: 1) for each water-body the maximum abundance in a year is established, 2) the maximum abundances are summed.

Bufo calamita and *Hyla arborea* are an exception to this option. The index for this species is based on the maximum abundance per sample site per visit. This option takes into account the possible migrations of choruses within one season. For other species this behaviour is omitted. Tests performed by Statistics Netherlands did not indicate a dependency between waters within sample sites. Therefore a calculation based on presence/abundance in individual waters is also an option. This results in a higher number of units (larger sample size) and will increase the reliability (options 3 and 4).

Species	Red List	Habitats Directive	Occurrence
<i>Salamandra salamandra</i>	Threatened		Few localities
<i>Triturus cristatus</i>	Vulnerable	Annex II, IV	Nationally
<i>Triturus helveticus</i>	Vulnerable		Regionally
<i>Alytes obstetricans</i>	Vulnerable		Regionally
<i>Bombina variegata</i>	Endangered	Annex II, IV	Few localities
<i>Pelobates fuscus</i>	Threatened	Annex IV	Regionally
<i>Bufo calamita</i>		Annex IV	Nationally
<i>Hyla arborea</i>	Threatened	Annex IV	Regionally
<i>Rana arvalis</i>	Vulnerable	Annex IV	Nationally
<i>Rana lessonae</i>	Vulnerable	Annex IV	Nationally

Table 1. Target species of the Dutch Amphibian Monitoring Program and their status. Red List categories refers to the national Red List of the Netherlands.

Table 2. The four options for calculating indices with *Rana arvalis*.

1997	1998	1999	2000	2001	2002	2003	2004	n	slope	stdev
Indices based on presence per site										
100	116	122	122	104	124	128	102	42	1.0063	0.0246
Indices based on abundance per site										
100	116	121	118	89	128	128	97	42	1.0016	0.0230
Indices based on presence in all waters										
100	116	122	122	104	124	128	102	125	1.0064	0.0203
Indices based on abundance in all waters										
100	116	121	118	89	128	128	97	125	1.0016	0.0195

Table 3. The indices over 1997–2004 calculated over the total number of waters (*Triturus cristatus*, *Rana arvalis*, *R. lessonae*) or total number of sample sites (*Bufo calamita*).

Species	1997	1998	1999	2000	2001	2002	2003	2004	slope	stdev
<i>Triturus cristatus</i> 160 waters (54 sample sites)										
	100	115	121	102	96	104	104	136	1.0138	0.0224
<i>Triturus cristatus</i> in NATURA 2000 55 waters (16 sample sites)										
	100	115	140	82	80	103	75	161	1.0032	0.0341
<i>Rana arvalis</i> 125 waters (42 sample sites)										
	100	116	121	118	89	128	128	97	1.0016	0.0195
<i>Rana lessonae</i> 266 waters (sample 55 sites)										
	100	124	134	128	125	108	114	105	0.9916	0.0099
<i>Bufo calamita</i> 76 sample sites (251 waters)										
	100	91	98	111	93	81	80	80	0.9653	0.0387

Results 1997 - 2004

The Amphibian Monitoring Program has grown steadily from 61 sample sites in 1997 to 253 in 2004. The total numbers of waters that has been surveyed so far adds up to 2,200. Not all sample sites are visited annually. Some observers stop monitoring for private reasons. Of all sites that have so far been surveyed, about 50% were visited in 2004. Below we present the results of four more widely distributed target species and the contribution of volunteers to data collection.

The four options for calculating the indices mentioned above are given for *Rana arvalis* in table 2 as example. *R. arvalis* is monitored at 42 sites, in a total of 125 waters. If the standard deviation is below 0.02, we consider the indices to be reliable. The standard deviation using all water-bodies is better in comparison to the site indices. The last index, the total abundance of all waters, is the most reliable. We will use this approach to analyse the other Annex IV species.

Rana arvalis.—The development of the indices over the last eight years (table 3) shows a steady increase from 1997 to 2003 and low figures in 2001

and 2004. For the overall period the population of *Rana arvalis* is considered to be stable. The index is representative for its eastern distribution areas. Not all major areas where *R. arvalis* occurs are yet included.

Triturus cristatus.—The development of the indices at national level (table 3) shows an increase since 1997 with the highest index for 2004. The standard deviation is 0.0224. There is no trend in the indices at national level (Table 3). The indices for NATURA 2000 sample sites show a strong variation. The number of sample sites within NATURA 2000 is 16, observed in 55 water-bodies and can be considered to be still too limited for reliable trend detection.

Rana lessonae.—For most people it is not feasible to distinguish the three types of *R. esculenta* synklepton. People just monitor ‘green frogs’. The index of *Rana lessonae* is based on sample sites where, to our knowledge, *R. lessonae* is present. The index increased in the first period, but for the overall period the situation is considered to be stable. The standard deviation is 0.0099. All major distribution areas are included. The index is not corrected for over and under sampling. We intend to apply such corrections in the future.

Bufo calamita.—The coverage of the sample sites improved strongly since the introduction of routes that focus only on calling animals. The indices shows a decrease since 2002. The standard deviation is 0.0387. All major distribution areas are included. The index is not corrected for over and under sampling. We expect to detect reliable trends within the next two years of monitoring.

Discussion

The national Amphibian Monitoring Program exists since 1997. Reliable indices are available for species that are monitored within the framework of Species Protection Plans. Reliable indices are also available for the common species *Triturus vulgaris* (624 waters), *Bufo bufo* (703 waters), *Rana temporaria* (754 waters) and *R. esculenta* synklepton (806 waters). The number of surveyed waters for the target species *T. cristatus*, *B. calamita*, *R. arvalis* and *R. lessonae* is less than 25% of that of the common species. The limited set of data for target species affects the reliability of the indices. This reliability has improved considerably over the years with the increasing number of sample sites and longer data series per sample site.

The sample sites are not yet evenly distributed over the major distribution areas of the four species. Improvement of the coverage of the sample sites is one of the main targets for the near future. Weighing the indices of sample sites according to the surface of the area it represents can be applied to correct for over and under sampling. However, for the available data of the four species, applying weight factors results in a reduction of reliability. A substantial increase in the number of sample sites is still necessary to present national indices.

The reliability of the indices is affected by the nature of the data. A relatively high proportion of the field data is incidental. This is especially the case for *Triturus cristatus*. During subsequent visits the species is often only occasionally observed. This is assumed to be caused by low detection probabilities due to low densities or possibly inappropriate field methods. Observers will receive stricter instructions and novice observers will receive more intensive field training.

What is the conclusion for the implementation of the NATURA 2000 as a new target for the Amphibian Monitoring Program? Although national indices can be expected in the near future, this is not to be the

case for NATURA 2000 with the present collection of sample sites. The target is based on a subset of sample sites. The number of sample sites within NATURA 2000 needs to increase substantially to approximately 50 sites. In the larger areas several sample sites are necessary. The local availability of observers (volunteers) may be limiting for monitoring within NATURA 2000 areas. For the NATURA 2000 target professional input will be required for fieldwork. A cost-effective approach is proposed with at least one sample site per area that is surveyed annually and additional sites which are surveyed in a circulating program and frequency of once every three years.

Acknowledgements. We would like to thank all volunteers who contribute to the national Amphibian Monitoring Program. These are the people who collect most of the data. In total more than 250 people participate into this program on voluntary basis. We thank the Dutch Ministry of Agriculture, Nature and Food Quality and bureau Statistics Netherlands for funding this program. And thanks to Ben Daemen for calculating the indices and trends.

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The effect of movement on survival – a new method with an application in the arboreal gecko *Gehyra variegata*

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Abstract. Mortality during movement between habitat patches is the most obvious cost of migration, but it rarely has been demonstrated empirically. An approach is presented, which uses capture-mark-recapture data of an arboreal gecko species to determine the effect of individual movement on survival in a structured population. Because capture-mark-recapture data are widely available for a range of animal species, it should be possible to extend its application to other species. The advantage of our approach is that only one additional parameter has to be estimated for describing movement. The study revealed a strong negative effect of movement on survival. Hence animals that moved more often between trees, had a lower survival rate, which can be explained by a higher predation risk when travelling on the ground. Interestingly, the mean movement rate for males was significantly higher than for females, which should lead to a biased sex ratio towards females in the population. However, males had a higher basic survival rate than females and the combination of both effects resulted in a similar survival rate of both sexes. The findings are in accordance with the predictions of the theory of the evolution of mating systems.

Introduction

In recent years it has become increasingly clear that movement is a key process in landscape ecology. Turchin (1998) states “The understanding of how an animal is affected by moving through a given landscape is a basic need for an adequate description of population dynamics in heterogeneous landscapes”. The statement is based on the observation that fragmentation of habitat (hence alteration of habitat structure) can have significant effects on population dynamics (Andrén, 1994; Fahrig, 1998). Understanding the factors influencing the survival and the spatio-temporal distribution of a population can be helpful for rebuilding threatened populations (Newman, 1998). For example, population viability analysis (PVA) is used to predict the fate of populations of threatened species (e.g. Shaffer 1990). Even if extensive background information for the modeled species is available, accurate predictions are difficult (Wiens, 1996; Lindenmayer et al., 2000). Lindenmayer et al. (2000) tested the predictive power of a PVA by comparing it with the known fate of three different species and concluded that valuable predictions could only be made when dispersal and movement rates are known. Therefore, approaches which allow assessing movement rates and the effect of movement on survival rates are necessary. Often it is assumed that movement in the hostile matrix results in an increased mortality risk (Fahrig, 1998). However, this is very hard to measure empirically (Ims & Yoccoz, 1997; Fahrig, 1998).

The focus of this study was to develop an approach to test the hypothesis that movement in a structured population results in higher mortality and apply it to a population of the arboreal gecko *Gehyra variegata*. As capture-mark-recapture data are widely available for a range of animal species, it should be possible to extend its application to other species.

Material and Methods

Study Site

The study was carried out in Kinchega National Park (32°28' S, 142°20' E) in western New South Wales, Australia, from February 1994 until March 2001. Kinchega is located at the eastern border of the arid zone. The study site contained 60 black box trees (*Eucalyptus largiflorens*) in a plot of approximately 150x100 m of riverine woodland. Every year at the end of February, except 1995, a population of the arboreal gecko *Gehyra variegata* was studied for 9-21 days by mark-recapture. Population estimates are based on geckos living on 41 trees located in the centre of the study site and surrounded by a circle of 19 trees. Geckos were captured by hand and marked with a permanent ink for short-term and by toe-clipping for long-term identification. As it is not possible to determine sex before the geckos reach maturity (at 2-3 years of age), only adults were included in the analysis.

Population size, movement rates, and survival rates. The study used the robust design of Pollock (1982) and Henle (1988). There are seven primary periods (years) which include 9-21 secondary periods (days). This approach assumes a closed population during a short-term period (several days) and an open population during a long-term period (one year). Population size was estimated using program CAPTURE (Otis et al. 1978).

Survival estimates were calculated with MARK (White & Burnham, 1999). This computer package allows to fit different Cormack-Jolly-Seber (CJS) models to capture-mark-recapture data and to determine the most appropriate model. The bootstrap approach for goodness-of-fit tests (Lebreton et al., 1992) was used to investigate the fit of models. Model selection was based on the corrected Akaike Information Criterion (QAICc) (Anderson et al., 1994).

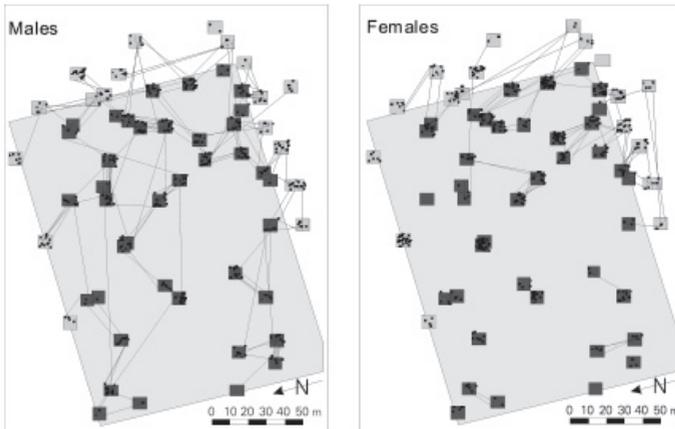


Figure 1: Recorded captures and movements of males ($n=455$) and females ($n=488$) among trees during the study period. Each dot represents a captured animal and a line indicates a movement between trees.

Territorial index t_i . Since mark-recapture regimes do not allow a continuous monitoring of individuals and because individuals in our population do not fall into two distinct categories "floater" or "territorial", we had to develop an approach for quantifying site fidelity that accounted for the different numbers of captures of individuals across years. A territorial index (t_i) was calculated from the individual capture history of each animal and used as an individual covariate. The territorial index for each individual was estimated by calculating the probability an animal was met at the same tree between recaptures. This was achieved by counting the number of events an animal was captured at the same tree between recaptures divided by the number of all captures minus one. Hence, the territorial index ranged from 0 to 1. A territorial index of 0 was achieved by an individual that was never met at the same tree when recaptured and a territorial index of 1 characterize an animal that occupied the same tree during the whole study. A likely source of bias resulting from the use of the territorial index is the uncertainty of the calculated value of t_i for animals that are rarely caught. For example an animal that is only caught twice at the same tree is less likely to be a territorial animal than an animal that was caught 5 times at the same tree, but both animals do get a territorial index of 1. It is known there are more territorial animals in the population (Gruber & Henle, 2004), thus, this bias makes the estimate of the effect of territoriality on survival more conservative. The reasoning is as follow: As the frequency of floaters in the population is lower than that of territorial individuals, there are more territorial animals that are rarely caught and regarded as floaters as there are floater that are rarely caught and mistakenly recorded as territorial animals. This leads to the potential effect that the higher number of wrongly assigned floaters lowers the estimate of the effect of territoriality on survival, so again if it still can be found, the actual effect of site fidelity on survival is even stronger.

Results

Population size

Between 29-48 animals were captured in each study period, which means that between 60% to 86% of the estimated population was captured. Estimated numbers were very similar between years. On average

55 geckos were estimated in the habitat which equals about one pair of geckos on each suitable tree. The sex ratio was 1:1.

Sex-specific territorial index (t_i)

All recapture events of males and females are mapped in Figure 1 ($n = 455$ for males and $n = 488$ for females). A straight line between trees represents movements between two recapture events. Some groups of trees were almost completely isolated from the rest of the population, and on some trees no geckos were found. Males (179 of 455 recaptures) moved significantly more often than females (121 of 488 recaptures), $n=943$, $\chi^2 = 22.3$, $df = 1$, $p < 0.001$). The mean territorial index t_i was higher for females (0.63) than for males (0.41), hence females tend to stay more often at the same tree (Mann-Whitney U-Test, $Z = -2.456$, $p = 0.014$).

Sex-specific survival rates

The initial goodness-of-fit tests suggested no violation of the basic CJS assumptions for both sexes (test2+test3 of RELEASE for males $\chi^2=3.77$, $df=13$, $p=0.993$, for females $\chi^2=5.33$, $df=13$, $p=0.967$). The bootstrap test (Lebreton et al. 1992) showed no lack of fitness for the most general CJS-model [$\phi(\text{sex} \times t)$, $p(\text{sex} \times t)$] and the variance inflation factor \hat{c} was 1.34. The most parsimonious model with the lowest QAICc was the model with constant recapture rate $p(\cdot)$ and different yearly survival rates for males and females plus the individual covariate t_i . All models that included t_i as an individual covariate performed better than any other model tested. The individual covariate t_i has quite a strong effect on survival (Fig. 2), but the estimated survival rate at mean t_i for males (0.71) was the same as for females (0.70). Males have a higher basic survival

rate than females, but as the average ti for males is higher than for females the realized survival rate is the same for both sexes.

Discussion

Population size

The population size was very constant among years. Even the flooding of the whole area that occurred between December 1999 and January 2000 had no effect on population size. On average 55 geckos were caught on 41 trees.

Sex-specific survival rates

The capture effort during the study was high, resulting in a high pooled capture probability for a complete study period. It was possible to detect differences in sex-specific survival rates by the capture-mark-recapture study and by the simulated data (not shown). The sex ratio of males to females was very close to 1:1 as found by Henle (1990a). When movement dependent mortality is included, survival rates should be the same between sexes, due to the even sex ratio.

Movement and survival

As mentioned above, the commonly used assumption that movement is associated with a higher mortality risk has rarely been demonstrated empirically (Fahrig, 1998). The multi-state/strata CJS-model approach of Arnason (1972) is not possible to use, as it cannot separate the effect of movement and survival. To overcome this problem, we used CJS-models with an individual covariate which reflects the amount of movement of an individual animal. The idea of using individual covariates was discussed by Skalski et al. (1993) and used by Kingsolver & Smith (1995) for addressing other ecological problems. The approach can be used for every capture-mark-recapture study, where the location of captured animals is recorded. Its advantage is that there is just one additional parameter to estimate, which allows to address this kind of questions and test hypothesis with field data. A necessary assumption is that the effect of movement on survival is constant during the study and for each animal, because with available models it is not possible to update values of a time-varying covariate in a mark-recapture analysis (Skalski et al., 1993).

Comparing the sex-specific survival rates, females have a lower basic survival rate but this is compensated by the lower movement rate. The lower basic survival rate of females possibly can be attributed to higher costs of reproduction, a relationship observed in many

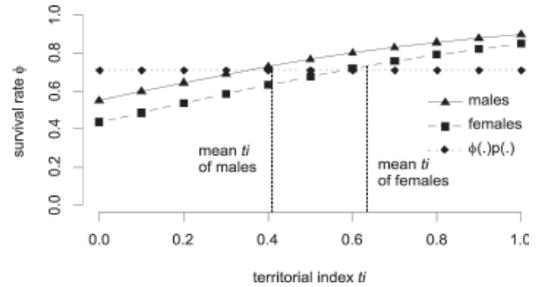


Figure 2: Relationship between survival rate for males and females and the territorial index ti estimated for model $\phi(\text{sex}+ti)p(.)$. Note that survival rate is the same for both sexes if the mean ti (0.41 for males and 0.63 for females) is used to calculate the sex specific survival rate.

ectotherm species (Roff, 1992; Sutherland, 1996; Madsen & Shine, 2000).

Predation risk most likely accounts for the additional mortality when moving on the ground (Henle, 1990a; Henle, 1990a,b; Gruber, 2004). In line with this hypothesis is the much lower yearly survival rate of syntopic, terrestrial geckos of the same size (0.33, <0.20, and 0.28 for the three terrestrial gecko species *Diplodactylus damaeus*, *Diplodactylus tessellatus* and *Heteronotia binoei*: Henle, 1990b).

From the theory of mating systems it is known that the distribution of females determines the distribution of males. Females themselves are distributed by resources (Davies, 1991). This is expected on theoretical reasons. In species in which females contribute more to the offspring, male reproductive success is determined by the number of mates and the female reproductive success is determined by the resources (Trivers, 1972). The most important resource for this arboreal gecko species are trees (Bustard, 1968). Trees provide resting places, food, and protect from predators. Females have only one option, they will try to find suitable trees and stay there once settled, hence representing the females' way to optimize resources. For males there are two options. Either stay at a suitable tree with a female or try to increase the number of mates by looking for other females on other trees. Hence, it could pay for males to move between trees, in spite of a higher risk of dying. We plan to study this further by applying genetic techniques of parental analysis, to test whether these alternative strategies for males are indeed equivalent.

Conclusion

Using an individual covariate in CJS-model allowed estimating the effect of movement on survival. The

approach presented here is in accordance with the existing and well established framework of CJS-models. A rich literature and many computer programs support such analyses (e.g. Anderson et al., 1994; Burnham and Anderson, 1998). The approach can be used in every capture-mark-recapture study where individual movement rates can be summarized in an individual attribute that expresses the amount of movement of individuals.

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The influence of habitat characteristics on amphibian species richness in two river basins of Romania

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Abstract. We present the distribution and aquatic habitat use of amphibian communities in two river basins of Transylvania (Romania) on a medium spatial scale: the middle section of Târnava Mare Valley and the Upper section of the Olt Valley. We used between 13 and 15 variables to characterize the breeding ponds and their surrounding terrestrial areas. A total number of 14 amphibian species and a species complex were identified in the two areas. In Târnava, the average species richness is larger in permanent ponds than in temporary ones. No significant differences were found between the species richness in permanent and temporary ponds in Ciuc. In permanent ponds of both areas the presence of high traffic roads in the vicinity (i.e. within an 800 m radius) explained more of the variation in species richness than the other habitat factors, whereas the presence of dirt roads accounted for the highest variation in species richness in temporary ponds.

Introduction

Recent studies show that amphibians are in global decline (Houlahan et al., 2000, Stuart et al., 2004). The causes of this phenomenon are very diverse (see Corn, 2000 for a review). Habitat reduction caused by destruction and fragmentation is often cited as being the most significant anthropogenic cause of amphibian declines in Europe (Corn, 2000; Stuart et al., 2004). Understanding habitat requirements and habitat use of different species is a major challenge in conservation biology. Species with complex life cycles such as pond breeding amphibians need special attention because of the spatial heterogeneity of the habitats they require for living (Dodd and Smith, 2003).

Romania still has extensive areas with minimum impact from human activities. We focused on two areas (Târnava Mare and Upper Olt Valleys) which have a high diversity of aquatic habitats that support species rich amphibian communities (Hartel et al., 2005; Demeter et al., 2006). There is little background information about the habitat availability and habitat use of amphibians in these two areas. Our aims during this study were: (i) to inventory habitat diversity and habitat use by amphibians and, (ii) to identify the most important anthropogenic factors influencing the amphibian species richness in the areas investigated.

Materials and methods

The Târnava Mare and the upper Olt valleys are situated in central Romania. An area of approximately 101 km by 26 km was studied in the middle section of the Târnava Mare valley, and an area of 70 km by 25 km in the upper Olt valley (Ciuc Basin). The middle section of Târnava Mare Valley (hereafter Târnava) is dominated by hills with an altitude of 600-800 m, although the lower hills (600-750 m) are only found to the west of the valley. Temperature values decrease from an annual mean of 9 °C in the west to an annual mean of 6.5 °C in the east. The upper Olt valley (hereafter Ciuc) is a mountain basin in the Eastern Carpathians. The mean altitude of the basin is 700 m, and the surrounding mountains have an altitude of 1000-1800 m. Yearly average temperatures are between 1-4 °C in the mountains and 3.7-7.5 °C in the basin.

The study period was from 2000-2004, with the great majority of the ponds being surveyed in 2004 in both areas. Amphibians were detected by dipnetting (both adults and larvae), torch count at night, and call surveys for anurans. Each permanent pond was surveyed 3-4 times in 2004, and the temporary ponds 1-3 times, including night observations. The surveys were carried out between March and August. Each pond and its surroundings in a 800 m radius was characterised using a number of habitat variables: 15 variables were used for permanent ponds and 13 for temporary ponds (tables 1-2). The permanent and temporary characters of the ponds were established through repeated surveys effectuated during the whole season (Hartel et al., 2005), the size and vegetation.

The average values regarding species richness and habitat variables between the two areas investigated were compared using Kruskal-Wallis ANOVA by Ranks and Mann-Whitney U test, depending on the differences between the homogeneity of variances (tested using Levene test).

We used hierarchical partitioning (HP) (Chevan and Sutherland, 1991) to evaluate the independent influence of habitat factors on species richness. In HP the goal is to compare the influence of each predicted variable on a response over a hierarchy of all possible 2^N models for N predictors instead of identifying a single best model (MacNally, 2002). We used the *hier.part* package (Walsh and MacNally, 2004) in the statistical software R version 2.0.1 (R Development Core Team, 2004) to carry out HP for permanent and temporary ponds in both Târnava and Olt valleys.

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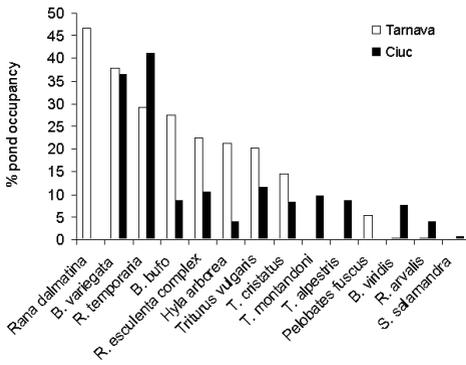


Figure 1. The percentage of pond occupancy of the amphibian species in the two regions. The data for permanent and temporary ponds are pooled.

Significance of the independent contribution to variance (I) for the predictors was assessed using Z-scores, from a comparison of the observed I s to I s resulting from 100 permutations on randomized data (MacNally, 2002).

The following habitat variables were considered for permanent ponds (every variable scored as 1 if present and 0 if absent): arable land (the presence of arable land around the pond, coded as present/absent), intensively managed arable land (with chemicals and heavy machineries being used), negligible arable land (traditional land use), road, main road (roads with high traffic volume), main urban areas (combination of buildings, industrial units, roads etc.), and pasture/grass land. For temporary ponds we considered as habitat parameters: main road, dirt road, arable land, intensively managed arable land, negligible arable land and pasture.

	Târnava	Ciuc
Elevation (m a.s.l.)***	399.2 (278-625)	821.18 (638-1301)
Area (m ²)	72156.4 (100-1500000)	87074.9 (24-810000)
Maximum depth (cm)**	233 (30-250)	411.18 (6-3800)
Depth < 30 cm (%)	29.53 (1-100)	not estimated
Macrophyte cover (%)*	29.25 (1-100)	24.6 (0-95)
pH	7.56 (6.02-8.94)	7.75 (6.14-10.45)
Conductivity (µS/cm)	637 (66.2-1828)	328 (107.4-683)
Age (years)	16.9 (2-40)	17.5 (1-40, and 5 natural ponds)
Distance from forest (m)**	231.69 (0-1200)	1312.27 (0-3000)
Green corridor (%)	77.42	54.55
Arable land (%)	54.84	22.73
Intensively managed arable land (%)	6.45	4.55
Negligible arable land (%)	48.39	22.73
Road (%)	51.61	45.45
Main road (%)	30.65	13.64
Main urban areas (%)	19.35	18.18
Pasture/grass land (%)	79.03	77.27

Table 1. Comparison of the variables describing habitat characteristics of the permanent ponds and their surroundings. Values in parentheses: minimum and maximum. Area, pH, conductivity and the distance from the forest were compared using ANOVA. Elevation, maximum depth, macrophyte cover and age, were compared using Kruskal-Wallis ANOVA by Ranks. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Results

A total of 513 ponds were surveyed during this study, of which 84 are permanent (16%). Of these, 248 ponds are situated in Târnava (62 permanent and 186 temporary), and 265 ponds in Ciuc (22 permanent and 243 temporary). Permanent ponds in Târnava are situated at lower altitudes, have a smaller depth, a larger macrophyte cover, and are closer to the forest than permanent ponds in Ciuc. Green corridors are more frequent in Târnava than in Ciuc. Temporary ponds in Târnava are situated at lower altitudes, have smaller areas, and are closer to the forest than temporary ponds in Ciuc (table 2).

Eleven species were found in Târnava and 12 species in Ciuc, including a species complex (*Rana esculenta*). There are differences between the two regions in species pond occupancy patterns. *Rana dalmatina* and *Pelobates fuscus* are absent in Ciuc, while *T. alpestris* and *T. montandoni* are absent in Târnava. In Ciuc hybrids between *T. vulgaris* and *T. montandoni* were also found. *Rana temporaria* is the most common species in Ciuc, while *Bombina variegata* is widely distributed in both areas. *Bufo bufo*, *Hyla arborea*, *R. esculenta* complex, *Triturus vulgaris* and *T. cristatus* have a higher frequency of occurrence in Târnava than in Ciuc, whereas *B. viridis* and *R. arvalis* are more common in Ciuc (fig. 1). In Târnava there are permanent ponds with 6 to 9 amphibian species, the percentage of ponds with 8 species being relatively large. In Ciuc 14% of the permanent ponds are not used by amphibians, and there are no permanent ponds with more than five species. A larger proportion of

	Târnava	Ciuc
Elevation (m)***	528.1 (304-840)	758.57 (634-1505.4)
Size (m ²)***	34.01 (0.2-504)	654.32 (0.35-30000)
Depth (cm)***	20.23 (3-150)	36.5 (0-150)
pH	6.98 (5.69-8.95)	Not estimated
Conductivity (µS/cm)	506 (113-1448)	Not estimated
Distance forest (m)***	111.75 (0-1000)	1649.69 (0-4000)
Green corridor (%)	90.32	29.64
Macrophytes (%)	Not estimated	32.97 (0-100)
Pasture/grassland (%)	40.32	71.94
Arable land (%)	9.14	40.71
Dominant arable land (%)	8.60	10.67
Negligible arable land (%)	0.54	28.65
Main road (%)	0.54	10.28
Dirt road (%)	45.16	27.67
Main roads and urban areas (%)	-	9.09

Table 2. Comparison of the variables describing habitat characteristics of the temporary ponds and their surroundings. Values in parentheses: minimum and maximum. All comparisons were made using Kruskal-Wallis ANOVA by Ranks. *** $P < 0.001$.

the temporary ponds are not used by amphibians in Târnava than in Ciuc (fig. 2).

In Târnava, the average species richness is significantly higher in permanent ponds (average 4.66, SD = 3.28) than in temporary ponds (average 1.28, SD = 1.58) (Mann-Whitney U test, $P < 0.001$). No significant differences were found between the species richness in permanent and temporary ponds in Ciuc (average 1.86, SD = 1.42 vs. 1.49, SD = 1.20) (Mann-Whitney U test, $P = 0.18$). The species richness of permanent ponds is higher in Târnava than in Ciuc, whereas temporary ponds have larger species richness in Ciuc than in Târnava (Mann-Whitney U test, $P < 0.01$ for both pond types).

The hierarchical partitioning analysis indicates that the presence of high traffic roads within an 800 m radius explains more variation in species richness than the other habitat parameters considered in the case of permanent ponds. The presence of dirt roads within a 800 m radius is the most important habitat factor in the case of temporary ponds (fig. 3).

Discussion

There are differences between the amphibian species composition in the two areas studied. Thus Târnava is out of the geographical range of *T. alpestris* and *T. montandoni* (Cogălniceanu et al., 2000). The reasons for the absence of *P. fuscus* and *R. dalmatina* from Ciuc was not studied yet. Possible factors may include the colder climate and geographic isolation, and shortage of suitable habitat. This aspect needs further research. The large species number in Ciuc (12) is the result of the topographical heterogeneity of this area which consists of a mountain area with 6 species and a lower altitude area with 8 species.

Species that prefer large permanent ponds for reproduction, such as the *T. cristatus*, *B. bufo*, and *R. esculenta* complex (Laurila, 1998; Cogălniceanu et al., 2000; Babik and Rafinski, 2001) are more common in Târnava, where this habitat type is well represented. Species that prefer temporary ponds for reproduction, such as *B. variegata* (Barandun and Reyer, 1997 a; b) and *B. viridis* (Ghira et al., 2002) occupy a greater percentage of ponds in Ciuc. The significantly larger distance of ponds from the forests in Ciuc combined with a colder climate may be the reason for the very low pond occupancy of *H. arborea* in Ciuc compared with Târnava, as this species prefers forested areas as terrestrial habitat (Fuhn, 1960; Cogălniceanu et al., 2000). In Târnava, the species richness of permanent ponds is higher than that of temporary ponds, most probably due to the higher diversity of microhabitats

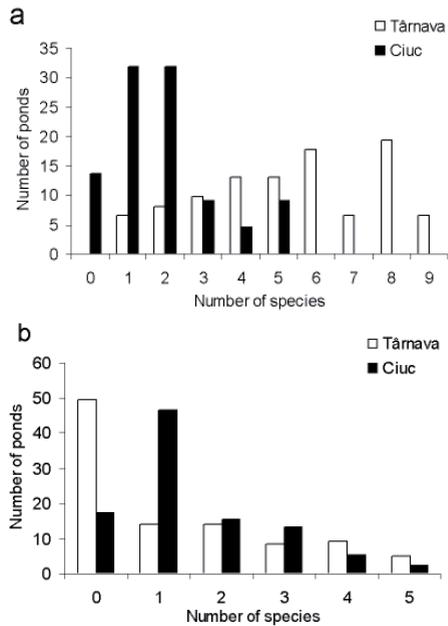


Figure 2. The distribution of the ponds with different species richness in the two regions: (a) permanent ponds, (b) temporary ponds.

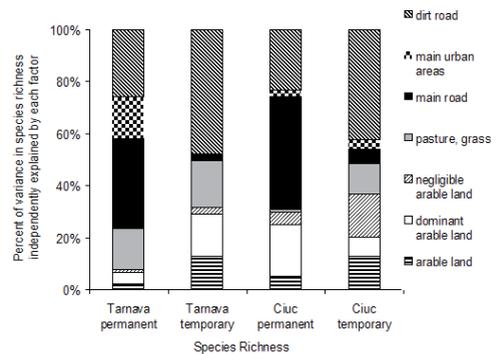


Figure 3. Proportion of explained independent variance (relative Is) associated with the anthropogenic factors for species richness in permanent and temporary ponds in Târnava and Olt valleys based on hierarchical variance partitioning modelling.

(larger structural complexity) provided by permanent ponds. In Ciuc, the lower species diversity of both permanent and temporary ponds could be explained by the lower overall species richness within the two altitude zones (6 and 8 species), the relatively low age and low number of permanent ponds. Temporary ponds typical to the low altitude zone of Ciuc have a relatively long hydroperiod (mean 10.4 weeks in 2004, n=48) (Demeter, 2005).

Roads have a huge impact on the environment, with high amphibian mortality caused by traffic being reported in the literature (e.g. Ashley and Robinson, 1996; Lodé, 2000; Smith and Dodd, 2003). Our results indicate that the roads represent the most important source of variation in species richness in the two areas. Similar results were reported in a study of North American salamanders (Porej et al., 2004). Road traffic across the investigated areas will increase in the near future, due to economic development and since many of the local villages are tourist destinations. The negative impact of the roads will also increase with the construction of the motorway, that will cross Târnava at a length of about 15 km. Forman and Deblinger (2000) showed that the significant ecological effects of roads on plants and animals, including amphibians, averages 600 m outward from a road.

In conclusion, the areas investigated still hold a high diversity of habitats and amphibian species. The pond occupancy of individual species shows regional differences. The majority of species in Ciuc have low pond occupancy. Species richness is highest in the permanent ponds from Târnava, whereas there is no significant difference between permanent and temporary ponds in Ciuc regarding species richness. Roads represent the principal source of variation in species richness in both areas.

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Experimental examination of the combat behaviour of the snake *Lampropeltis mexicana* (Garman, 1884)

Thomas Kölpin

Abstract. This paper examines the combat behaviour of the mexican kingsnake *Lampropeltis mexicana*. The results base on 149 experiments, for which in each case two males were put together in an experimental arena. The test animal group existed of 13 males. A description of the combat behaviour with its most important motor patterns is given and a classification in three phases is made. Three different variations of combat behaviour are described. The mean duration of combat is for the typical combat 16 minutes and 33 seconds and for escalated battles 85 minutes and 53 seconds. The existence of escalated combats is described, from which one would have a lethal ending without engagement from outside. A significant positive correlation of body-size and body-weight with the number of the won combats is established. There is no significant difference of the quantity of biting between the winner and the loser of the combat. Agonistic behaviour for food between males is described.

Introduction

Combat behaviour exists in many animal species. The reasons for the combats can be territories, food sources or breeding mates. Two different kinds of combat behaviour exist, the ritualised combat and the escalated combat (Franck, 1979). In the escalated combat the participants try to harm or even kill the rival, while the ritualised combat prevents severe damage. The ritualised combat is very common in animals with dangerous “weapons” like ungulates with horns, and venomous snakes. Combat behaviour occurs between young, females and animals of both sexes for different resources, but in most cases it is a ritualised combat of sexual mature males for receptive females. Because of a strong relation between the fighting strength and the body size and body weight, in many species with an intensive combat behaviour a sexual dimorphism in body size and body weight between males and females exists (Alcock, 1996), males being larger than females. Combat behaviour is common in many snake species (Gillingham, 1980; Shine et al., 1981; Gillingham et al., 1983; Osborne, 1984; Hammond, 1988; Hersek et al., 1992; Aldridge, 1993; Drobny, 1993; Heimes, 1995; Firmage & Shine, 1996). For many species and subspecies of the genus *Lampropeltis* combat behaviour has been described (Shaw, 1951; Mohen, 1967; Carpenter & Gillingham, 1977; Murphy et al., 1978; Clark et al., 1983; Secor, 1990).

Lampropeltis mexicana was described in 1884 by Garman (Garman, 1884). It is a moderately sized colubrid snake of about one metre in length. The males

are larger and heavier than the females. *L. mexicana* lives in the mountains south of the Saladan Region of the Chihuahua Desert in Mexico (Garstka, 1982). Three subspecies have been described: *L. mexicana mexicana* (Garman, 1884), *L. m. greeri* (Webb, 1961) and *L. m. thayeri* (Loveridge, 1924). *L. mexicana* has a strictly seasonal breeding phenology. After a hibernation of four to five months (Trutnau, 2002) the breeding season takes place from early March to the end of May (Hilken & Schlepper, 1998). The combats of the males for receptive females occur during this breeding period.

In this study, I describe the combat behaviour of the males of *Lampropeltis mexicana*. The goal was to address the following questions: (1) How long is the duration of the combats? (2) Does an escalation of combat behaviour exist and can damage be a consequence of these escalations? (3) Do larger or heavier males always win the combat? (4) Does the frequency of biting differ between the winner and the loser of the combat? (5) Do battles among males for food occur?

Material and methods

The test animal group consisted of 13 adult males of *Lampropeltis mexicana*. For each experiment two males of *L. mexicana* were put together in an experimental arena and the interactions were recorded by videocameras. The examination took place during the natural breeding season of *L. mexicana* in the years 1998, 2000, 2001 and 2002.

Results

The results are based on 192 experiments, in 149 of which a combat behaviour could be recorded. The motor patterns of the combat behaviour of *Lampropeltis mexicana* were as follows, following the terminology described by Gillingham (1980) for *Pantherophis obsoletus*:

(a) Touch (TO): an act of initial contact where one male

Combat variation	Number of experiments	% of experiments
0	43	22
1	73	38
2	65	34
3	11	6
total	192	100
1, 2 and 3	149	78

Table 1. Combat variations observed in the experiments performed.

Combat duration	Number of experiments	% of experiments
-10 min	36	55
10-20 min	8	12
20-30 min	9	14
30-60 min	9	14
More than 60 min	3	5
Total	65	100

Table 2. Duration of typical combats (combat variation 2).

Combat duration	Number of experiments	% of experiments
-60 min	3	27
60-120 min	5	45
More than 120 min	3	27
Total	11	100

Table 3. Duration of escalated combats (combat variation 3).

	Short aggressions	Typical combats	Escalated combats
Number of observations	73	65	11
Mean duration	few seconds	16 min 33 sec	85 min 53 sec
Maximal duration	few seconds	1 Std 28 min	2 Std 37 min
Maximal quantity of bites of the winner	12	20	68
Maximal quantity of bites of the loser	0	19	105
Maximal duration of bites	4 sec	5 sec	112 sec

Table 4. Comparison of different combat variations

physically touches the second with the snout or body. (b) Mount (MT): one male crawls on the opponents back from the rear. (c) Dorsal Pin (DP): the initiator creates a short U-shaped loop with the anterior one third of his body, elevates this above the opponent and uses it to forcefully push his head region down to the substrate.

(d) Hover (HV):

one male forms a rigid S-shaped loop with the anterior one third of his body and holds this posture above the same region of the opponent. (e) Push-Bridge (PB): an upward body-bridging movement of one male in order to get rid of the mounted opponent or a bite of him. (f) Biting (BT): one male bites the other into his body, head, tail or neck. (g) Twist (TW): the rear one-third of the body of both males get twisted in each other like a cork-screw. (h) Avoid (AV): an anteriorly, posteriorly or laterally directed gliding movement to avoid further aggressive interactions. (i) No Response (NR): one male shows no response on the behaviour of the opponent.

Phases of the combat behaviour were as follows: (1) Phase: Phase of contact: The initial phase with the first contact between the males in form of the motor pattern Touch. (2) Phase: Phase of the real combat: Starts with a first agonistic motor pattern like Mount, Dorsal Pin or Biting. (3) Phase: Phase of ending: In this phase one male takes to flight to Avoid further aggressions of the opponent and gets pursued by him, or both males show No Response.

Variations of the combat behaviour observed were as follows: (1) Variation 1: Short aggression: After the first agonistic motor pattern like Mount, Dorsal Pin or Biting of one male the opponent takes to flight. (2) Variation 2:

Table 5. Combat balance, body size and body weight of ten males in the experiments.

Specimen	Body length (cm)	Body weight (g)	Total experiments	Experiments with agonistic behaviour	Combats won	% of combats won
M 1	106	245	9	8	6	75
M 2	110	370	9	9	9	100
M 3	102	390	9	3	0	0
M 4	84	200	9	7	5	71
M 5	79	105	9	7	3	42
M 6	80	180	9	8	5	63
M 7	75	115	9	6	1	17
M 8	64	90	9	7	1	14
M 9	64	75	9	5	0	0
M 10	61	75	9	6	0	0

Typical combat: The real combat of *L. mexicana* with the aggressive motor patterns Mount, Dorsal Pin, Hover and Biting and the defensive motor pattern Push-Bridge. Often the rear one third of the bodies of the opponents get twisted. (3) Variation 3: Escalated combat: It shows the same motor patterns like the typical combat. More Biting occurs and the combat is more intensive and damages take place.

The duration of the combats varied from a few seconds up to more than two and a half hours. Combat Variation 1 (short aggressions) had a durations of a few seconds. Combat Variation 2 (typical combats) varied from 34 sec to 88 min (middle duration: 16 min 33 sec). Combat Variation 3 (escalated combats) varied from 36 min to 157 min (middle duration: 85 min 53 sec).

In 11 experiments an escalated combat was observed. Typical attributes of the escalated combat are a distinct longer duration, a higher quantity of bites and a longer duration of biting than in the other combat variations. In one case an escalated combat, without engagement from outside, came to a lethal ending. Such an occurrence is

also described by Perry-Richardson (1991) for *Morelia viridis*. Until now no description of a combat of snakes with a lethal ending in nature exists. Escalated combats do not only occur among similarly sized males.

Relationship between the body length and the body weight and won combats are shown in Table 5. The calculation of the Spearman-Rank-Correlation-Coefficient (r_s) shows a significant positive correlation between the body length of the males and the combats won ($r_s = 0,7214$, $N = 10$, $p < 0,05$), and a significant positive correlation between the body weight of the males and the combats won ($r_s = 0,5326$, $N = 10$, $p < 0,2$). Hence, larger and heavier males are more successful in combats.

In 71 of 149 combats biting of the opponents was observed. The Wilcoxon-Test shows no significant difference of the quantity of biting between the winner and the looser of the combat ($T = 623,5$, $N = 65$, $z = 0,299$, $p > 0,1$).

Outside the breeding season the males competed for food items. Not only short aggressions (combat

Combat variation	Quantity of experiments	Quantity of experiments with biting	Percentage of experiments with biting
0	43	0	0
1	73	23	32
2	65	37	57
3	11	11	100
total	192	71	37
1, 2 and 3 (experiments with agonistic behaviour)	149	71	48

Table 6. Occurrence of biting in different combat variations.

variation 1) but also typical combats (combat variation 2) with the typical motor patterns Mount, Dorsal Pin, Push-Bridge and Twist occurred. In no case the motor pattern Biting was observed. Also no escalated combats (combat variation 3) for food items could be seen.

Discussion

The combat behaviour of *Lampropeltis mexicana* with its typical motor patterns is very similar to that of many other colubrid species (Gillingham, 1980; Drobny, 1993; Heimes, 1995) especially of other species in the genus *Lampropeltis* (Carpenter & Gillingham, 1977; Murphy et al. 1978; Clark et al. 1983).

The combat duration of up to 157 min is very long for snakes. Most descriptions of combats in literature give a duration of some minutes to one hour (Carpenter & Gillingham, 1977; Gillingham, 1980; Shine et al., 1981; Clark et al., 1983; Hammond, 1988; Hersek et al., 1992; Drobny, 1993; Firmage & Shine, 1996). Only Murphy et al. (1978) described a combat with a duration longer than one hour. This combat had a duration of 305 min and was observed in of *Lampropeltis alterna*, a species closely related to *L. mexicana*.

No occurrence of escalated combats of snakes in nature has been described until now indicating that escalated combats may be an artifact of captivity.

The significant positive correlation between the body length and body weight of the males and the combats won possibly explains the reason for the sexual dimorphism in length and weight, with larger and heavier males, in *L. mexicana*.

Food induced combat behaviour has been described for a few snake species (Kelleway, 1982; Firmage & Shine, 1996). The food induced combats of *L. mexicana* occur only between two males or between two females but never between one male and one female.

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Reptiles in the Red Data Book of Ukraine: a new species list, status categories, and problems arising from conservation legislation

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Abstract. A list of reptiles, proposed by Ukrainian herpetologists for inclusion in the third edition of the Red Data Book of Ukraine (RDBU), is given. The third edition is planned to be enriched with five taxa: *Darevskia armeniaca*, *D. lindholmi*, *Lacerta viridis*, *Elaphe dione* and *Vipera berus nikolskii*; recently discovered *Darevskia dahli* should be also included. All eight species, which were present in the second edition of RDBU (*Mediodactylus kotschyi*, *Pseudopus apodus*, *Hierophis caspius*, *Coronella austriaca*, *Elaphe sauromates*, *Zamenis longissimus*, *Z. situla*, *Vipera renardi*), will remain in the third edition. Status categories of species and problems of RDBU compiling, which arise from conservation legislation, are discussed.

Introduction

The Red Data Book of Ukraine (RDBU) had two editions (Red, 1980; Shcherbak, 1994). According to the Ukrainian legislation, every 10 years a new revised edition should be published, the Ministry for Environmental Protection of Ukraine being responsible for its organization. In 2004 the project No 7/1040/25 of the Ministry “The study of species diversity with the aim of keeping the Red Data Book of Ukraine” was implemented by scientists mainly from the National Academy of Sciences of Ukraine. Under this project the series of species outlines have been prepared as a basis for corresponding outlines in the third edition of RDBU (RDBU-3). The compilation of the database on each species has been started as well. Most of the species outlines have been published (Kotenko and Kukushkin, 2005a, b, c; Kotenko and Sytnik, 2005; Kotenko et al., 2005; Kukushkin and Kotenko, 2005; Kukushkin and Sviridenko, 2005; Kukushkin and Tsvlykh, 2005; Kukushkin and Sharygin, 2005; Zinenko and Kotenko, 2005), giving possibility to Ukrainian herpetologists to discuss the data presented, and to supplement them before the preparation of final versions of species outlines for the third edition of the RDBU. The outlines on *Darevskia armeniaca* (authors I. Darevsky and I. Dotsenko) and *Zamenis longissimus* (author S. Tarashchuk) have not been published up to now, and on *Elaphe dione* only new data on the species distribution in Ukraine have been presented (Kotenko and Kondratenko, 2005). RDBU is an official document, and the protection of species included in this book is regulated by a special decree of 1992 of the Supreme

Soviet of Ukraine, and the Law “On the Red Data Book of Ukraine” (Law, 2002). In this communication, a species list of reptiles proposed for RDBU-3, species status categories and problems arising from conservation legislation are considered and discussed. The main aim of this paper is to inform foreign herpetologists about the situation with a new national red list of reptiles and about the state of threatened reptile species in Ukraine.

Materials and Methods

For the outlines on amphibians and reptiles a uniform scheme (structure) has been used, taking into account a scheme accepted in RDBU-2, with some changes and additions made by E. Pisanets and T. Kotenko. Each outline of a species included such points (some explanations are given in brackets):

- Ukrainian and Latin names,
- systematic position,
- taxonomic characteristics,
- description (short description of external appearance),
- protective status (category),
- distribution,
- habitats,
- abundance/numbers and the trends of their change (available data on population density and total numbers, with mentioning factors influencing them),
- arguments for including to RDBU (based on the Law and other grounds),
- peculiarities of biology (different aspects of biology and ecology, practical significance etc.),
- prospects and examples of conservation of artificially created populations in natural conditions,
- undertaken and necessary measures of conservation,
- sources of information (published and unpublished),
- authors.

Specialists who have been studying the species for a long time prepared the outlines. Those were based on personal authors’ data and summarised information from literature and museum collections on relevant species within the territory of Ukraine. The species distribution in Ukraine is not discussed in detail, as in RDBU-3 all outlines will be supplemented by schematic maps of the species localities.

Results and Discussion

Ukraine is inhabited by 22–23 species of reptiles (depending on the taxonomic status of the Nikolsky's viper), six of which were included in the first edition of RDBU (Red, 1980), eight — in the second edition (Shcherbak, 1994). According to the Ukrainian legislation (Law 2002, paragraph 14), such species should be included to the third edition of RDBU:

- relict and endemic species;
- species of which Ukrainian populations are at the range border;
- species which have particular scientific value;
- species, declining under the impact of human economic activities.

The protective categories envisaged by the Law (2002) for the species, included to RDBU-3, are (the corresponding IUCN categories are given in brackets, if they differ from the RDBU-3 categories): extinct, extinct in the nature (= extinct in the wild), in danger of extinction (= critically endangered and endangered), vulnerable, rare (no IUCN equivalent), not evaluated, imperfectly studied (= data deficient). The category “rare” refers to species with small populations, which now are not vulnerable or in danger of extinction, but are threatened.

Taking into account mentioned legislation, the Ukrainian herpetologists proposed to include 12 species and one subspecies in the third edition of RDBU (Table 1), one more species (recently discovered *Darevskia dahli*) should be included as well. In such case, 59 or 56% of 22–23 reptile species of Ukraine fauna will be included in the RDBU.

Introduced species are usually not included in the red lists (and to RDBU as well), but an exception was made for *D. armeniaca*. This species was introduced to Ukraine in 1963 (Darevsky and Shcherbak, 1968), it inhabits only a small isolated rocky area near Zhytomyr (North Ukraine) and cannot harm the aboriginal Ukrainian herpetofauna. This population is unique and has an outstanding scientific significance, what meets one of the criteria of including species to RDBU, envisaged by the Law (2002). In the outline on *D. armeniaca* prepared for RDBU-3 (Darevsky and Dotsenko, unpubl.), the species was attributed to the categories of “not evaluated” or “data deficient”. Actually, this species with only one local, but stable population well meets the category of “rare species”, regardless of the level of its study. After the termination of the project, *D. dahli* was revealed in the population of *D. armeniaca* (Dotsenko and Darevsky, 2005). It should

be also included to RDBU under the category of “rare species”, although formally it can be now attributed to the category of “data deficient”.

Resulting from the current Ukrainian legislation (Law, 2002), three main groups of problems exist regarding RDBU reptiles (and some other groups of animals). They can be indicated as problems on:

- criteria of the including of species to RDBU (“criteria problem”);
- level of animal organization (species, subspecies, population), which can be protected by RDBU (“level problem”);
- choosing the proper system of protective categories (“categories problem”).

As it follows from the Law (2002), the priority is given to endemic species. In general, it is the correct approach – endemic taxa should be protected. According to this, the only endemic species of reptiles in Ukraine — *D. lindholmi* — has been proposed to be included to RDBU-3. However, this species is in a good condition: it is abundant, inhabits rocky landscapes of the Crimean Mountains, and is not intensively collected. Therefore, neither the species itself, nor its habitats are in any danger. Being proposed for RDBU, this species caused another problem — the identification of its protective status (see below). In my opinion, the main criterion of including a species to the red list should be the real state of its populations.

Subspecies are not the subjects of the Law-2002, so they are not conservation units of RDBU. If a species is represented in Ukraine by one subspecies and it is threatened, it is included to RDBU (as, for example, *Mediodactylus kotschy danilewskii*). But if a species in Ukraine has two or more subspecies and at least one of them is widely distributed and successful, the needs of conservation of a threatened subspecies confront with the letter of the law: a species as a whole cannot be included to RDBU, because it is not threatened, and a threatened subspecies cannot be included because this contradicts the legislation. Such situation is topical regarding the adder (*Vipera berus*) in the case, if the Nikolsky's viper (*V. nikolskii*) is considered as a subspecies of the adder. The Crimean mountain populations of *Emys orbicularis*, which were formerly designated as *E. o. hellenica* (Fritz, 1992, 2001), but now are considered as specific aboriginal populations within *E. o. orbicularis*, are threatened or endangered and need strict protection (Kotenko et al., 2005). Another situation: a species is in RDBU, but its different populations have different state and need different protective measures. Such

Table 1. Reptiles of the fauna of Ukraine in the national Red Data Book.

No	Taxon	Red Data Book of Ukraine, category of protective status ¹		
		I edition (1980)	II edition (1994)	III edition (proposal of 2004) ²
1	<i>Emys orbicularis</i> (Linnaeus, 1758)	–	–	–
2	<i>Mediodactylus kotschy</i> (Steindachner, 1870)	Rare	III (Rare)	Vulnerable (II)
3	<i>Anguis fragilis</i> Linnaeus, 1758	–	–	–
4	<i>Pseudopus apodus</i> (Pallas, 1775)	Rare and In danger of extinction	I (In danger of extinction)	Vulnerable (II)
5	<i>Darevskia armeniaca</i> (Méhely, 1909)	–	–	Not evaluated (IV) or Data Deficient (V)
6	<i>Darevskia dahli</i> (Darevsky, 1957)	–	–	– ³
7	<i>Darevskia lindholmi</i> (Lantz et Cyrén, 1936)	–	–	Restored (VI) ⁴
8	<i>Eremias arguta</i> (Pallas, 1773)	–	–	–
9	<i>Lacerta agilis</i> Linnaeus, 1758	–	–	–
10	<i>Lacerta viridis</i> (Laurenti, 1768)	–	–	Vulnerable (II)
11	<i>Podarcis taurica</i> (Pallas, 1814)	–	–	–
12	<i>Zootoca vivipara</i> (Jacquin, 1787)	–	–	–
13	<i>Coluber (=Hierophis) caspius</i> (Gmelin, 1789)	–	II (Vulnerable)	Vulnerable (II)
14	<i>Coronella austriaca</i> Laurenti, 1768	–	II (Vulnerable)	Vulnerable (II)
15	<i>Elaphe dione</i> (Pallas, 1773)	–	–	In danger of extinction (I)
16	<i>Elaphe sauromates</i> (Pallas, 1814)	Rare	II (Vulnerable)	Vulnerable (II)
17	<i>Zamenis longissimus</i> (Laurenti, 1768)	Rare	III (Rare)	In danger of extinction (I)
18	<i>Zamenis situla</i> (Linnaeus, 1758)	Rare	I (In danger of extinction)	In danger of extinction (I)
19	<i>Natrix natrix</i> (Linnaeus, 1758)	–	–	–
20	<i>Natrix tessellata</i> (Laurenti, 1768)	–	–	–
21	<i>Vipera berus</i> (Linnaeus, 1758)	–	–	–
22	<i>Vipera berus nikolskii</i> Vedmederja, Grubant et Rudaeva, 1986	–	–	Not evaluated (IV) or Data Deficient (V)
23	<i>Vipera renardi</i> (Christoph, 1861)	In danger of extinction	II (Vulnerable)	II (Vulnerable)

¹ Categories of protective status are given according to the species outlines in RDBU-1 (Red, 1980), RDBU-2 (Shcherbak, 1994) or according to categories presented in the Law (2002); in brackets the corresponding names or numbers are given.

² List of taxa is given according to the report on the project No 7/1040/25 (2004) of the Ministry for Environmental Protection of Ukraine, categories of the protective status are given in accordance to opinions of authors of the species outlines.

³ *Darevskia dahli* for Ukraine fauna was discovered after the project termination.

⁴ Category “restored species” is not envisaged for RDBU-3, but was present in RDBU-2 as category VI.

populations should be attributed to different protective categories. For example, *Vipera renardi* is relatively abundant and widely distributed (category II), while the population of Orlov Island (the Black Sea) is unique and very small, and the island is continually diminishing in size (so the category I is appropriate for this population). The special concern and protection are also needed for the Crimean mountain populations or subspecies of *V. renardi* and, probably, *Lacerta agilis*; but current law, as it was said before, considers only species.

The “level problem” has another aspect as well. The taxonomic revisions change the status of forms in both directions. On the one hand, the black adder, which was treated for some time as a separate species *V. presteri*, later *V. nikolskii* (Grubant et al., 1973; Vedmederja, Grubant and Rudaeva, 1986), and was reasonably proposed for RDBU-3 (Kotenko, 1999), is now considered as a subspecies *V. berus nikolskii* (Joger et al., 1997; Milto and Zinenko, 2005). This is the only case when a threatened subspecies of a widespread species has been proposed to be included in the RDBU (anyway, it is against the current legislation). On the other hand, a former subspecies of the rocky lizard — *Lacerta saxicola lindholmi* — is now regarded as a full species *Darevskia lindholmi* (Ryabinin et al., 1996; Arribas, 1999). I believe that the official, supported by the legislation, protection of an animal should not depend on changes in its taxonomic status. So both species and subspecies levels should be valid for RDBU, and this should be reflected in the law.

One of the “categories problems” has been mentioned above: in RDBU-3 (and in the Law-2002) there is no proper category for *D. lindholmi*. Authors of a relevant outline (Kukushkin and Sviridenko, 2005) used the category of “restored species”, though nobody ever restored this species. Moreover, such category was present in RDBU-2, but is not envisaged for RDBU-3. A more general problem is the discrepancy of the national system of categories (see Law, 2002) with the IUCN system (see IUCN, 1994). Authors of the Law-2002 tried to follow categories, used in RDBU-2, and made only two changes: included an additional category “species extinct in nature” and excluded “restored species”. In the Ukrainian system the IUCN category of “Lower risk” is absent, with its subcategories “near threatened”, “conservation dependant” and “least concern”. And it is just the LR/nt category, which describes the most adequately the state of *Lacerta viridis* in Ukraine (Kotenko, 1999). Because of the absence of such category, this species has been attributed to the “vulnerable” category (Kotenko, 2005).

In comparison to RDBU-2, in the draft of RDBU-3 the protective category has been changed for three species (Table 1). This results from the better knowledge of some species population state, on the one hand, and from continuing negative impact of human activities, on the other hand. Detailed investigations on *M. kotschyi danilewskii* and *Pseudopus apodus* have shown that they are wider distributed and more abundant than it was supposed before. Significant decline of *Zamenis longissimus* populations bear witness to the necessity of attributing this species to the category I.

Attention should be drawn to the fact that *Elaphe dione*, one of the rarest snakes of Ukraine fauna, was missing in RDBU-2, although since 1983 this species was repeatedly proposed to be included in the RDBU (Kotenko, 1983, 1987, etc.). Biology and ecology of this species in Ukraine is almost not studied, and even the data on its distribution are not numerous (Kotenko and Kondratenko, 2005). At the present time the worst state of reptile species in Ukraine is typical of three colubrid species (*E. dione*, *Z. longissimus* and *Z. situla*), which have been attributed to the I category (table 1). However, there are no species of reptiles in real danger of extinction in Ukraine, and only some species of birds and mammals can be attributed to this category. For reptiles the I category is used to indicate the most rarely occurring species with the lowest population numbers (in comparison to the other species of reptiles).

Summarising, we can say that the Ukrainian legislation should be further elaborated (adjusted) concerning the criteria of species including to RDBU, the possibility to include subspecies (and even separate populations) and the system of protective categories.

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Morphological peculiarities and their possible bearing on the taxonomic status of the Crimean montane populations of the Steppe Viper, *Vipera renardi* (Christoph, 1861)

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Abstract. The external morphology of more than 1000 specimens of *Vipera renardi* (Christoph, 1861) from the Crimean peninsula and from the eastern and southern continental parts of Ukraine was examined and analyzed by statistical, multivariate methods. It turned out that, in contrast to central and northern Crimean as well as continental lowland populations, which correspond to the diagnosis of *V. renardi*, the South Crimean, montane populations have lower scale counts. According to the results of the multivariate analysis, the latter populations form a separate group, the level of divergence from the former being comparable with subspecies or even species-level differences within the *V. renardi* complex. The populations most distinct from typical *V. renardi* (s. str.) are those from the northern macroslope of the main range of the Taurian ridge. Compared with typical *V. renardi*, the montane populations are characterized by smaller size, lower numbers of circumocular and supralabial shields, ventral plates, intercanthal, parafrontal and loreal shields, a lighter body colouration and a less frequent colour pattern with fragmented zig-zag bands. These morphological peculiarities together with the partly isolated distribution of the Crimean montane population suggest that their subspecific recognition may be warranted.

Introduction

Data on the morphological variation of steppe vipers (*Vipera renardi*) in the Crimean peninsula are scanty and dispersed. The first paper devoted to the geographical variation of *V. renardi* in Crimea was published comparatively recently (Karmyshev 1999) and the data presented in this paper concern only the Sivash region populations (without separation of Crimean and South Ukrainian mainland material) and Kerch peninsula. Due to the low number of specimens, they do not reflect the peculiarities of variation in this species in Crimea. Comparison between steppe vipers from Crimea and Povolzhje (European Russia) shows that Crimean populations are characterized by lower ventral counts (Karmyshev, Tabachishchin, 2003). Before, Shcherbak (1966) had pointed out, that some Crimean specimens of *V. renardi* possess a low number of ventrals (min. 129). These researchers referred the Crimean steppe vipers to the widely distributed eastern Europe and Central Asian subspecies *V. ursinii renardi* (Christoph, 1861). In present time, this subspecies is considered as the nominotypic subspecies of the polymorphic species *V. renardi* (Kotenko et al. 1999; Nilson, Andrén 2001).

In the last years, detailed morphological descriptions of some Crimean peripheral populations (Kukushkin 2004 b, 2005 a, 2005 b) were published, but attempts to summarize the available data have not been made.

Therefore, the aim of this paper is a general analysis of morphological variation in the steppe viper in Crimea based on extensive material. For the first time, data on the least known and the southernmost populations from Crimean mountains were used. Even the presence of steppe vipers there was uncertain for a long period. For instance, Brauner (1905) neglected the presence of steppe vipers in Crimean mountains, and for the first time it was found on the northern macroslope of the main range only in 1928 (Puzanov 1931). Only after the second half of the 20th century it was established, that *V. renardi* is comparatively widely distributed in the central and eastern parts of the northern macroslope of the main range (Shcherbak 1966; Kukushkin 2004 a). Besides, we present data on the morphological variation of vipers from the Black sea coast of Kerch peninsula. The south-western part of Kerch peninsula is part of the alpine Crimean fold mountains and is presently considered as part of the Crimea Mountain (Atlas Krym, 2003), however its relief has a more hilly or even flat character.

Materials and Methods

25 morphological characters describing pholidosis, body proportions and coloration pattern, both previously used (Vedmederya 1989; Nilson, Andrén 2001) and proposed by us, were recorded from 1000 specimens of *V. renardi* from different populations of Crimea and

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eastern and southern regions of mainland Ukraine and adjacent regions of Russia (Kursk, Belgorod) (Fig. 1). 23 characters were used in a multivariate analysis (Table 1). The morphology of the majority of the snakes, collected by the first author in 1992-2005 years in Crimea, in the Kujuk-Tuk island (Kherson region) and in the vicinities of Tokmak (Zaporizhzhja region) was studied in living specimens. Material from southern and eastern mainland regions of Ukraine in collections of the Museum of Nature at Kharkiv National University (MNKNU) was studied by the second author (Fig. 1).

The statistical treatment was performed using the software package Statistica 6.0. Patterns of geographical variation were investigated by principal component analysis (PCA) on 23 standardized characters, based on discrete, meristic and continuous variables (Table 1). The specimens were grouped on the basis of geographical origin and within the same habitats (see Fig. 1). Since *Vipera renardi* shows significant sexual dimorphism in many characters, the data from male and female specimens were analyzed separately in parallel analyses. All characters were standardized to zero mean and unit standard deviation.

Character	PC 1, males	PC 2, males	PC 1, females	PC 1, females
L. – body length, mm	-	-	-	-
L. cd. – tail length, mm	-	-	-	-
Vent. – number of ventral scales (including preventral)	0.219	0.249	0.299	-0.141
S. cd. – number of subcaudal scales	0.192	-0.016	0.175	-0.265
Gul. – number of gular scales	0.129	0.279	0.189	0.052
Sq. – number of scales around midbody	0.224	0.231	0.228	-0.126
Lab. – number of supralabial scales	0.247	0.192	0.281	-0.175
SLab. – number of sublabial scales	0.154	-0.087	0.081	0.229
Or. – number of scales around eye (without supraocular)	0.272	0.022	0.313	0.005
Ic. – number of scales between apical, caudal and frontal shields	0.097	-0.318	0.304	0.072
PF. – number of parafrontal scales, in both sides together	0.286	-0.127	0.296	-0.141
S. sf. – number of scales in contact with frontal and supraocular shields simultaneously	-0.135	0.331	-0.029	0.199
Lor. – number of loreal scales	0.259	-0.240	0.240	0.246
A2 – presence of two apical scales, in %	-0.060	-0.332	-0.116	-0.131
ON – presence of contact between upper preocular and nasal shields, in %	0.257	0.188	0.251	-0.200
S. pn. – number of small scales, touched to the posterior edge of nasal shield, not including upper preocular	0.297	0.084	0.067	-0.120
O.L. – number of subocular in contact with supralabial scales, (right+left)/2	-0.218	0.271	-0.288	-0.136
G.3/4 – presence of granule between third and fourth supralabial and subocular row, in %	0.302	-0.013	0.262	0.258
G.4/5 – presence of granule between fourth and fifth supralabial and subocular row, in %	0.161	-0.325	0.139	0.192
Fpr. – presence of fragmented parietal scales	0.271	-0.019	0.260	-0.232
Fr-Par. – break of normal contact between frontal and parietal shields, in %	-0.061	-0.134	0.028	-0.398
FZ – cases of fragmentation of ventral stripe (break at least in one point), in %	0.232	0.071	-0.088	-0.331
Inf. – number of sublabial scales in contact with the first mandible scale, (left+right)/2	0.201	-0.043	0.069	0.344
Scr. – presence of small keels on lateral (adjacent with belly) row of body scales, in %	-0.037	0.321	-0.041	-0.175
ZW – number of zigzag band windings	0.133	0.134	0.188	0.136

Table 1. Characters and their eigenvector coefficients in relation to the first and second principal components of the male and female PCAs.

Results

The principal component analysis gives similar results for males and females. The ordinations of sample means along the first two principal components, resulting from analyses using 23 characters are shown in Fig. 2. The ordination plots show an indication of geographical variation, with southern populations from mountainous regions of Crimea (Low Mountains, Chatyrdag) and Kerch peninsula (Chauda) having the lowermost first component scores. The highest scores have samples from north-western pre-Sivash parts of Crimea (N. Sivash), Kujuk-Tuk island in Sivash and continental steppe populations from Zaporizhzhja region (Tokmak). Populations from central parts of Crimea and from steppe and forest-steppe territories of Ukraine have intermediate scores. The second principal component differentiates Low Mountain and mainland steppe populations (Steppe) in females against all the rest populations, and all continental populations against all Crimean populations in males. Eigenvector coefficients given in Table 1 show, that the list of characters responsible for this separation slightly differ between males' and females' PCAs (listed according descending



Figure 1. Localities of samples: “Forest-Steppe” (not shown), Kharkiv and Poltava regions, Ukraine; Kursk and Belgorod regions, Russia; “Steppe” (not shown), Kharkiv, Kherson, Zaporizhzhja, Dnipropetrovsk, Donetsk regions, Ukraine; “Tokmak” (not shown), Tokmak vicinities, Zaporizhzhja region; “Kujuk-Tuk”, Kujuk-Tuk island, Kherson region; “Northern Sivash”, northern pre-Sivash part of Crimea, Dzhankoy and Krasnoperekopsk districts; “Southern Sivash”, South-eastern part of pre-Sivash part of Crimea, Nizhnegorod, Sovetsky and Kirovskoe districts; “Centre”, Krasnogvardejsky district; “Sasyk”, western coast of Crimea, Sasyk lake, Saki district; “Chauda”, Cape Chauda, Kerch peninsula, Leninsky district; “Low Mountains”, Low mountains of the northern macroslope of the main range of Taurian ridge, Simferopol district; “Chatyrdag”, Middle mountains of the northern macroslope of the main range of Taurian ridge, northern slopes of Chatyrdag jayla, Simferopol district.

of absolute value of Eigenvector coefficient character and first PC axis: males – G.3/4, S.pn., Pf., Or., Fpr., Lor., ON, Lab., FZ, Sq., Vent., O.L., Inf., etc.; females – Or., Ic., Vent., Pf., O.L., Lab., G.3/4, Fpr., ON, Lor., Sq., etc.).

Data on the morphology of some Ukrainian mainland and Crimean populations of steppe vipers are presented in Table 2. In comparison with typical *V. renardi*, occupying continental Ukraine and the western part of the Crimean coast of Sivash lagoon, vipers from the southern part of Crimea are characterized by rather small size (L. ♂ < 500 mm, L. ♀ < 600 mm; L. cd. ♂ < 70 mm, L. cd. ♀ < 60 mm), less number of ventral, orbital, supralabial, intercanthal, parafrontal and loreal scales, frequent absence of contact between upper preocular scale and nasal shield and not fragmented parietal shields. Thus, the pholidosis of steppe viper populations from southern Crimea is oligomerized. The most prominent differences in almost all characters exist between viper samples from the southernmost population Chatyrdag mountain massif and Kujuk-Tuk island, which corresponds with the diagnosis of *V. renardi* according to Nilson and Andrén (2001).

The body coloration of the steppe viper in the investigated territory is rather variable. In continental Ukraine and pre-Sivash region of Crimea specimens with typical coloration of *V. renardi* prevail. They have sand-gray ground colour of dorsum and dark-brown or black zigzag bands. In the mountainous forest territory of Crimea the percentage of specimens with pale

yellow-brown dorsal coloration and bright-brown or light-brown zigzag bands is increased, while greenish-gray coloured specimens, which are present in small numbers in almost all the remaining populations, are absent. More than a half of all snakes in the Chauda population possess a pronounced yellowish or reddish shade of dorsal coloration. The general tone of coloration can frequently be yellowish-gray with ochre, citric, yellowish-olive or reddish shade. In southern Crimea the frequency of specimens with fragmented zigzag is significantly lower, in particular if compared with the Kujuk-Tuk island population. In all populations studied, most individuals have dark bellies with light dots, but in the southern populations of Crimea the belly is lighter, and there are also no black-bellied individuals, which in turn form a rather large part of continental Ukraine populations (up to 53 % males and 14 % females). Only in the mountainous forest zone (Low Mountains, Chatyrdag) and in Cape Chauda isolated specimens with a light pink-gray and black-white (without dots) belly could be found.

Discussion

The originality of the montane Crimean population (Chatyrdag) and their edge position in the range of *V. renardi* allow to speculate about the existence of an undescribed subspecies of the steppe viper in this region. Similarities of several pholidosis and coloration features of montane Crimean *V. renardi* with Caucasian species of the *Vipera ursinii* group – *Vipera lotievi*

Character	Sample											
	Forest-steppe		Kujuk-Tuk		Nothern Sivash		Chauda		Low Mountains		Chatyrdag	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
L. max	463	600	561	595	527	637	434	537	467	543	441	541
L. cd. max	64	65	76	67	77	63	61.5	52	69.5	52	63	54
Vent.	(24)	(30)	(28)	(31)	(28)	(78)	(29)	(43)	(10)	(10)	(25)	(15)
	137-147	139-151	137-147	142-151	138-147	139-150	136-146	139-149	139-146	140-146	134-142	137-142
	141.3±0.5	144.4±0.6	144.2±0.3	147±0.3	141.8±0.5	144.5±0.3	140.9±0.5	143.0±0.4	141.2±0.1	142±0.6	138.4±0.4	139.7±0.4
S. cd.	(25)	(29)	(35)	(31)	(28)	(62)	(27)	(44)	(10)	(10)	(24)	(15)
	28-38	20-29	32-42	24-31	32-39	24-31	31-37	22-28	33-38	26-29	33-37	24-27
	34.0±0.5	25.7±0.3	36.3±0.3	28.2±0.3	36±0.3	26.5±0.2	33.6±0.3	25.0±0.2	34.6±0.5	27.3±0.3	34.8±0.2	25.3±0.30
Or.*	(25)	(30)	(65)	(60)	(36)	(93)	(29)	(47)	(10)	(10)	(24)	(15)
	8.5-11	8-10.5	7-10.5	8-11	8.5-11	8-11	8-11	7.5-12	7-9	8-9.5	7-9	8-9.5
	9.4±0.1	9.0±0.1	9.5±0.08	9.8±0.1	9.6±0.1	9.6±0.1	9.2±0.1	9.2±0.1	8.9±0.2	8.8±0.1	8.3±0.1	8.5±0.1
Lab.*	(25)	(30)	(30)	(23)	(30)	(75)	(29)	(42)	(10)	(9)	(24)	(15)
	9-10	8.5-10	8-10	8-10.5	8.5-11	7.5-10	8.5-9.5	8-9.5	7-9	8.5-9.5	8-9	8-9
	9.2±0.1	9.1±0.1	9±0.08	9.3±0.1	9.1±0.1	9.1±0.04	8.8±0.17	8.9±0.1	8.9±0.1	9±0.1	8.6±0.1	8.6±0.1
Ic*	(25)	(29)	(64)	(60)	(36)	(91)	(29)	(46)	(10)	(10)	(24)	(15)
	3-8	2-9	3-9	3-12	3-10	3-11	2-7	2-9	3-7	3-5	2-7	2-5
	4.2±0.3	5.3±0.3	4.4±0.2	5.3±0.2	4.8±0.3	5.5±0.2	4.2±0.3	4.7±0.2	4.3±0.4	3.8±0.3	4.3±0.2	3.7±0.2
Pf	(25)	(29)	(62)	(57)	(33)	(95)	(27)	(47)	(10)	(10)	(25)	(15)
	2-11	3-9	4-15	4-19	3-14	3-18	3-10	2-13	2-10	4-11	2-8	2-8
	5.8±0.5	6.2±0.3	7.8±0.3	10.2±0.4	7.2±0.5	8.4±0.6	5.7±0.4	6.8±0.4	5.5±0.7	6.8±0.8	4.7±0.3	4.7±0.5
Lor.*	(25)	(30)	(55)	(52)	(74)	(28)	(45)	(28)	(10)	(10)	(23)	(14)
	3-6	2-7	3-7	3-8	4.5-8	2.5-7.5	2.5-6	2.5-7	2-5	2.5-6	2-6	4-5.5
	4.1±0.1	4.5±0.2	4.9±0.1	5.5±0.1	5.5±0.2	5.5±0.1	4.4±0.2	5.2±0.1	3.5±0.4	3.6±0.3	4.2±0.2	4.6±0.1
Sq.	(25)	(32)	(62)	(54)	(53)	(106)	(30)	(50)	(10)	(10)	(24)	(15)
	21-22	17-23	19-22	21-24	20-23	21-23	20-21	20-21	20-21	21	19-21	21-22
	(58)	(58)	(168)	(168)	(114)	(232)	(80)	(100)	(18)	(20)	(50)	(36)
Fpr. %	14.0±4.9	27.6±5.9	18±3.0	38.1±3.8	11.4±3.0	33.6±3.1	2.5±1.8	10±3.0	0	20±8.9	0	0
	(21)	(25)	(88)	(79)	(61)	(116)	(40)	(46)	(10)	(10)	(26)	(17)
FZ. %	42.9±10.8	26.1±8.8	62.5±5.2	50.6±5.6	52.5±6.4	17.2±3.5	15±5.7	15.2±5.3	10.0±9.5	0	30.8±9.05	0
A2. %	(58) 1.8±1.7		(161) 5.6±1.8		(178) 18.5±2.9		(95) 3.2±1.8		(20) 20.0±8.9		(42) 14.3±5.4	
ON. %	(112) 63.4±4.5		(348) 84.8±1.9		(320) 85.3±2.0		(188) 41±3.6		(38) 60.5±7.9		(84) 42.9±5.4	

Table 2. Morphology of some Ukrainian populations of *V. renardi* (n; lim.; X+Sx). * – (left+right)/2

Table 3. Climatic characteristics of the *V. renardi* localities in Crimea and Ukraine (Andrienko et al. 1977, Vazhov 1977, Marynych 1982, Podgorodetskiĭ 1988, Atlas Krym 2003). *KH – coefficient of moistening Ivanov-Vysotsky (relation annual precipitation and annual evaporation).

Climatic characters	Region of Crimea (altitude, m above sea-level)				
	South of forest-steppe, Eastern Ukraine (50-200) (Forest-steppe and Steppe)	Northern pre-Sivash region (< 20) (Northern Sivash)	Kerch Black sea bank (< 120) (Chauda)	Western bank (< 20) (Sasyk)	Mid Mountains of Main range (600 – 1100) (Chatyrdag)
Average temperature, °C:					
July (August)	20 – 21	22.5 – 23.2	23.3 – 23.4	22.1 – 23.2	15.4 – 17.0
January (February)	-6.0 -8.0	-3.0 -1.7	+0.3 +0.8	-0. . +1.0	-3.7 -3.0
Whole year	6.0 – 7.0	10 – 10.3	10.6 – 11.5	11 – 11.5	6.4 – 8
Annual precipitation, mm	400-500	339 – 370	329 – 376	342 – 358	595 – 960
KH*	0.6-1.4	0.38 – 0.40	0.38 – 0.40	0.40 – 0.46	0.80 – 1.80
Length of period with positive temperature, days	150-160	170 – 193	214 – 227	220 – 230	147 – 165

Nilson, Tuniev, Orlov, Höggren et Andrén, 1995 and *Vipera erivanensis* (Reuss, 1933) can be explained with high probability as convergent adaptation to mountainous conditions. But, at least basing on general external morphology, these populations are most closely related to the western group of *V. renardi*.

Populations of low Mountains and Kerch peninsula (Chauda) together with populations of the southern part of steppe Crimea (Southern Sivash, Sasyk) are less distinct from typical *V. renardi* and in some cases demonstrate an intermediate position between it and the southern Crimean populations (Table 2, Fig. 2). In our opinion, such situation could be the result of at least two stages of colonization of Crimean peninsula by steppe vipers and their subsequent intergradation: a relict, which remained in the mountains and the southern part of plain Crimea during the Neopleistocene, and *V. r. renardi*, which according to Shcherbak (1966) came to Crimea from the north during the “xerothermic” stage of the Holocene. Introgressive hybridization is widely represented in small European vipers, in particular in the *V. kaznakovi* (Orlov, Tuniyev 1990) and *V. berus* complexes (Milito, Zinenko 2005). Under laboratory conditions, *V. renardi* is successfully hybridized with related Caucasian species – *V. erivanensis* and *V. lotievi* (Shyriaev 2005).

Gradually increasing differences in a southward direction from typical *V. renardi* can hardly be considered as clinal variation. The high level of similarity between Main range populations (Chatyrdag, Low Mountains) and southern Crimean steppe populations (Chauda, Sasyk) is impossible to explain by climatic or other habitat parameters, as the investigated populations live under

very contrasting ecological conditions (Table 3). In the Main range region (Chatyrdag, Low Mountains) vipers occur in bushes (*Pyrus eleagnifolia*, *Prunus stepposa*, *Crataegus* sp., *Rubus* sp., *Juniperus oxycedrus*), in the open sites in broadleaved forest (*Quercus petraea* + *Carpinus betulus*, *Fraxinus excelsior* + *Acer campestre*, *C. betulus* + *Fagus orientalis*), in stony forest-steppe near mountain plateaus with dominance of European-Mediterranean, Fore Asian, Crimean-Caucasian and endemic Crimean elements (*Quercus pubescens*, *Carpinus orientalis*, *Sorbus graeca*, *Cornus mas*, *Cotinus coggygria*, *Acer steveni*, *Cotoneaster tauricus*, *Ligustrum vulgare*, *Spiraea hypericifolia*, *Jasminum fruticans*, *Juniperus hemispherica*, *J. foetidissima*, *Asphodeline taurica*, *Cerastium bibersteinii*, *Thymus tauricus*, *Stipa lithophila*, etc.), in mountain-meadow steppe (*Festuca rupicola* + *Carex humilis*) up to 900-1100 m above sea level, but do not go further then 1 km into mountain plateaus. Morphologically similar with mountainous populations, the Kerch population of Cape Chauda inhabits semidesert and meadow steppes with dominance of *Artemisia taurica*, *A. lerchiana*, *Festuca valesiaca*, *Lynosiris villosa*, *Limonium meyeri*, *Achillea nobilis*, *Ferula caspica*, *Malabaila graveolens* and little participation of the Mediterranean species (*Capparis spinosa*) and climatically more similar to habitats of pre-Sivash populations (Table 3). In the pre-Sivash and Western regions of Crimea *V. renardi* occupies typical habitats: lowland semidesert steppes and halophytic meadows, more rarely – psammophytic steppes.

It should be mentioned, that in the northern macroslope of Main range of Crimean mountains, together with *V. renardi*, two other endemic species of Caucasian origin

dominate in the herpetocenosis: *Lacerta agilis tauridica* Suchow, 1927 (sensu Kalyabina et al. 2004) and *Darevskia lindholmi* (Lantz et Cyrén, 1936) (Shcherbak 1966, Kalyabina-Hauf et al. 2004, Kalyabina-Hauf, Ananyeva 2004). It is supposed, that *Emys orbicularis* L., 1758 from Mountainous Crimea and Kerch peninsula is also represented by an endemic form (Kotenko et al. 2005).

It is interesting, that the geographical variation of the sand lizard, which is syntopic with the steppe viper in Crimea, shows the same tendencies: oligomerization of the pholidosis in south-western direction, low levels of differences between mountainous populations and northern Crimean and southern Ukrainian populations as compared with differences between other subspecies or species in the complex, intermediate character states between *L. a. exigua* (Eichwald, 1831) and *L. a. tauridica* in populations of pre-mountainous and southern plain parts of Crimea (Sviridenko, Kukushkin 2005; Peskov, Brovko 2005). It is rather probable, that stages of colonization of Crimea and its routes in *L. agilis* and *V. renardi* were similar.

The evidence presented here suggests a late Pleistocene origin of the steppe viper populations of the mountainous and adjacent plain parts of Crimea. During the Wurm glacial on Crimean jajas, only weak glaciation was developed (Ena et al. 1991), however, reptile populations could survive and persist cold periods in refuges on slopes and in southern parts of the plains. Remains of relict thermophilic vegetation (*Juniperus excelsa*, *Taxus baccata*) in gorges of the eastern slope of Chatyrdag confirm such assumption. However, further analyses of possible correlations between morphology and habitat parameters have to be carried out, which could

alternatively explain the observed interpopulational differences, before taxonomic conclusions can be drawn; a molecular genetic approach seems indispensable.

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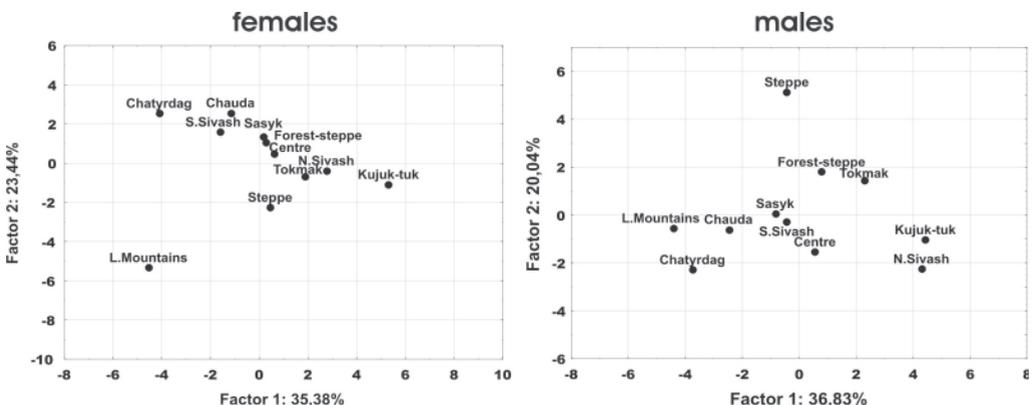


Figure 2. The ordination plots of the sample means along the first two principal components. Left – females, right – males. Sample names as in Fig. 1.

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Karyotype diversity of the Eurasian lizard *Zootoca vivipara* (Jacquin, 1787) from Central Europe and the evolution of viviparity

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Abstract. We sampled seven populations of *Zootoca vivipara* from Central Europe (6 Austrian, 1 German) and found several chromosomal forms inhabiting this region: (1) oviparous *Z.v. carniolica*, (2) ovoviviparous form from Hungary and southern Austria with the same karyotypic characters, (3) ovoviviparous western form, and (4) ovoviviparous form in the NE Alps including the type locality, which is karyologically similar to the NE Austrian Pannonian lowland populations and the ovoviviparous Russian form. Austria is characterized by high chromosomal diversity in *Z. vivipara*, housing 3 different ovoviviparous chromosomal forms plus an oviparous one. Together with other data sets, our results corroborate the hypothesis of a multiple origin of viviparity in this species.

Introduction

The widely distributed Eurasian lizard *Zootoca vivipara* (Jacquin, 1787) (Reptilia, Lacertidae) is represented in Europe by a mosaical array of populations diverging in karyological and molecular features as well as in reproduction mode. Now it becomes clear that *Z. vivipara* represents a complex including several forms and subspecies. All of them are morphologically only weakly differentiated. However they differ in the mode of reproduction and in several mitochondrial DNA and karyotype features (Heulin et al. 1993, 1999; Kupriyanova 1990, 2004; Kupriyanova & Böhme 1997; Mayer et al. 2000; Odierna et al. 1993; 1998; 2001; Surget-Groba et al. 2001). In the karyotype markers they differ in the female diploid number ($2n=36$; $2n=35$), in the type of the female sex chromosomes (ZW, $Z_1 Z_2 W$), as well as in size, constitution and morphology of the W chromosome (w- acrocentric microchromosome, W- macrochromosome: A/ST, A- acrocentric, ST- subtelocentric; SV- submetacentric). At present at least four different karyotypes have been described from females of ovoviviparous *Z. vivipara* from geographically distant populations in Europe. There are two karyologically different oviparous forms, one from the Pyrenean region in western Europe with $2n=35$, $Z_1 Z_2 W$ (the Pyrenean form) and another one, the subspecies *Z. v. carniolica*,

with $2n=36$, Zw recorded from some localities in southern-central Europe. The taxonomic status of these distinctive chromosomal forms with parapatric or allopatric distributions remains uncertain. Some of them inhabit only small areas, others are rare in a country and therefore may need protection there. High genomic diversity has been detected in central Europe (Mayer et al. 2000; Puky et al. 2004; Surget-Groba et al. 2006; Mayer et al. in prep.) but only few chromosomal data are available.

The objectives of this research were (1) to study the karyotypes of some ovoviviparous and oviparous populations from Austria (including the terra typica of *Z. vivipara*) and Germany, (2) to compare their features with those of chromosomal forms already described and (3) to compare the data with available mtDNA data.

Materials and methods

14 specimens from six localities in Austria and two specimens from one locality in Germany were collected (see Tab. 1). According to mitochondrial DNA data these specimens are representatives of four mitochondrial haploclades (Mayer et al. in prep.). Some of these localities are close to a haploclade border (Fig 1).

Chromosomes were obtained according to the scraping and air-drying method (Olmo et al. 1987; Odierna et al. 1993) from intestine, blood and lung tissues. Specimens were injected with phytohemagglutinin M (Difco) and then with colchicine. The slides were stained for 10 min. with 5% Giemsa solution in phosphate buffer pH 7. C-banding of the chromosomes was carried out according to Sumner (1972). To identify the mode of reproduction in the respective populations, pregnant females were kept in a terrarium up to giving birth to offspring.

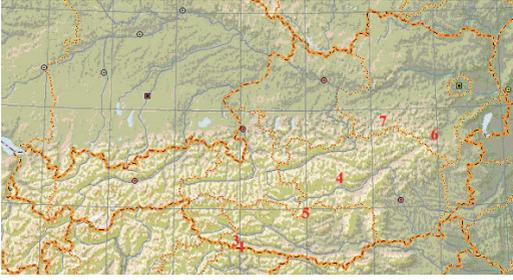
Results and discussion

Chromosome sets of all females and two males of *Z. vivipara* (and the mode of their reproduction) from all localities have been studied. Main

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Figure 1. Map showing sampling localities.

characters of karyotypes have been analyzed, i.e. the diploid number ($2n$), the size (M , m) and the rough morphology of chromosomes, the number of chromosomal arms (NF), number and system of sex chromosomes (ZW ; $Z_1 Z_2 W$), and the morphology of W sex chromosomes (A/ST , SV , a) (Tab. 2). Amount and localization of C -bands in W sex chromosomes of specimens from the terra typica of *Z. vivipara* have been studied for the first time.

Males possessed 36 acrocentric chromosomes, a karyotype correlating with male karyotypes of all chromosomal forms of *Z. vivipara* investigated so far. Female samples differed in karyotype markers and/or in the mode of reproduction. These populations appear to correspond to several already

described chromosomal forms in different regions of Europe.

The karyotype of females of *Z. vivipara* from the Klein-Kordin Alm, southern-most Austria, (population 1; “western” haploclade) and from Gänsehals near Maria Laach in Rhineland, western Germany (pop. 2) shows $2n = 35$, 34 acrocentric (A) and 1 submetacentric (SV) chromosomes, $NF=36$, with $Z_1 Z_2 W$ sex chromosome system. The W sex chromosome is a biarmed submetacentric macrochromosome (Fig. 2 a, b). These females are characterized by ovoviviparous reproduction. They belong to the “western” haploclade (like *Z. v. pannonica* from Botany, Slovakia, Mayer et al. in prep.) and correspond karyologically to the known western chromosomal form of *Z. v. vivipara*. This type has a rather wide distribution area mainly in western and central Europe and inhabits many countries (e.g. Bulgaria, Denmark, Germany, the Netherlands, France, Italy, Poland, Sweden, Switzerland). Recently it has been found in the western region of Russia, too (Kupriyanova 2004). This paper reports the first record of the western chromosomal form in Austria.

The karyotype of females from the Strainiger Alm, southern Austria (pop. 3; haploclade “south”) shows

Taxon	Samples	Haplotype	Locality
<i>Z. v. vivipara</i>	1♀/1♂ population 1	Type "west"	Klein-Kordin Alm, about 20 km W of the village Hermagor, Carinthia, Carnian Alps, near the Austrian-Italian border
<i>Z. v. vivipara</i>	2♀ population 2		Gänsehals near Maria Laach in Rhineland, western Germany
<i>Z. v. carniolica</i>	2 ♀ population 3	Type "south"	Straniger-Alm, about 100 m below Klein-Kordin Alm, Carnian Alps, near the Austrian-Italian border
<i>Z. v. vivipara</i>	2♀ population 4.	Type "east"	Neunkirchner Hütte, about 20 km N of the village Oberwölz, Styria, Niedere Tauern
	2 ♀ population 5		Turracher Höhe, about 30 km N of Villach, Styrian-Carinthian border, Gurktaler Alpen
<i>Z. v. vivipara</i>	3♀/1♂ population 6	Type "north"	Schneeberg, about 50 km S of Vienna, northeastern Calcareous Alps, terra typica
	2 ♀ population 7		Ötscher region, about 100 km SW of Vienna, northeastern Calcareous Alps

Table 1. Samples and localities of *Z. vivipara* (haplotype is given according to Mayer et al. (in prep.).)

Table 2. Results of analyses of *Z. vivipara* from central Europe

Mitochondrial haplotype	2n/♀	System of sex chromosomes	Morphology of W chromosome	Mode of reproduction	Chromosomal form
type "west" pop. 1, 2	35	Z ₁ Z ₂ W	SV	Ovoviviparous	<i>Z. v. vivipara</i> , western form
type "south" pop. 3	36	Zw	m, a	Oviparous	<i>Z. v. carniolica</i>
type "east" pop. 4, 5	36	Zw	m, a	Ovoviviparous	<i>Z. v. vivipara</i> , Hungarian form
type "north" pop. 6, 7	35	Z ₁ Z ₂ W	ST/A	Ovoviviparous	<i>Z. v. vivipara</i> , similar to Russian form

2n=36 acrocentric (A) chromosomes, (NF=36), with ZW system of sex chromosomes. The W sex chromosome is an unpaired acrocentric (a) microchromosome (m) (Fig. 2 c). These females of the "southern" haploclade belong to the oviparous subspecies *Z. v. carniolica*. (Mayer et al. 2000). According to the comparative analysis these specimens of the "southern" haploclade correspond to the oviparous subspecies *Z. v. carniolica*. This subspecies has a rather small distribution range in the southern part of central Europe: northern Italy, Slovenia, northwestern Croatia and southern Austria.

The karyotype of females from the Neunkirchner Hütte (pop. 4; haploclade "east") and Turracher Höhe (pop. 5; haploclade "east") in southern central Austria has also shown 2n=36 acrocentric (A) chromosomes, NF=36, with a ZW system of sex chromosomes. Like in *Z. v. carniolica* its W sex chromosome is an unpaired acrocentric (a) microchromosome (m) (Fig. 2 d). However, these females are characterized by ovoviviparous reproduction. We should stress that in spite of the similar karyotype structure, *Z. v. carniolica* (pop. 3) and this clade of *Z. v. vivipara* (pop. 4 and 5) belong to different haploclades (types "south" and "east", respectively) and are characterized by different reproduction mode. According to combined analyses (karyotype features: 2n=36, NF=36, Zw, w-m and viviparous reproduction) these specimens from southern central Austria correspond to those recently described from one relict population in central Hungary (Odierna et al. 2004; Puky et al.

2004). The present paper reports the second record of this form (Hungarian form).

The populations from Schneeberg (pop. 6) and from the Ötscher region (pop. 7) in north-eastern Austria studied for the first time are representatives of the mitochondrial type "north" (Tab. 1), which include not only the terra typica of the species but also Austrian viviparous populations usually assigned to the subspecies *Z. v. pannonica* (Mayer et al. in prep.). The female karyotype has shown 2n=35, 34 acrocentric (A) chromosomes and one unpaired subtelocentric/acrocentric (ST/A) macrochromosome (M), NF=35, a Z₁ Z₂ W sex chromosome system, where the W sex chromosome is unpaired ST/A (Fig. 3 a). The analysis discovered high similarities by several karyotype markers (2n=35, Z₁ Z₂ W, W-ST/A) between these samples and those of Austrian so-called *Z. v. pannonica* (viviparous), the Pyrenean form (oviparous, Portalet population) and the viviparous Russian form of *Z. v. vivipara*. The latter was discovered by karyological methods in several geographically very distant populations across European and Asian Russia (Kupriyanova 1986), the Transcarpathian region of Ukraine (Kupriyanova, 1990), eastern Hungary (Odierna et al., 2004; Puky et al., 2004), in central Estonia (Kupriyanova, 1997) and eastern Finland (Kupriyanova et al., 2005a). Comparative C-banding analysis of W chromosome of representatives of the terra typica and the Russian form exhibits some similarities in the localization of constitutive heterochromatin blocks. Most of the autosomes and the subtelocentric W chromosome

possess conspicuous centromeric and telomeric C-bands. Additionally, the W sex chromosome has one remarkable interstitial C-band (Fig. 2 b). Nevertheless, there are some differences in the amount of heterochromatin in centromeric C-blocks - namely prominent centromeric and interstitial C-bands in the terra typica form but smaller bands in the Russian form (Fig. 5 c). Presently we have no information from some additional chromosomal markers in order to characterize more precisely and better to differentiate the specimens from Austria. Hence for comparison we still need additional information. According to mtDNA analysis these viviparous populations belong to different haploclades: the former to the haploclade "north" whereas the latter one to the "Russian" haploclade

(Mayer et al. in prep.). Future cytogenetical and molecular researches may provide us with valuable information to resolve the relationships of these chromosomal forms belonging to different haploclades. Extensive interpopulation karyotype analyses as well as detailed investigations of the chromosome structure may also help in solving several open questions concerning the centres of formation and differentiation of the forms and their glacial refugia, postglacial colonizations and the evolution of viviparity. From just presented and available chromosomal and cytogenetical data the situation in *Z. vivipara* is more complex than we thought earlier. Combined analyses of chromosomal, reproduction and mitochondrial DNA data support the hypothesis that viviparity arose more than once

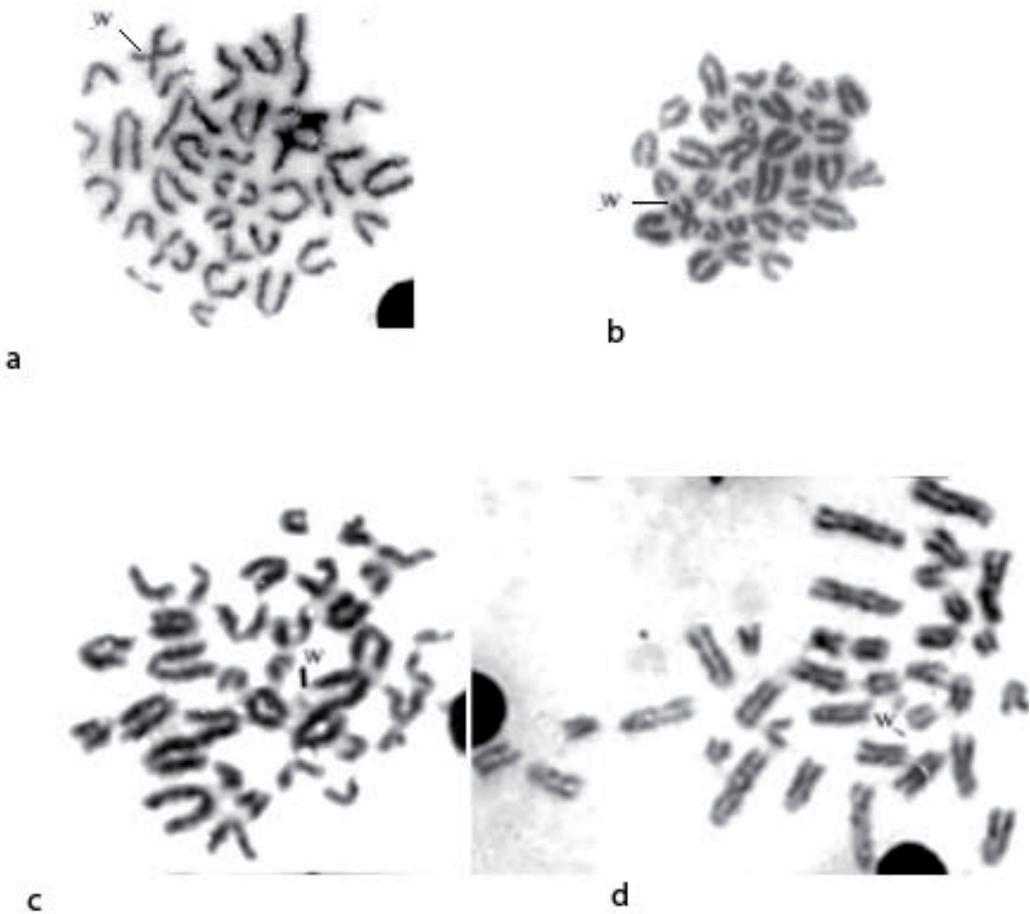


Figure 2. Giemsa stained female metaphase plates of *Zootoca vivipara*. a, b - specimens from populations 1 and 2. $2n = 35, Z_1 Z_2 W, W-SV$: *Z. v. vivipara*, western form; c - specimen from population 3, $2n = 36, Zw, w-m$, a: *Z. v. carniolica*; d - specimen from population 4, $2n = 36, Zw, w-m$, a: *Z. v. vivipara*, Hungarian form. Arrows point to W - sex-chromosome.

independently in various populations of *Z. vivipara* (Odierna et al. 2004; Kupriyanova et al. 2005b; Surget-Groba et al. 2006). Probably viviparity arose at least in oviparous populations with females possessing $2n=36$, Zw (like the oviparous *Z. v. carniolica*) and in another oviparous population with females displaying $2n=35$, $Z_1 Z_2 W$ (like the oviparous Pyrenean form of *Z. v. vivipara*). The molecular data do not rule out a possibility of reversal back to oviparity (Surget-Groba et al. 2006). The taxonomic status of all forms of *Z. vivipara* characterized by different karyotypes, reproduction mode and mitochondrial DNA sequences needs clarification. Presently there are no indications of introgression in specimens from localities close to borders of different mitochondrial or chromosomal forms.

Conclusions

As a result of karyological investigations of seven populations of *Z. vivipara* from Central Europe we found that several chromosomal forms of *Z. vivipara* inhabit this region. They are (1) the oviparous *Z. v.*

carniolica and (2) an ovoviviparous form of *Z. v. vivipara* (Hungarian form) with the same karyotype characters, ($2n=36$, $NF=36$, Zw, w-m), which is probably endemic in southern Austria and parts of Hungary (3) an ovoviviparous form (the western form) of *Z. v. vivipara* with $2n=35$, $NF=36$, $Z_1 Z_2 W$, W-SV and (4) an additional ovoviviparous form in the northeastern-most Alps (including the terra typica population) karyologically rather similar to so-called *Z. v. pannonica* (ovoviviparous) from the northeastern Austrian lowlands and to the ovoviviparous Russian form with $2n=35$, $NF=35$, $Z_1 Z_2 W$, W-ST/A, the W sex chromosome with three remarkable C-bands.

Finally it has been demonstrated that (i) central Europe (Austria) is characterized by high diversity of chromosomal forms of *Z. vivipara*, (ii) three different chromosomal forms of *Z. v. vivipara* occur among ovoviviparous populations (iii) an additional ovoviviparous population group of *Z. v. vivipara* (the Hungarian form) lives in Hungary and parts of Austria in restricted areas and may need protection, at least in Hungary, (iv) there is some correlation

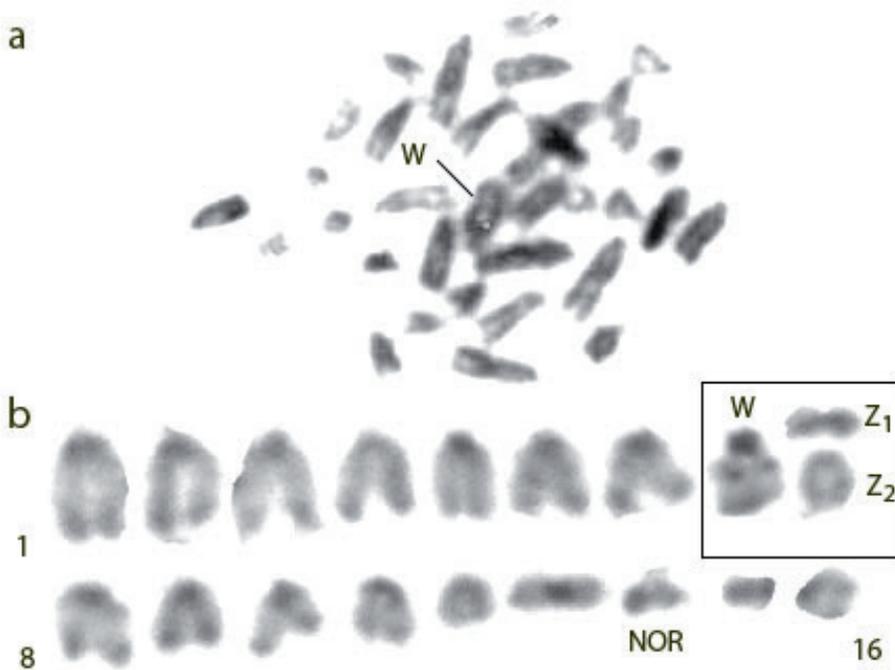


Figure 3. Giemsa stained female metaphase plate (a) and C-banded karyotype (b) of *Z. v. vivipara* (population 7) $2n = 35$, $Z_1 Z_2 W$, W- ST/A. Arrow points to W-sex chromosome

between chromosomal and mtDNA data, and (v) comparative analyses of chromosomal, molecular and reproductive data from central Europe support a previous hypothesis (Odierna et al. 2004; Kupriyanova et al. 2005b; Surget-Groba et al. 2006) that viviparity arose more than once in different populations of the species.

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Reproductive cycles of the Siberian newt *Salamandrella keyserlingii* Dybowsky, 1870

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Abstract. Females of *Salamandrella keyserlingii* with deposited or fertilized eggs from the previous year spawn in the end of April- beginning of May. In females that have not spawned in spring maturing eggs are found in the oviduct by the end of May and copulation takes place in the beginning of July. It seems that both variants of breeding cycle exist side by side. If females could not get spermatozooids during summer then eggs are reserved for the next breeding season.

Introduction

The genitals and the means of fertilization in *Salamandrella keyserlingii* (Hynobiidae, Caudata, Amphibia) are poorly known. There exists an opinion that fertilization in this species is external and precedes courting (Bannicov et al., 1977; Kuzmin, 1999). A number of studies confirm that females of *S. keyserlingii* are polygamous, i.e. copulation with several males takes place at the same time. On the basis of these assumptions the conclusions about primitive nature of reproduction in *S. keyserlingii* are made (Berman et al., 1983; Sytina et al., 1987; Ishchenko et al., 1995; Kuzmin et al., 1995; Borkin, 1999). Studies of the breeding ecology in *S. keyserlingii* have been conducted near Tomsk, south-east of Western Siberia since 1975. Earlier we have described the genitals, internal fertilization and three year sexual cycle of the siberian newt (Kuranova, 1991; Saveliev et al., 1991, 1993). The results of these studies are presented below.

Material and methods

The study carried out in Tomsk Oblast' (West Siberian), in 2003-2005. Totally 97 specimens (46 females and 51 males) were investigated. The snout-vent length (SVL) and total length (TL) of 48 specimens (31 females and 17 males) captured in spawning ponds in first-second decade May was measured to the nearest 1 mm; the length of testicles was measured in 25 sexually mature males captured between May and August 2005. Age and seasonal dynamics of testicles are described by index L_{tes}/SVL (where L_{tes} – length of testicle). The fecundity defined on number of eggs in laying ($n = 296$). We have studied the reproductive system of 24 Siberian newts (including 15 females) by means of histological and histochemical methods from April till August,

2003. Species were captured during migration before breeding and during spawning (the third decade of April - May) and also during summer. For histological research the urinogenital system was fixed in 10 % formaldehyde, for scanning and transmission microscopy the urinogenital system was fixed in 2 % paraformaldehyde. Ovarium, various parts of oviductus, testicles with vas efferens, ren with Ductus Wolfi were separated urinogenital system. Sections were stained according to Mallori method. The spermatozoa in the oviducts of female *S. keyserlingii* were traced indirectly with marked antibodies. The antibodies of 2 classes were used: A (IgA) and G (IgG). The marked material was studied by means of scanning electronic microscope Hitachi S-500 and Jeol 100 In, and a photomicroscope Leitz Ortholux 2 Pol BK. The statistical processing of material was conducted with the spreadsheets MS Excel 7.0 and statistical package STATISTICA 6.0. Differences of means were estimated by criterion of Mann-Whitney (*U*-test).

Results and discussion

Sexually mature males with SVL of 59.7 ± 0.8 mm ($n=17$) and females with SVL of 59.6 ± 1.0 mm ($n=31$) can be found in spawning ponds in spring. The sexual dimorphism express in TL: males (mean \pm SE) = 112.5 ± 2.5 mm (range 88-123), females = 106.3 ± 1.8 mm (range 91-126) ($p \leq 0.05$). The average fecundity in Tomsk population varied from 98.0 ± 11.3 to 171.6 ± 16.3 eggs in different years. The average long term fecundity is 134.2 ± 2.6 eggs (limit 37-254, $n=296$).

The sample of *S. keyserlingii* females can be divided into two groups based on histological studies of their genitals. The first group consists of individuals that have spawned in spring. In April-May females spawned eggs which had been already fertilized in the previous breeding season. After spawning ovaries contain evidence left by of disintegration mature unfertilized eggs (Fig. 1). Eggs are subjected to involution in 2-4 weeks time. Disintegration is accompanied by forming the morphogenetic trace of the egg. This trace is comprised of the nucleus that has been subjected to rapid chromatolysis and of the yolk filled cytoplasm which persists for a longer time period. In 7-10 days

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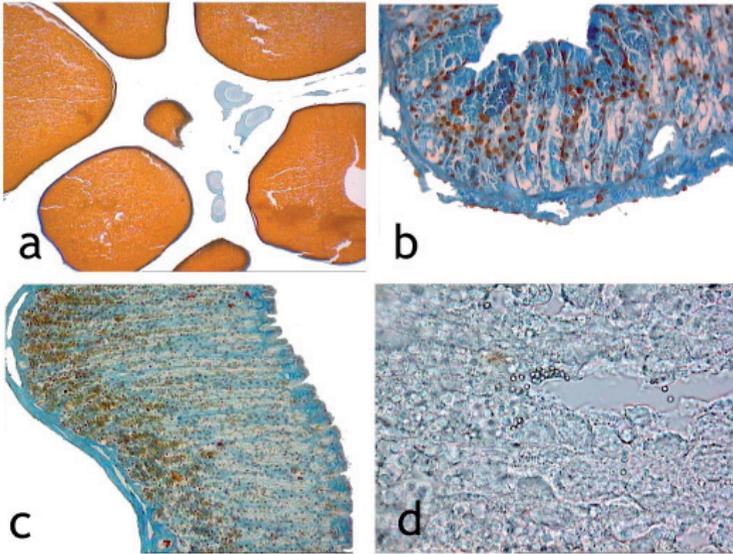


Figure 1. (a) Reproductive system in female *Salamandrella keyserlingii* before spawning, (b) Mature eggs in the ovary, (c) active secretory glands in oviducts, and (d) spermatozoa marked with monoclonal antibodies.

the active degeneration of secretory epithelium starts in oviducts of spawned females (Fig. 2). In spawning females cells of oviduct walls are autolysed and multiple necrosis centers are found. The oviduct is filled with cellular detritus. It consists of disintegrated secretory cells, fragments of nuclei and fibroblasts of degraded epithelium. In Siberian newts this process continues until the end of May. No necrosis of the epithelium is to be found in the oviducts from the beginning of June.

However there is a certain problem. Time is needed to restore the secretory epithelium after degeneration. We did not find females with traces of spawning in ovaries and with undegenerated epithelium of the oviduct.

Hence in the end of May their ovary has not been restored after the spring spawning and the epithelium of the oviduct is in the state of morphological regeneration. Females recycle sexual system within 40-50 days. Sexual system's preparation of females takes much more time than the maturation of male spermatozoa.

The second group consists of specimens with other state of ovaries and oviducts. These are the females which have not spawned in the year of collection. There are no fertilized eggs in ovaries. In the middle of May ovaries contain some ovocytes of various ages with signs of

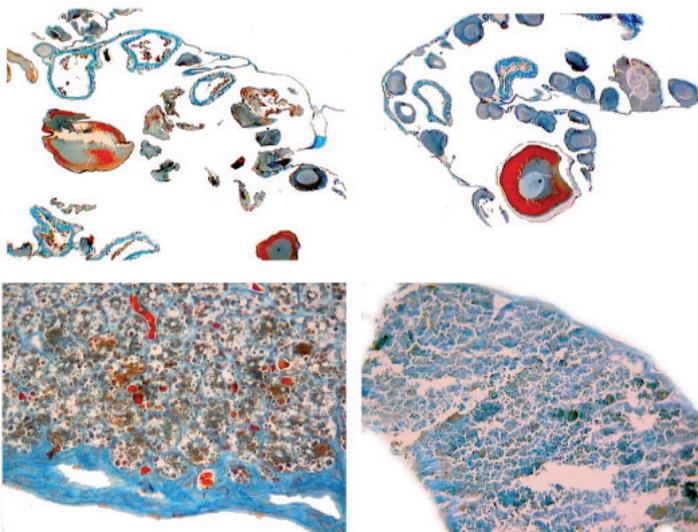
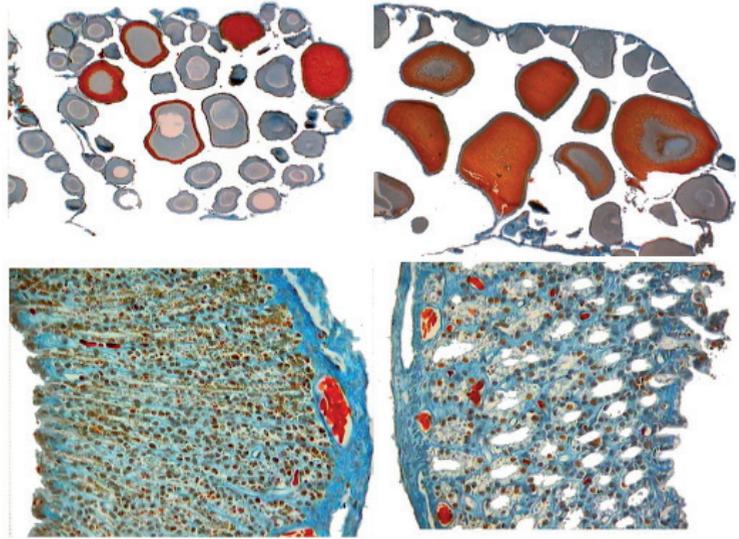


Figure 2. The ovaries and oviducts in females after spawning. Unfertilized eggs are being destroyed in the ovaries. Secretory glands of oviducts are subjected to destruction.

Figure 3. The ovaries and oviducts in the females of *Salamandrella keyserlingii* in July. The maturation of the eggs and the regeneration of oviducts are started.

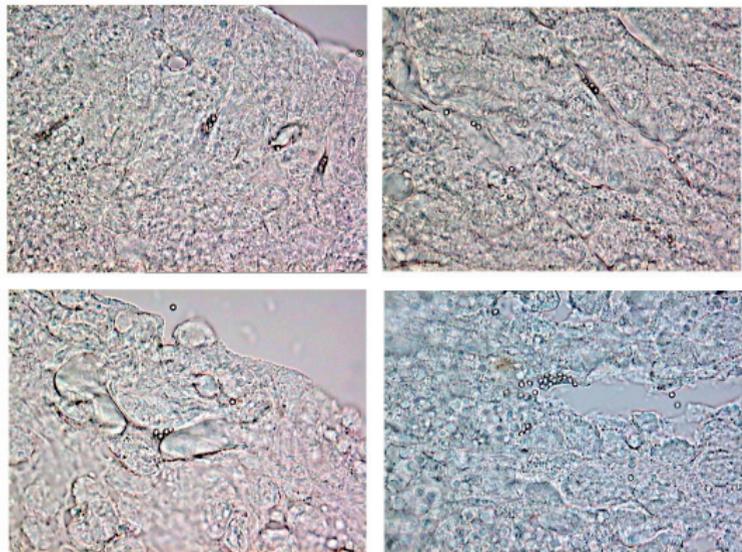


primary maturing yolk in the cytoplasm. Oviducts are normal but signs of degeneration and necrosis of epithelium are not present. Such situation is typical for females with mature but unfertilized eggs (Fig. 3). In this case the ovary is filled with healthy large eggs, but the epithelium of the oviduct is not degenerated. It indicates that the unspawned females do not abort the unfertilized eggs and the latter are reserved for the next year. Presumably they are able to spawn these mature eggs in the next breeding season. Maturing eggs in this group of females are noticed in the end of June. At the same time the epithelium of the oviducts is well developed and vascularized. In the beginning of July spermatozoa have been found in oviducts of these

females using common histochemical methods and antibodies. These spermatozoa loose terminal filament and are deposited in folds of secretory epithelium. From this moment on the maturation of ovaries is accelerated. In the end of July the ovary is filled with maturing eggs by 40-50%.

Research of the reproductive system in males shows the following results: early in May the testicles display sporadic spermatides but mature spermatozoa are absent. The number of spermatides is increased only by the end of May. The mature spermatozoa are found in the beginning of July (Fig.4, 5). Spermatozoa are characterized by a complex morphological structure (Fig. 5). They possess a long head, thick body, the tail

Figure 4. Marking of the spermatozoa in the oviducts of females with monoclonal antibodies. Spermatozoa are marked with antibodies joined to microspheres.



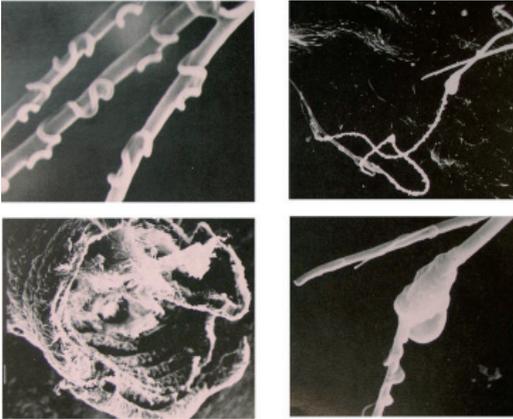


Figure 5. Spermatozoa in *Salamandrella keyserlingii* carry a large reserve of proteins and hydro carbonates which allows them to survive through winter.

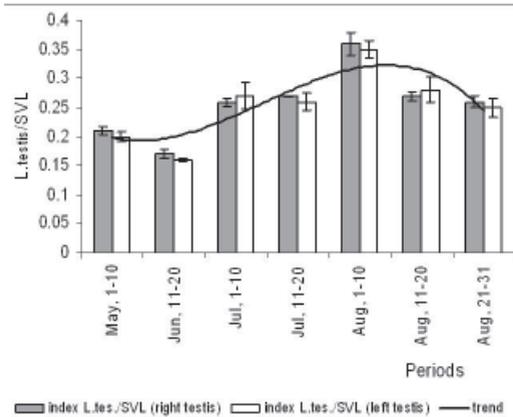


Figure 6. Seasonal dynamics of the testicles of males in *Salamandrella keyserlingii* (L_{tes}/SVL , $n=25$; Tomsk area, 2005)

adjacent cylinder and a terminal filament. The general length of the sperm cell is 115.0 ± 6.0 μm ($n=270$). During the active spermatogenesis the size of testicles is increased (Fig. 6). The seasonal dynamic of length in left and right testicles is similar. The size increases in second half of July with maximum in first decade of August.

It is possible to assume the following scheme of egg laying and reproduction in Siberian newts. Females with deposited or fertilized eggs from the previous year spawn between the end of April and the beginning of May. In their ovaries there remain degrading unfertilized eggs. After spawning the epithelium degenerates and can not be restored by June. These females will be ready for reproduction next year. They will restore oviducts and ovaries in the present season. However it can not be excluded that during favorable

seasons a female will have a chance to restore quickly and to reserve spermatozoa in the end of July - beginning of August. By this time males have enough mature spermatozoa.

In females that have not spawned in spring the epithelium does not show signs of degeneration and maturing eggs are found in the oviduct by the end of May. They are ready to accept mature spermatozoa in the beginning of July. Copulation takes place in the beginning of July. This fact is proved by presence of spermatozoa in oviducts of females of bylm's time. Females reserve spermatozoa or fertilized eggs until spring. It seems that both variants of breeding cycle exist side by side. If females could not get spermatozoa during summer then eggs are reserved for the next breeding season.

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Bioacoustics in the genus *Adenomera* (Anura: Leptodactylidae) from Santa Catarina, southern Brazil

Axel Kwet

Abstract. Bioacoustic variation recorded from different *Adenomera* populations in southern Brazil is presented and discussed. Recent recordings of advertisement calls from Santa Catarina and southern Paraná states are compared with calls published from other regions in the southern Atlantic Rain Forest domain, including those of *Adenomera araucaria* and *Adenomera marmorata*. Our data allow to differentiate between at least four species of *Adenomera* occurring in Santa Catarina.

Introduction

Eight species are currently recognized in the leptodactylid genus *Adenomera*. Three nominal species are distributed in the Atlantic rain forest domain of southern Brazil. *Adenomera araucaria* is restricted to northeastern Rio Grande do Sul and southeastern Santa Catarina (Kwet & Angulo, 2002), whereas both *Adenomera marmorata* and *A. bokermanni* have a wide distributional range and are suspected to be composites of several cryptic species (Heyer, 1973, 1977, 1984). Heyer (1984) and Angulo et al. (2003) suggested the use of bioacoustic data as a means of resolving the systematics in this group. Advertisement calls of *Adenomera* from several localities in Santa Catarina, Rio Grande do Sul and Paraná were recorded and analysed. These preliminary data revealed the occurrence of different taxa and suggest that the species diversity of *Adenomera* in this limited region is much higher than currently acknowledged.

Materials and methods

Advertisement calls of *Adenomera* were recorded from 1995-2004 at 16 different localities in the Atlantic rain forest of southern Brazil (states of Rio Grande do Sul, Santa Catarina and Paraná; for localities see Fig. 1 and Table 1). Recordings were made with a Sony WM-D6C tape recorder and a Sennheiser Me-66 microphone with K6 module. Acoustic analysis was performed using a Macintosh-based signal analysis software (Canary 1.2, Cornell University) at a sampling frequency of 44.1 kHz. Voucher specimens are deposited in the Museu de Ciências e Tecnologia da PUCRS (MCP, Porto Alegre, Brazil).

Results

The bioacoustic data presented from 16 localities in southern Brazil revealed six different call types representing at least five distinct, partly sympatrically occurring taxa of *Adenomera* (Table 1, Figs. 2-6).

(1) The advertisement call of *Adenomera araucaria* (Fig. 2) from Rio Grande do Sul and southern Santa Catarina consists of single notes, each of which presenting some amplitude modulation that can take the form of 6-11 weak pulses (Table 1, Fig. 7 left; see also Kwet & Angulo, 2002). The call duration is 85-140 ms and call rate is 26-45 calls per minute. The call is further characterized by its high dominant frequency of 4.6-5.4 kHz and a notable upward frequency modulation of 500-1200 Hz.

(2) Two different call types were recorded from the island of Ilha de Santa Catarina and nearby continental localities. One of these calls differs only in subtle nuances from the call of *Adenomera araucaria*, mainly by a shorter call duration (65-100 ms) and a lesser frequency modulation of about 500-700 Hz (Table 1, Fig. 7 right). Pending further studies, individuals of these presumably conspecific populations are referred to as *Adenomera* cf. *araucaria* (Fig. 3). A recording from Blumenau with similar characteristics but differing in a significant longer call duration (173-220 ms) is most likely an aggression call of this species.

(3) The second call type from Ilha de Santa Catarina (Table 1, Fig. 8 left) and nearby continental localities has a much lower dominant frequency (3.5-4.3 kHz) without a notable frequency or amplitude modulation. The call rate lies between 10 and 24 calls per minute, the call duration is 95-160 ms. This recording belongs to an undescribed

Figure 1. Map of southern Brazil showing recording localities.



species (*Adenomera* sp. I, Fig. 4) which is also morphologically distinguishable from *Adenomera araucaria* by its differently shaped snout ending in a small spatula and toe tips that are more expanded. (4) Populations of *Adenomera* from Jaraguá do Sul near the type locality of *Leptodactylus nanus* Müller, 1922 in northern Santa Catarina (Humboldt = Corupá) are currently assigned to *Adenomera marmorata*. However, they belong to a different taxon (*Adenomera* sp. II, Fig. 5) which is morphologically similar to *Adenomera* sp. I. but having a smaller snout-vent length. *Adenomera* sp. II differs from *Adenomera* sp. I. also in call parameters, i.e., the higher dominant frequency of 4.6-5.4 kHz and the shorter call duration of 70-120 ms (Table 1, Fig. 8 right). For these populations the name *Adenomera*

Identification	Temp. air (°C)	Number calls (indivi.)	Call duration (ms)	Calls/min	Pulse per call	Dominant frequency (Hz)	Fundam. frequency (Hz)	Other frequencies (Hz)	Frequen. modulation
(1) <i>A. araucaria</i>									
São Francisco Paula	16-26	40 (4)	119.2 (85-137)	26-45	6-11	4620-5400	2200-2640	6990-7960	500-1200
(2) <i>A. cf. araucaria</i>									
São Bonifácio	28.5	10 (1)	71.2 (68.0-90.5)	57	8-12	5300-5640	2650-2980	7700-7800	600
Taquaras	25.5	10 (1)	89.2 (84-96)	56	6-10	4840-5330	2400-2800	7200-7600	650
Santo Amaro	21	10 (1)	88.9 (85-93)	46	6-8	5220-5650	2600-2800	—	620
Ilha de Santa Catar.	21-23	30 (3)	75.7 (64.0-99.6)	46-72	6-13	4750-5540	2400-2830	7200-7950	450-600
Blumenau	21	10 (1)	189.5 (173-220)	22-35	14-18	4200-5050	—	—	700
(3) <i>Adenomera</i> sp. I									
Taquaras	25.5	10 (1)	147.0 (134-155)	18-20	1	3780-3860	—	5700-5850	0-50
Águas Mornas	17	10 (1)	130.0 (122-145)	18	1	3460-4150	—	5400-6040	30-50
Santo Amaro	20	3 (1)	129.7 (123-133)	12-14	1	4130-4290	—	6200-6400	20-40
Ilha de Santa Catar.	22-23	20 (2)	115.6 (96-133)	18-24	1	3850-4240	—	6000-6240	0
São Pedro Alcântara	17	10 (1)	138.3 (112-163)	10-12	1	3820-4050	—	5800-6200	0-20
Porto Belo	20.5	9 (1)	124.6 (118-134)	14	1	3970-4190	—	6020-6200	60
(4) <i>Adenomera</i> sp. II									
Blumenau	21	20 (2)	101.6 (83-113)	22-26	1	4870-5280	—	7300-7400	0-60
Morro do Baú	17	8 (1)	78.1 (67.2-84.5)	20	1	4980-5220	—	7500-7700	10
Ibirama	25	8 (1)	85.9 (76-95)	18-20	1	4750-4900	—	6900-7300	0-20
Rodeio	22	10 (1)	88.1 (82-93)	24-30	1	4620-4750	—	6900-7200	0-80
Guaramirim	—	12 (1)	88.8 (72.7-99.4)	22	1-3	4800-5050	2300-2600	7200-7450	60
Jaraguá do Sul	20	20 (2)	110.7 (98-122)	20-24	1	4620-5320	2400-2700	6900-7900	0-80
Pirabeiraba (call II*)	21.5	10 (1)	75.9 (70.1-80.8)	37	1	5210-5440	—	7800-7900	0-20
(5) <i>Adenomera</i> sp. III									
Guaratuba	23	10 (1)	31.6 (30.6-32.3)	120	6	4000-4200	—	—	300
(6) <i>Adenomera</i> sp. IV									
Pirabeiraba (call I*)	24.5	10 (1)	71.6 (67.7-79.1)	93	1	3030-3070	1440-1610	5950-6120	400

Table 1. Acoustic parameters for different populations of *Adenomera*. * Data from Kwet & Angulo (2002).



Figure 2. *Adenomera araucaria* from São Francisco de Paula, RS.



Figure 3. *Adenomera* cf. *araucaria* from Ilha de Santa Catarina, SC.

nana should be revalidated (see also discussion in Kwet & Angulo, 2002).

(5) A call (Table 1, Fig. 9) from Guaratuba, southern Paraná, with high call rate (about 120 calls/min) and short call duration (30-35 ms) most likely belongs to another undescribed species (*Adenomera* sp. III, Fig. 6). The call of this species, which is larger (about 27 mm) than and morphologically distinct from all other *Adenomera* in southern Brazil, has a dominant frequency of about 4-4.2 kHz without harmonics.

(6) A distinct call (Table 1, published as type I call in Kwet & Angulo, 2002) with uncertain identity was recorded from Pirabeiraba, northern Santa Catarina, by Ronald W. Heyer. This unpulsed call without frequency modulation is also unique in having a very low dominant frequency of about 3 kHz. This call probably belong to another undescribed species (*Adenomera* sp. IV).

Discussion

Extensive intra- and interpopulational morphological variation has rendered the genus *Adenomera* a taxonomically difficult group. Heyer (1974, 1977, 1984) stated a broad overlap in morphological characters between different populations of *Adenomera* and suggested that bioacoustic data are needed for resolving the systematics in this group. Recently published comparisons of call recordings from different localities in southern Brazil (Kwet & Angulo, 2002) and Peru (Angulo et al., 2003; Angulo & Icochea, 2003) confirmed that the species diversity in these regions is much greater than currently recognized. The preliminar data presented here demonstrate that populations of *Adenomera* in Santa Catarina currently assigned to *Adenomera marmorata* actually belong to several (at least four) different species. Some of the recorded calls are similar to those published from the Amazon or



Figure 4. *Adenomera* sp. I from Águas Mornas, SC.



Figure 5. *Adenomera* sp. II ("*Adenomera nana*") from Jaraguá do Sul, SC.



Figure 6. *Adenomera* sp. III from Guaratuba (23°C), Paraná.

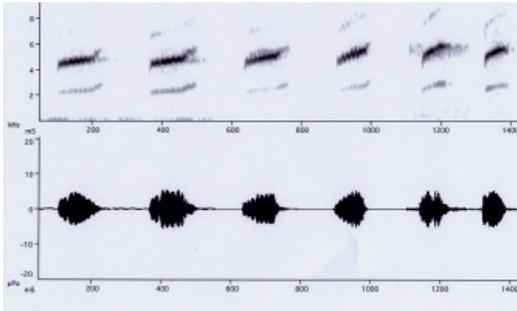


Figure 7. Audiospectrogram (above) and oscillogram (below) of advertisement calls of four males of *Adenomera araucaria* from São Francisco de Paula, RS (left), air temperatures: 19°C, 16°C, 16°C, 26°C); and two males of *Adenomera* cf. *araucaria* (right) from São Bonifácio (28.5°C) and Ilha de Santa Catarina (22°C), SC.

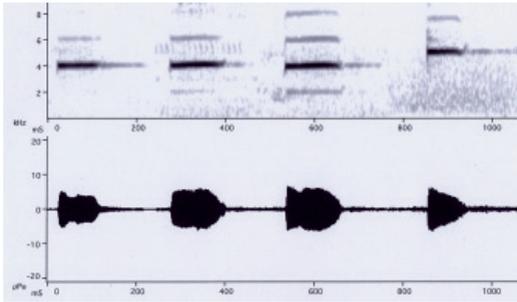


Figure 8. Audiospectrogram (above) and oscillogram (below) of advertisement calls of three males of *Adenomera* sp. I (left) from Porto Belo (20.5°C), Ilha de Santa Catarina (22°C), and Águas Mornas (17°C), SC; and one male of *Adenomera* sp. II (left = "*Adenomera nana*") from Morro do Baú (17°C), SC.

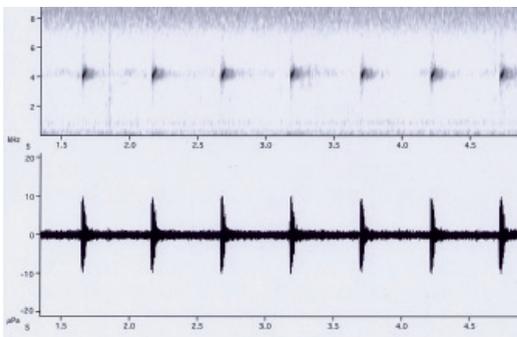


Figure 9. Audiospectrogram (above) and oscillogram (below) of the advertisement call of *Adenomera* sp. III from Guaratuba (23°C), Paraná.

Paraguay river basin, i.e., *Adenomera hylaedactyla* and *Adenomera andreae* (Angulo et al., 2003; Angulo & Icochea, 2003; Heyer, 1973; Marquez et al. 1995; Zimmerman & Bogart, 1984) and *Adenomera diptyx* from Bolivia (published as *Adenomera andreae* by Marquez et al. 1995). However, they have different acoustic parameters and are also distinctive to the human ear. Further investigations are needed to clarify the species' identity of *Adenomera* in southern Brazil and to make taxonomic decisions.

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Road killing of lizards and traffic density in central Italy

Marco Lebboroni, Claudia Corti

Abstract. The use of roads and the mortality by traffic collision were assessed for three lizard species (*Lacerta bilineata*, *Podarcis sicula*, *Podarcis muralis*) in a hilly area of central Italy. Roads were chosen to include two different habitats and two different traffic levels, resulting in four categories. For both habitats, the density of lizards using roads was significantly higher at lower traffic levels; the density of road killing was significantly higher on roads at low traffic too. Among the three species, the mortality of *Lacerta bilineata* was higher on low traffic roads crossing pasture with shrubs.

Introduction

Road traffic affects wildlife in several ways, among which direct killing of animals as a result of collision with cars is one of the main causes of mortality for many species of vertebrates in the European countries. The intensity of this impact on wildlife populations depends on many factors, concerning both ecological traits of different taxa and road features. For birds, it seems that the density of traffic strongly affects collisions with vehicles, but detailed studies are scanty (Erritzoe *et al.*, 2003). The occurrence of reptiles is generally documented in lists of road-killed species, but specific studies are scanty too. Concerning snakes, Bonnet *et al.* (1999) showed a risk of road-killing for adult males during the mating season and for juveniles during dispersion. Lizards use open habitats created by management of road sides: moreover, asphalt used as a substrate can result in increasing the basking performance. In this work we investigate the influence of traffic density on (1) the use of roads by lizards and (2) the intensity of road-killing for lizards.

Materials and methods

The field work was carried out in a hilly area of central Italy (Marche) bordering the Apennines. The landscape was dominated by corn fields interspersed with hedges and woods in lowland (habitat 1), while at higher altitudes pastures with moderate shrubbery were common (habitat 2). Roads were chosen among asphalt ones to include two different traffic densities for these two main habitat types, resulting in four road categories: (1a) field-hedges mosaic with high traffic (2a) crop-hedges mosaic with low traffic (2b) pastures-shrubbery with high traffic (2c) pastures-shrubbery with low traffic. Roads were considered at high traffic (> 30 cars/hour) or at low traffic (< 5 cars/hour) according to a preliminary count of cars in the central hours of the day. A total length

of 30 km was surveyed by car at slow speed during late morning (about 10.00 – 12.00 a.m.) in August 2004, repeating the counts three times a week. For each survey we report on maps both lizards observed using the road, and – once stopping to allow identification – lizards found dead on the same route. Count of lizards was standardized to number / km: statistical analysis was based on a non-parametric comparison between pair of roads (Mann-Whitney test).

Results

We observed three species of lizards along roads (*Lacerta bilineata*, *Podarcis sicula* and *Podarcis muralis*) ($n = 36$). Almost all the observations (95 %) resulted in lizards basking at short distance from the road sides, with a few individuals observed crossing the road. All the three species occurred among those lizards found dead ($n = 16$), with the highest relative frequency for *Lacerta bilineata* (Fig. 1). Density of observed versus killed lizards along each road was not directly compared for the different detectability of the two samples.

First, we analyse differences between values of lizard counts at high and low traffic level. The mean number / km of lizards observed was significantly higher along roads with low traffic, both in habitat 1 ($z = 2.94$, $df = 1$, $P < 0.005$) and in habitat 2 ($z = 3.05$, $df = 1$, $P < 0.005$, no lizards observed in pastures-shrubbery with high traffic).

In the same way, the mean number / km of lizards found dead was significantly higher along roads with low traffic in habitat 1 ($z = 2.68$, $df = 1$, $P < 0.01$) and in habitat 2 ($z = 2.58$, $df = 1$, $P < 0.01$) (Fig. 2). Among the three species, the mortality of *Lacerta bilineata* was higher on low traffic roads crossing pastures with shrubbery. On these roads, in a few cases this species was also observed catching grasshoppers (Acrididae), that were found dead by road-killing in a great number. When basking on the road, *Lacerta bilineata* stays restless for long periods, although escaping if approached by walking. On maps the distribution of the collisions showed that most of

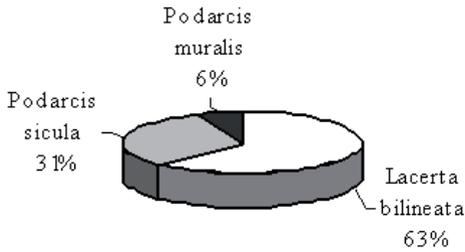


Figure 1. Relative frequencies of lizard species found dead by road-killing.

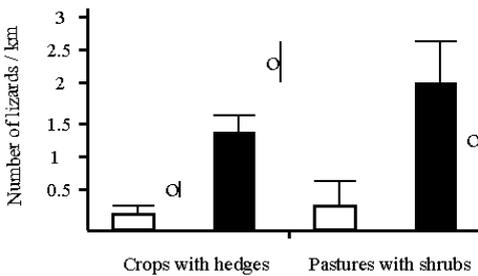


Figure 2. Comparison of lizards' abundance in the four road types. Bars (white: high traffic; black: low): mean values of number of road-killed lizards / km (with SD). Circles: mean values of number of observed lizards / km (with SD). No lizards were observed on high traffic roads in habitat 2.

the road-killings were located on the side immediately after a bend, when cars arrive suddenly at fast speed, probably not allowing the lizards to be avoided.

Discussion

Roads with lower traffic volumes were more used by lizards for basking and feeding than roads with higher car densities. Although our data refer to a short part of the annual activity period and the sample could be too small, the concordance shown by our results for the two different habitats seems to reinforce our findings, and not to be related to a general higher density of lizards in a particular habitat. The occurrence at higher densities

in low traffic roads could be explained because lizards were not immediately disturbed when entering the road, or by being not familiar with the cars' passage. Roads with higher traffic can make lizards avoiding them totally, or produce a learning effect. However, density of lizards could be already lower in the surroundings of these roads because of indirect disturbance such as noise, pollution or resources availability.

Road-killing affecting lizards has been poorly investigated, and comparisons are possible only with studies concerning vertebrates as a whole. In a study carried out in Tuscany, values of 0.35 lizards dead / km / year were found (Scoccianti *et al.*, 2001); these data are similar to ours for high traffic roads but are lower if considering the different duration of the study. For lizards, the frequency of road-killing on roads at low passage of cars could be a consistent risk for the population viability if considering the large development of roads in the Apennine countryside of central Italy. *Lacerta bilineata* in particular seems to be the most sensitive species to collision (although it is easily detectable, because of larger size, when compared to *Podarcis* sp.). *Lacerta bilineata* is reported as a species of interest in many regional Red Lists: moreover, it has been also suggested as an ecological indicator for the agri-environments in Tuscany (Corti and Lebboroni, 2004). A little more attention provided by everyone driving should be sufficient to avoid further decline of this species.

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Geographical and within-population variation of larval life-history traits in *Rana temporaria* and *R. arvalis*

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Abstract. The variance components of larval traits of two brown frog species in two widely separated populations were estimated using full-sib/half-sib breeding design and raising the tadpoles under laboratory conditions. The means of length of larval period (LP) in two species were nearly identical in each of two localities. The counter-gradient variation was revealed in size at metamorphosis (SM), LP and growth rate (GR) in *R. temporaria* and in LP and GR in *R. arvalis*. In *R. temporaria* from both localities, the heritability in SM was lower than in LP. In *R. arvalis*, the heritability in SM was higher than in LP. In both species, the heritability in GR in northern and southern populations was similar or some higher in northern population. In *R. temporaria*, the stronger (than in *R. arvalis*) influence of parental body length (including maternal effects) on SM, LP and GR reflects the importance of these traits for fitness at postmetamorphic stages of life cycle.

Introduction

The variation in larval life-history traits in temperate zone anurans depends on: (1) adaptation to local ecological conditions; (2) climatic factors, primarily the length of activity season and ambient temperatures in a given locality; (3) parental influences, including genetic and non-genetic effects. The magnitude of these three groups of factors differs in geographically remote populations (Laurila et al., 2002; Laugen et al., 2003). In addition, the dependence of larval traits on parental body size is connected with the fact that adult body size is subject to natural and sexual selection (Lyapkov et al., 2004 a, b). Therefore, in related species that differ in adult body size, this dependence can be subject to change. A comparison between two species of the brown frogs, *Rana temporaria* and *R. arvalis* permits us to test this possibility: while being different in their adult body size (Kabardina, 2002), these species are quite similar in duration of the embryonic and larval development and in body size at metamorphosis (Lyapkov, 1995).

The aim of the present study is to compare two geographically separated populations of *R. temporaria* and *R. arvalis*, with separating the components of phenotypic variation in larval life-history traits and evaluating the effects of parental body size within each population.

Materials and Methods

The adult amplexant *R. temporaria* and *R. arvalis* were collected in populations from two localities: in Moscow province, near Zvenigorod Biological Station of Moscow State University, 55 km westward of

Moscow (55°44' N, 36°51' E, hereafter called "ZBS"); and in Kirov province, near Kipenevshchina village, 40 km westward of Kirov (58°40' N, 49°5' E, hereafter called "Kirov"). An incomplete factorial breeding design (Laurila et al., 2002; Sommer, Pearman, 2003) was used to separate additive genetic variation (V_A) from non-additive genetic variation (V_N), maternal effects (V_M) and environmental variation (V_E). The males and females were anesthetized and then males' testes were removed and crushed in amphibian Ringer solution (after Laugen et al., 2002). Eggs from each of two females were fertilized by sperm suspension from two males. The tadpoles obtained from artificial fertilizations were raised in common garden experiment. Each full-sib group was reared in two aquaria (replications). The standard laboratory conditions included the density of 20 tadpoles per 18 l aquarium, feeding *ad libitum* and raising by constant temperature of 20°C until metamorphosis.

In each species and each of populations, different numbers of males (and correspondingly females) were used for mating. Unfortunately, the *R. temporaria* progeny of some matings died before hatching and therefore the numbers of successful mating "matrices" (i.e., progeny of 2 females mated by 2 males) were low: one mating "matrix" from ZBS and one from Kirov population in *R. temporaria*, 2 "matrices" from ZBS and 1 "matrix" from Kirov population in *R. arvalis*. For these full schemes, the cross-classified 3-way ANOVA design (with factors "females", "males" and "replications") was used in each species and each population. In addition, in *R. temporaria*, the whole data set (with excluding at random the progeny of one of two males in the cases of full "matrices") were used in nested 3-way ANOVA design ("females" were nested in "males"). In this nested design, the data on progeny of 5 males and 10 females from ZBS population (i.e. 10 full-sib groups), and on progeny of 3 males and 6 females from Kirov population (6 full-sib groups) were used. The causal components of variance in each larval trait were obtained by the restricted maximum-likelihood (REML) options in module VARCOMP in STATISTICA 6.0 (Statsoft Inc.). The standard interpretations (Falconer, Mackay, 1996) are used for results of both ANOVA-designs for estimating components of phenotypic variation, with residual causal variation component consisting of residual variance ($\sigma^2_{\text{residual}}$) and replication variance (σ^2_{rep}).

The genetic correlations were estimated by calculating product-moment correlations among male means (Laurila et al., 2002). The significance

of differences of product-moment correlations from zero was determined directly from correlation coefficients values and sample sizes.

The influence of parental body size on larval traits was estimated by cross-classified 2-way ANOVA (factors “female size” and “male size”, both with 2 levels: “large” and “small”), separately for each population of each species (*R. temporaria*: altogether 440 metamorphs from ZBS and 318 from Kirov; *R. arvalis*: 326 and 229, respectively). Body length was determined in each individual with caliper, to the nearest 0.1 mm. In each female, the egg diameter was determined by measuring 20-30 eggs with eyepiece micrometer, to the nearest 0.05 mm.

In each species, the data on metamorphs raised in laboratory conditions were compared with data of those from natural ponds within the same populations (Lyapkov, 1995 and unpubl. data).

Results and Discussion

Metamorphs from lab vs. those from natural ponds

In metamorphs from experimental crosses, the mean length of embryonic and larval periods (from fertilization up to the end of metamorphosis, **LP**, days) in two species was nearly identical in each locality

(Table 1, upper part). In *R. temporaria*, the mean size (body length) at metamorphosis (**SM**, mm) was higher in metamorphs from Kirov as compared to those from ZBS. The reverse differences between localities were revealed in LP. As a result the mean growth rate (**GR**, mm/day) in metamorphs from Kirov was much higher than in those from ZBS. In *R. arvalis*, mean SM and LP were lower in metamorphs from Kirov than in those from ZBS, while mean GR was higher. These differences between two localities within each species indicate on countergradient variation in all three traits in *R. temporaria* and in LP and GR – in *R. arvalis*.

In *R. temporaria* (in both localities), mean SM and GR in metamorphs from natural ponds were lower (and mean LP – higher) than in those raised under laboratory conditions (Table 1). In *R. arvalis* from ZBS, the mean LP in metamorphs from natural ponds were higher (and the mean GR – lower) than in those from laboratory. These results revealed the physiological constraints

Locality	Trait	Species: Origin:	<i>Rana temporaria</i>		<i>Rana arvalis</i>		
			Lab	Nat	Lab	Nat	
ZBS	SM		<u>15.77</u>	<u>13.37</u>	15.50	15.90	
	LP		<u>58.83</u>	<u>77.22</u>	58.14	<u>78.28</u>	
	GR		<u>0.269</u>	<u>0.173</u>	0.268	<u>0.203</u>	
Kirov	SM		16.51	16.34	14.48		
	LP		<u>51.20</u>	<u>55.0</u>	51.67		
	GR		<u>0.323</u>	<u>0.297</u>	0.282		
ZBS	SM	Female body size:	small	large	small	large	
		Male body size					
	LP	small	<u>15.22</u>	<u>15.68</u>	15.93	<u>15.14</u>	
		large	<u>16.07</u>	<u>16.85</u>	15.83	<u>15.22</u>	
	GR	small	58.16	58.56	60.03	59.01	
		large	60.10	59.07	<u>56.17</u>	<u>58.96</u>	
	Kirov	SM	small	<u>0.262</u>	<u>0.269</u>	<u>0.267</u>	<u>0.257</u>
			large	<u>0.268</u>	<u>0.285</u>	<u>0.282</u>	<u>0.259</u>
		LP	small	16.74	<u>16.10</u>	14.47	13.60
			large	16.93	<u>16.48</u>	15.02	14.65
		GR	small	<u>52.06</u>	<u>50.39</u>	58.36	<u>51.50</u>
			large	<u>51.80</u>	<u>50.40</u>	<u>49.64</u>	<u>51.24</u>
		small	0.322	0.320	<u>0.248</u>	<u>0.265</u>	
		large	0.328	0.328	<u>0.303</u>	<u>0.287</u>	

Table 1. The mean values of larval traits from laboratory crosses (Lab) and natural breeding ponds (Nat) (upper part of the table), and the effects of parental body size on mean values of progeny larval traits (lower part of the table).

Notes. Within each species, the significant ($P < 0.05$) differences between metamorphs from Lab and Nat are shown by underlining and the differences between localities are shown by bold typing. The significant ($P < 0.05$) differences between progeny of small and large females are shown by underlining, and the differences between progeny of small and large males are shown by bold typing. See text for traits designation.

Table 2. The significance of factors' effects (upper part of the table); the proportions (%) of components of phenotypic variation in SM, LP and GR (median part of the table); and the genetic (above diagonal) and phenotypic (below diagonal) correlations among larval traits (lower part of the table).

Notes. The significant ($P < 0.05$) influence of factors are shown by "+", non-significant – by "-". The significant variation components and correlation coefficients are underlined. See text for traits and variation components designation.

Species	ANOVA design	Locality:	ZBS			Kirov				
		Trait:	SM	LP	GR	SM	LP	GR		
<i>R. t.</i>	Cross-classified	Factor								
		males (m)	-	-	-	-	-	-		
		m × f	+	-	+	+	-	+		
		females (f)	-	-	-	-	-	-		
	Nested	replications	-	-	-	-	-	-		
		males	-	-	-	+	+	+		
		females	+	+	+	-	+	-		
		replications	+	-	+	-	-	-		
<i>R. a.</i>	Cross-classified	males (m)	-	-	-	-	-	-		
		m × f	-	+	+	-	+	+		
		females (f)	-	-	-	-	-	-		
		replications	-	-	-	-	-	-		
<i>R. t.</i>	Cross-classified	Variation component								
		V _A	0	46.38	0	64.81	<u>99.07</u>	0		
		V _N	<u>59.99</u>	0	<u>65.80</u>	<u>35.19</u>	0.93	<u>32.56</u>		
		V _M	25.42	0	19.37	0	0	11.13		
		V _E	<u>14.60</u>	<u>53.62</u>	<u>14.83</u>	0	0	<u>56.31</u>		
		Nested	V _A	47.18	<u>100</u>	60.10	<u>57.77</u>	<u>79.44</u>	<u>53.56</u>	
			V _N	<u>52.82</u>	0	<u>39.90</u>	0	0	0	
			V _M	0	0	0	0	0	0	
			V _E	0	0	0	<u>42.23</u>	<u>20.56</u>	<u>46.44</u>	
		<i>R. a.</i>	Cross-classified	V _A	<u>80.99</u>	8.71	25.82	49.53	16.36	58.21
				V _N	10.03	<u>82.02</u>	<u>58.96</u>	0	<u>83.64</u>	<u>41.79</u>
				V _M	0	4.58	4.53	0	0	0
V _E	<u>8.98</u>			<u>4.69</u>	<u>10.69</u>	<u>50.47</u>	0	0		
<i>R. t.</i>	Correlation coefficients:	Trait								
		SM	×	-0.273	<u>0.856</u>	×	0.552	0.313		
		LP	-0.108	×	-0.730	0.115	×	-0.618		
<i>R. a.</i>	Correlation coefficients:	GR	<u>0.865</u>	<u>-0.589</u>	×	<u>0.706</u>	<u>-0.620</u>	×		
		SM	×	0.561	<u>0.832</u>	×	-0.972	0.993		
		LP	0.064	×	0.008	-0.035	×	-0.993		
		GR	<u>0.789</u>	<u>-0.557</u>	×	<u>0.822</u>	<u>-0.594</u>	×		

imposed on SM, LP and GR in both species and within each of two localities.

Variance components in larval traits and correlations between traits

In *R. temporaria* (both in ZBS and Kirov), the proportion of V_A (i.e. heritability) in SM and in LP (Table 2, median part) was relatively high (including results of nested ANOVA). At both localities, the heritability in SM was lower than in LP. The results of nested ANOVA

confirmed that the heritability in GR was also high in both localities.

In *R. arvalis* (unlike in *R. temporaria*), the heritability in SM was higher than in LP, both at ZBS and Kirov. The proportion of V_N in LP was high and the proportion of V_E in all traits was relatively low. At each locality, the heritability in LP was lower in *R. arvalis* than in *R. temporaria*. The heritability in SM was higher in *R. arvalis* than in *R. temporaria* (in ZBS) or similar in both species (in Kirov). The heritability in GR was high at

both localities (as in *R. temporaria*). This indicates that additive variation in GR did not deplete under strong selection pressure in northern populations of both species.

In *R. temporaria*, genetic and phenotypic correlations coincided in each locality (Table 2, lower part). Both types of correlations between SM and GR were positive, while those between LP and GR were negative. The correlations between SM and LP were non-significant. In *R. arvalis*, both genetic and phenotypic correlations were similar with those in *R. temporaria*, except that the genetic correlation between LP and GR was non-significant in both localities. In the cases of negative or non-significant genetic correlations between SM and LP the directional selection can operate on both traits increasing the fitness of metamorphs. This possibility exists in both species and both localities. The significant positive (however weak) phenotypic correlation between SM and LP was revealed only in *R. temporaria* in Kirov, indicating the environmental constraints in northern populations. It is worth mentioning that in both species from natural ponds of ZBS the phenotypic correlations between LP and GR were generally positive (Lyapkov, 1995).

The non-genetic parental influence on larval traits

In *R. temporaria* (Table 1, lower part), the progeny of large males and large females (in ZBS) was characterized by higher mean values of SM and GR and by lower mean values of LP. In Kirov the same differences were revealed in progeny of large and small females in LP and in progeny of large and small males in SM. In *R. arvalis*, the similar effects were revealed in progeny of large and small males (in LP and in GR) but not in progeny of females (with only one exception – in GR, within small males, in Kirov). The stronger influence of parental body length on SM, LP and GR coincide with a stronger dependence of these traits on fitness at postmetamorphic stages of life cycle (both in juveniles and adults) in *R. temporaria* as compared to *R. arvalis*. In *R. temporaria*, these larval traits determine the body size of juvenile and immature individuals before the 1st and 2nd winterings, and thus – the survivorship during wintering (Lyapkov, 1997). Furthermore, larger males have higher breeding success while larger females have higher fecundity and reproductive effort (Lyapkov et al., 2004 a, b). The between-species differences in heritability in SM (see above) also coincided with the more tight interrelation between SM and fitness in *R. temporaria*.

The relatively high proportion of V_M in SM and GR was revealed in *R. temporaria* from ZBS only (Table 2). In all other cases, the proportion of V_M was low. The positive correlation between the egg size and SM in *R. temporaria* from ZBS ($r=+0.165$; $n=440$; $P<0.001$) fitted in well with this result, but in the Kirov population the correlation among these traits was negative ($r=-0.297$; $n=318$; $P<0.001$). The correlation between the egg size and LP was negative at Kirov ($r=-0.523$; $P<0.001$) but positive at ZBS ($r=+0.122$; $P<0.02$). The correlation between the egg size and GR was weakly positive (ZBS: $r=+0.087$; $P=0.069$; Kirov: $r=+0.139$; $P<0.02$). Surprisingly, in *R. arvalis* (both in ZBS and Kirov), the correlations between the egg size and SM (ZBS: $r=-0.114$; $n=326$; $P<0.05$; Kirov: $r=-0.204$; $n=229$; $P<0.01$) and between the egg size and GR (ZBS: $r=-0.305$; $p<0.001$; Kirov: $r=-0.110$; $P=0.097$) were negative, while the egg size positively correlated with LP (ZBS: $r=+0.312$; $P<0.001$; Kirov: $r=-0.122$; $P=0.065$). In general, the effects of egg size (on SM – at ZBS; on LP – at Kirov, and on GR – at both localities) were stronger in *R. temporaria* than in *R. arvalis*. Therefore, the maternal effects related to egg size appear to be stronger in *R. temporaria*.

The relatively low mean value of LP and high mean values of SM and GR revealed in common garden experiments at northern populations, the relatively high heritability, and the low proportion of maternal effects in all traits fit in well to the data obtained for Swedish populations of *R. temporaria* (Laurila et al., 2002; Laugen et al., 2002; Laugen et al., 2003) and *R. arvalis* (Räsänen et al., 2005) from different latitudes. The same trends were revealed in SM (but not in LP) in two Alpine populations from low and high elevation (Sommer, Pearman, 2003). But the other results of my study (the average values of SM, LP and GR, the between-population differences in proportion of variation components, in genetic and phenotypic correlations) do not coincide with the data mentioned above, indicating the distinctness among these widely separated populations. Apparently, the source of differences between southern and northern populations, in both species, concerns not only the relative contribution of variation components to single traits, but also the patterns of their interaction.

Conclusions

(1) The results of common garden experiment indicated the counter-gradient variation in SM, LP and GR in *R. temporaria* and in LP and GR in *R. arvalis*. The physiological constraints on SM, LP and GR were

revealed in both species and within samples from each of two localities.

(2) At both localities, the heritability in LP was lower in *R. arvalis* than in *R. temporaria*. In the southern population, the heritability in SM was higher in *R. arvalis*, while in the northern population it was similar in both species.

(3) In *R. temporaria*, at both localities, the heritability in SM was lower than in LP. In *R. arvalis*, the heritability in SM was higher than in LP.

(4) In both species, the heritability in GR in northern and southern populations was similar or somewhat higher in the northern population. This contradicts the hypothesis that the stronger selection pressure in northern populations can deplete the additive genetic variation in GR.

(5) In *R. temporaria*, the stronger (as compared to *R. arvalis*) influence of parental body length (including maternal effects) on SM, LP and GR reflects the importance of these traits for fitness at postmetamorphic stages of life cycle (both juveniles and adults).

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Notes on the taxonomy, nomenclature and distribution of the *Trachylepis* (formerly *Mabuia*) *aurata* (Linnaeus, 1758) complex

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Abstract. *Lacerta aurata* Linnaeus, 1758 is a composed taxon based on at least seven different species and the current usage of the name *aurata* is not stable. In order to stabilize the taxonomy we reconsider all the Linnaean types of *L. aurata*, designate and redescribe a lectotype of *L. aurata* and correct the type locality of *L. aurata* as Asia Minor. We present morphological and distributional evidence for the specific status of the nominal subspecies *Trachylepis* [*Mabuia* auct.] *aurata aurata* and *T. a. septemtaeniata* and report *T. septemtaeniata* from Afghanistan for the first time.

Introduction

The skink *Lacerta aurata* was described by Linnaeus (1758) on the basis of five earlier descriptions (Aldrovandi 1637, Seba 1734, Linnaeus 1749, Gronovius 1754, Linnaeus 1754). In all, four extant museum syntypes of *Lacerta aurata* Linnaeus, 1758 housed in the collections of the Swedish Museum of Natural History, Stockholm and the Museum of Evolution, Uppsala University, Uppsala are available. Andersson (1900) revised three of these syntypes and placed *L. aurata* into the genus *Mabuia* [= *Mabuya*]. Until recent time the name *Mabuya aurata* (Linnaeus, 1758) was commonly applied for one supposedly polytypic species distributed widely in the Middle East.

Generally, three subspecies of *Mabuya aurata* were recognized on the basis of colour pattern and number of gular and ventral scales: (i) *M. a. aurata* - having two longitudinal rows of large more or less rectangular dark spots on the dorsum (reported usually from Turkey and some adjacent Greek islands); (ii) *M. a. septemtaeniata* (Reuss, 1834) - with the dorsal pattern consisting of four more or less complete longitudinal rows of small dark spots (commonly assigned to populations from Eritrea, Syria, Iraq, southern Iran, Saudi Arabia, Bahrain, Qatar, United Arab Emirates and Oman); (iii) *M. a. transcaucasica* Černov, 1926 (formerly *M. a. affinis* [De Filippi, 1863]) characterised by the *septemtaeniata* pattern and higher number of gular and ventral scales (associated often with the individuals from Armenia, Azerbaijan [Nakhičevan], central and northern Iran and Turkmenistan) (for details and distribution see e.g.

Mertens 1924, Černov 1926, Schmidt 1939, Mertens 1952, Banikov et al. 1977, Yilmaz 1977, Achmedov and Ščerbak 1987, Leviton et al. 1992, Moravec 1998, Anderson 1999, Lymberakis and Kalionzopoulou 2003, Paysant 2005, Soorae and Al Hameiri 2005). Nevertheless, until recently, the definition of the individual subspecies remained unclear and different authors used different names for the same populations. Occasionally, also the trinomen *M. a. fellowsii* (Gray, 1845) was used for the western Turkish and adjacent Greek populations (e.g. Chondropoulos 1986).

Mausfeld et al. (2002) partitioned the genus *Mabuya* into four genera and restricted the application of the name *Mabuya* to the South American clade of these skinks. Therefore, we tentatively associate the skinks known formerly as *M. aurata* with the generic name *Trachylepis*, which was resurrected by Bauer (2003) for the Afro-Malagasy evolutionary lineage.

Our recent finding of sympatric occurrence of *T. a. septemtaeniata* with *T. a. aurata* in southern Turkey (surroundings of Birecik, voucher specimen ZFMK 13939) indicates specific status for both these taxa. However, serious questions concerning the validity of the name *Lacerta aurata* arose out from the study of the original description of this species and published revisions of its type specimens. The description itself is brief, general and largely uninformative. Type localities of *L. aurata* are given as "Jersea Anglorum, Cypro" [English island of Jersey and Cyprus] and lie outside the known ranges of today's *T. aurata* and *T. septemtaeniata*. The only species of *Trachylepis* living on Cyprus is *T. vittata* (Olivier, 1804) (see e.g. Schätti and Sigg 1989). Boulenger (1887) listed the populations from the Middle East under the name *Mabuia septemtaeniata* and used the specific epithet *aurata* for South American species. Lönnberg (1896, ex Andersson 1900) examined the Uppsala syntype of *L. aurata* and determined it as *Mabuia septemtaeniata*. Later, Andersson (1900) studied the remaining three Stockholm syntypes and associated them with names

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of three quite different skink species: *Mabuia bistriata* (Spix, 1825), *Mabuia homalocephala* (Wiegmann, 1828) and *Mabuia multifasciata* (Kuhl, 1820). This author also accepted Lönnberg's finding and concluded: "Lönnberg says that the type for Linnaeus description in Amoen. Acad. is a *Mabuia septemtaeniata* (Reuss), which thus first of all ought to bear the Linnean name." The above described situation apparently sheds doubt on the correctness of the existing usage of the name *aurata*.



Figure 1. Syntype of *Lacerta aurata* Linnaeus, 1758 (Uppsala Univ. Zool. Mus. Nr. 38). Designated as a lectotype of *Lacerta aurata* Linnaeus, 1758.



Figure 2. Syntype of *Lacerta aurata* Linnaeus, 1758 (NRM 136). Determined as *Trachylepis homalocephala* (Wiegmann, 1828).



Figure 3. Syntype of *Lacerta aurata* Linnaeus, 1758 (NRM 137). Determined as *Eutropis cf. multifasciata* (Kuhl, 1820).

In case that Lönnberg's determination is right, the name *Lacerta aurata* Linnaeus, 1758 would have priority over *Euprepis septemtaeniata* Reuss, 1834. In this case the Turkish and Greek populations currently known as *Trachylepis aurata* or *T. a. aurata* should receive a new name. *Euprepis fellowsii* Gray, 1845 would be available in this case.

To solve this problem, a thorough re-examination of the Linnaean types of *Lacerta aurata* appeared to be necessary.

Results

1. Type specimens of *Lacerta aurata*

Specimen described by Linnaeus (1749)

The first syntype of *Lacerta aurata* (Fig. 1) was originally described as *Lacerta aurata* by Linnaeus (1749). It originated from Linnaeus' own collection. Today it is deposited in the collections of the Museum of Evolution, Uppsala University, Uppsala (Catalogue number: Uppsala Univ. Zool. Mus. Nr. 38). This specimen is relatively well preserved; nevertheless, it is completely faded. Its general habitus and scalation correspond to both *Trachylepis aurata* and *T. septemtaeniata* (see e.g. Achmedov and Ščerbak 1987). However, the absence of the colour pattern does not allow to verify today if Lönnberg's identification (*T. septemtaeniata*) was correct.

Specimens described by Linnaeus (1754)

Three other syntypes of *Lacerta aurata* belonged to the collection of King Adolf Friderick of Sweden. They were described by Linnaeus (1754) as *Lacerta barbara* and they are housed in the collections of the Swedish Museum of Natural History, Stockholm (NRM 136-138). The re-examination of these specimens has generally confirmed the original Andersson's (1900) determination. The syntype NRM 136 (Fig. 2) represents *Trachylepis homalocephala* (Wiegmann, 1828) sensu Bauer (2003) and is conspecific with the two Linnean types of *Lacerta punctata* Linnaeus, 1758 catalogued as NRM 135. The specimen NRM 137 (Fig. 3) corresponds fairly well to Asian *Eutropis multifasciata* (Kuhl, 1820) or to some closely related species. The last Stockholm syntype (NRM 138) (Fig. 4) determined by Andersson (1900) as *Mabuia bistriata* can be better identified with today's *Mabuia nigropunctata* (Spix, 1825), which was until recently confused with the former species. This determination is supported by the following characteristics found in the syntype: five supraciliaries, tricarinate dorsal scales, wider snout and absence of light lateral stripe above the lateral dark band (see Avila-

Pires 1995). On the other hand, the palms of the syntype are covered by more or less subequal tubercles, which characteristic is more typical for *M. bistriata*.

Specimens described by Gronovius (1754)

The third source quoted by Linnaeus (1758) was Gronovius (1754: p. 75) who provided a description of a species named “*Scincus*” (Linnaeus added a question mark at this name). This relatively detailed description (pp. 75-76) is based on five earlier publications and apparently gathers several different reptile species under one name. The presented characteristics are mostly general and do not make possible a closer determination of the given taxon. In addition, it is reported to inhabit “Surinamam” [= Surinam].

Specimen described by Seba (1734)

This specimen was described and figured by Seba (1734: p. 141, pl. 89, fig. 3) as “*Lacerta, Americana*”. Both the text and the figure concern a representative of the family Teiidae - most probably *Ameiva ameiva* (Linnaeus, 1758).

Specimen described by Aldrovandi (1637)

The last source cited by Linnaeus (1758) was a figure of “*Lacertus Cyprius scincoides*” published by Aldrovandi (1637: p. 660). The given figure resembles Schneider’s skink *Eumeces schneideri* (Daudin, 1802) living also on Cyprus. Thus the fact that Aldrovandi mentioned Cyprus as a locality of this skink (p. 659) explains probably why Linnaeus (1758) erroneously believed that his *L. aurata* lives on this island.

2. Identity of *Lacerta aurata*

The above account shows that the description of *Lacerta aurata* is a composition based on at least seven different species and that the Uppsala syntype plays the key role in decision, which taxon should really bear the Linnean name. Regarding the fact that the faded colour pattern cannot be taken into consideration we examined the external morphology of 58 museum specimens and available photographs of *Trachylepis aurata* and *T. septemtaeniata* from Greece, Turkey, Syria, Iraq, Iran, Turkmenistan and Afghanistan (see Appendix) to find some more suitable discriminative character. It turned out that the position of frontal and third supraocular shield provides the needed key. These shields are separated from each other in *aurata*, whereas they are in contact in *septemtaeniata* (as well as the related *T. vittata*) (see Fig. 5).

In the Uppsala syntype the position of the frontal and third supraocular shield is the same as in *aurata*. Therefore, we believe that this syntype represents the taxon, which is presently called *Trachylepis aurata*. In

order to stabilize the taxonomy we take this opportunity to designate the Uppsala syntype (Uppsala Univ. Zool. Mus. Nr. 38) as the lectotype of *Lacerta aurata* Linnaeus, 1758. As a consequence of this, the remaining extant type specimens (NRM 136-138) as well as the specimens figured by Aldrovandi (1637) and Seba (1734) and described by Gronovius (1754) become paralectotypes. The Linnean type locality of *Lacerta aurata* (Jersey and Cyprus) is apparently incorrect. Thus, according to provisions of Article 76A.1.4. of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999) we correct the type locality of *L. aurata* as Asia Minor.

3. Description of the lectotype of *Lacerta aurata*

Adult specimen (sex undetermined) with moderate robust and slightly depressed body; snout-vent length 86mm; tail original, partly broken, round in cross section; tail length 126 mm; head length from rostrum



Figure 4. Syntype of *Lacerta aurata* Linnaeus, 1758 (NRM 138). Determined as *Mabuya nigropunctata* (Spix, 1825).

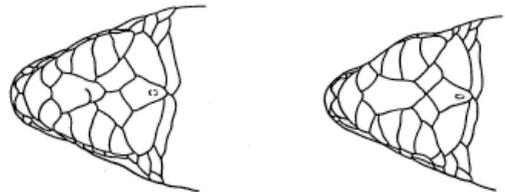


Figure 5. Difference in head scalation between *Trachylepis aurata* and *T. septemtaeniata*. Third supraocular shield separated from the frontal shield in *aurata* (left) and in contact with the frontal shield in *septemtaeniata* (right).

to the end of interparietal shield 15.4 mm; head length from rostrum to anterior margin of eye opening 18.1 mm; maximal head width 13.2 mm; four upper labials anterior and two upper labials posterior to subocular shield, three smaller scales between last upper labial and ear opening (same for left and right side); lower eyelid with a distinct transparent oval window; ear opening not covered by enlarged scales; area of the left mouth corner damaged; rostral, supranasal and frontonasal shields damaged; five supraciliary shields (left and right side); first and second supraocular shields in contact with frontale, third and fourth supraoculars in contact with frontoparietal shield (both on left and right side); postfrontal shields in a wide contact; parietal shields completely separated by interparietal shield; one pair of smooth nuchal shields; 33 scales around mid-body; dorsal scales with three slight keels; 61 gular plus ventral scales (from mental shield to vent), gular and ventral scales smooth; hind leg length (left/right) 30/30 mm; fourth finger length (from insertion of 3rd finger, claw included) 9.7/9.8 mm; fourth toe length (from insertion of 5th toe, claw included) 12.9/12.8 mm; lamellae under the fourth toe 18/18, flat and smooth; colouration (in alcohol) light brown, pattern completely faded.

Discussion and conclusions

The re-examination of the type material of *Lacerta aurata* Linnaeus, 1758 confirmed that the name *Lacerta aurata* can be fixed as a valid name. Thus, the name *Trachylepis aurata* (Linnaeus, 1758) can be correctly applied to populations having third supraocular shield separated from the frontal shield and dorsal pattern consisting of two longitudinal rows of large more or less rectangular dark spots. In consequence the name *Euprepis fellowsii* Gray, 1845 remains in the synonymy of *T. aurata*. According to our current knowledge this species is distributed in Turkey (including one locality in the European part; Bodenheimer 1944) and adjacent Greek islands: Kastellorizo, Kos, Rhodos, Samos, Simi (Chondropoulos 1986). Its occurrence in northern Syria and northern Iraq is expected (see e.g. Schmidt 1939).

Trachylepis septemtaeniata (Reuss, 1834) remains the valid name for the populations, which are characterised by third supraocular shield being in contact with the frontal shield and by pattern of four longitudinal rows of small dark spots on the dorsum (the spots can fuse anteriorly and disappear posteriorly). This species is known from Eritrea, Oman, United Arab Emirates, Qatar, Bahrain, Saudi Arabia, Syria, Iraq, Armenia, Azerbaijan, Iran and Turkmenistan. Populations coming from Armenia, Azerbaijan (Nakhičevan), central and northern Iran and Turkmenistan differ from the remaining ones by higher number of gular and ventral scales (e.g. Achmedov et Ščerbak 1987, Anderson 1999). They are traditionally recognized as a

distinct subspecies currently known under the name *T. s. transcaucasiaca* (Černov, 1926) (see Anderson 1999). Nevertheless, more detailed study is necessary to clarify the exact distribution and taxonomic status of this form. Our specimen (ZFMK 9064) collected in the vicinity of Sheva (Dar-e-Nur, Nangahar, Afghanistan) represents the first record of *T. septemtaeniata* for Afghanistan (occurrence expected by Leviton and Anderson 1970) and extends the known range from the easternmost Iranian locality (see Anderson 1999) for ca. 1200 km air distance to the east!

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Appendix

Abbreviations: NMP = National Museum Prague; ZFMK = Zoologisches Forschungsmuseum Alexander Koenig, Bonn

Trachylepis aurata

Greece: NMP 70284: Rhodos, Lindos.

Turkey: NMP 70285: 15 km E of Kas; NMP 70286: "Turkey"; ZFMK 7241: Izmir, Efes (Ephesus); ZFMK 59965: Gaziantep, Polateli; ZFMK 57086: Icel, Kizkalezi; ZFMK 57995: Püren Pass (Püren gecidi); ZFMK 68537: Mersin; 75863: Mardin, Derik; ZFMK 75873: Urfa, Halfeti; ZFMK 75880: Maras, Pazarcik.

Trachylepis septemtaeniata ssp.

Afghanistan: ZFMK 9064: Nangahar, Dar-e-Nur, vic. of Sheva.

Iran: NMP 34564: Qanar Marwan; NMP 35555: Markan, 8 km N of Ev Ogli; ZFMK 26842-44: Fars, Busheer; ZFMK 71601: Shiraz, Yasug.

Iraq: NMP 33089: Garraf at Baghdad; NMP 70613/1-19: Baghdad.

Syria: NMP 34788/1-3: Abu Kamal; NMP 34894: Abu Kamal; NMP 72492: Abu Kamal, wadi ar-Ratgah; ZFMK 71726: Abu Kamal.

Turkey: ZFMK 13939: Birecik.

Turkmenistan: NMP 70614: Kaakha; NMP 70615/1-3: "Turkmenistan"; NMP 70717/1-8: Kopet Dagh, Fijuza-Çubi; ZFMK 53810: Aşchabad.

Thermal Behaviour of the Malagasy Spider Tortoise *Pyxis arachnoides arachnoides* (Bell, 1827)

Sandra Moroni¹, Fabio Mattioli¹, Riccardo Jesu¹, Attilio Arillo²

Abstract. Five adult specimens of the Malagasy Spider Tortoise (*Pyxis arachnoides arachnoides*) collected in southern Madagascar have been housed at Acquario di Genova since April 1997. The 2.3 square metres wide terrarium in which they are hosted has been filled with limestone sand and placed close to a north facing window. UV and infrared bulbs have been positioned to allow temperature and light gradient along the longest terrarium side. During the first two years, the tortoises have adapted themselves to be active from April to October and to hibernate during the remaining part of the year. During the last two years a web-cam connected to a PC was used 24/24h in order to collect data on reproductive biology and activity patterns. Thermal preferences were investigated mainly by means of an infrared thermometer which avoided interference with tortoise behaviour. The two sexes showed significantly different thermal preferences and habitat use. Correlations between thermal behaviour and biological cycle are discussed.

Introduction

Pyxis arachnoides is a small tortoise restricted to coastal sandy soils of SW Madagascar. In the past this taxon has been considered as vulnerable by IUCN (Groombridge 1982), but in 2002 it has been moved to the “endangered” category because of several threats: habitat loss, over-collecting, human consumption, etc. Recently this species has been included in the Appendix I list of Cites.

In 1997 the Acquario di Genova established a cooperation with the Department of Animal Biology of the University of Antananarivo and the population of the subspecies *Pyxis a. arachnoides* has been investigated *in situ* (Jesu & Schimmenti 1995). On that occasion a few adult specimens were collected and transferred to Genova to establish a colony *ex situ* with the purpose of contributing to the knowledge of reproductive and thermal biology of this taxon.

Materials and methods

The five individuals (two males and three females) have been housed in a PVC open terrarium measuring 1.8 x 1.3 x 0.5 m³, placed in front of a wide window facing the north.

The terrarium, which has been filled with fine carbonatic sand, hosts some beach plants (*Rhoeo* sp.) which shade about one third of the entire surface; this has been divided into twelve quadrants (Fig. 1) grouped into

four sectors based on average temperature (Tab. 1). The photoperiod is naturally provided from outside, while the UV irradiation is guaranteed by an additional Osram Ultravitalux 300W lamp placed about one meter from the sand surface. A suitable daily temperature range is provided by means of two ceramic 150 W bulbs hanging about 15 cm from the sand surface within quadrant A1. All temperature data whether on substrate or tortoises have been gathered using an infrared thermometer Raytek MX4PTDG. During the thermal biology study period – extending from June to October 2004 - carapace temperature has been monitored every five minutes from 9.00 a.m. to 8.00 p.m. four days a week. It has been taken note of the behaviour observed in the moment in which each single temperature data has been collected. Data has been processed by the statistic program Minitab12.21.

Results

Figure 2 and 3 show the occurrence of females and males in five different times of the day within the four sectors in which the terrarium has been divided. It is evident that males prefer cooler areas of the terrarium during the night and most of the morning, moving to the basking area mainly in the second half of the day. Females, on the contrary, spend the night randomly and move to the basking area much earlier than males. This behaviour obviously conditions the body temperature of the tortoises during the day: as the analysis of variance clearly shows (table 2), the two sexes show significant different temperatures from 10.00 am to 1.00 pm, females being warmer than males. From the early afternoon, male temperatures tend to female ones because of their occurrence in the same areas (see again fig. 2 and 3).

For each category of behaviour identified, the two sexes (females vs. males) have shown significantly different average temperatures in two cases: feeding (27.13 vs.

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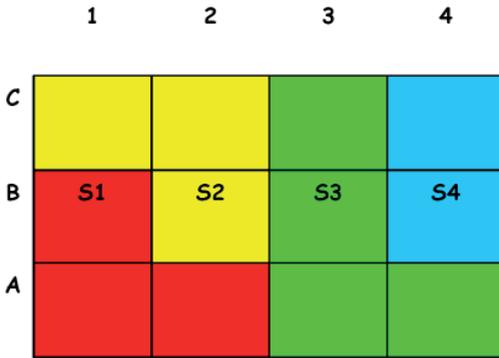


Figure 1. Terrarium area division.

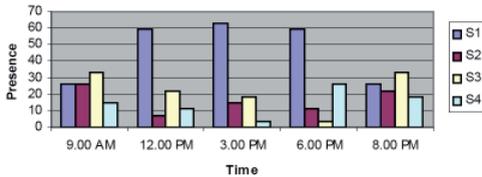


Figure 2. Female spatial distribution during the day.

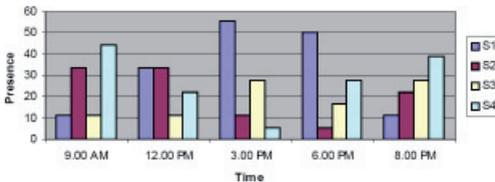


Figure 3. Male spatial distribution during the day.

27.95; $p=0.004$) and moving (28.74 vs. 30.22; $p=0.000$). Fig. 4 and 5 show the distribution of records respectively for feeding and moving within the ten temperature ranges recorded.

Discussion

The results show evidence for significant differences between males and females in terms of thermal behaviour and the activity rhythms. They can be summarized as follows:

(1) Females are used to start their daily activity long before males and with lower body temperatures, so they begin to eat and bask earlier. This behaviour

Sector	Quadrant	Temperature average
S1	A1,A2,B1	31
S2	B2,C1,C2	28
S3	A3,A4,B3,B4	25.5
S4	B4,C4	24

Table 1. Temperature average in all sectors.

Time	Male mean average	P	DF	Female mean average
9.00	24.84	0.143	1	25.19
10.00	25.32	0.001	1	26.85
11.00	26.74	0.001	1	28.55
12.00	27.58	0.000	1	29.50
13.00	29.92	0.032	1	31.76
14.00	30.70	0.066	1	32.19
15.00	32.38	0.277	1	33.33
16.00	32.19	0.382	1	33.15
17.00	31.00	0.469	1	31.58
18.00	30.73	0.441	1	31.34

Table 2. Comparison between male and female average body temperature (DF = degree of freedom).

can be explained assuming that females need a much higher energy income, compared to males, because of their different reproductive role. Such a difference is remarkable because although females have larger body volumes than males this is not enough to prevail in temperature conservation overnight but during the day it allows them to move less than males.

(2) Once gradually having reached their preferential body temperature (around 31-32°C), the females tend to maintain it rather steadily; on the contrary, males, once moved to the basking area, need less time to reach their preferential body temperature, but they need to move continuously from one sector to the other in order to adjust it. This is evidently due to their scarce thermal inertia, i.e. the higher heat dispersion of a small body, compared with a bigger one.

(3) These remarkable behaviour differences allow a correlation between thermal behaviour and reproductive activity. In particular, the early start of foraging activity characterizing the females can be correlated with the high energy cost for egg production; the same applies to their capability of keeping a rather constant high body temperature and related metabolism, which are presumably fundamental during ovulation. On the other side, the frequent movements of the males to keep their preferential temperature are also likely to favour their search for adult females.

(4) Concerning spatial distribution, males utilize constantly a specific area which at night is the cooler and vegetated one. Females, on the contrary, spend most of the nights in the areas used for basking, foraging and drinking. The present study cannot establish whether this behaviour is related to the restrictions of a terrarium or can also be observed in nature.

Acknowledgements. It would have been almost impossible to carry out this work without the precious contribution of: Aluigi F., Civitella D., Costa S., Ilaria D., Gili C., Poledri B., Salvio S., Signorelli S., Tarditi G., Zanzi D.

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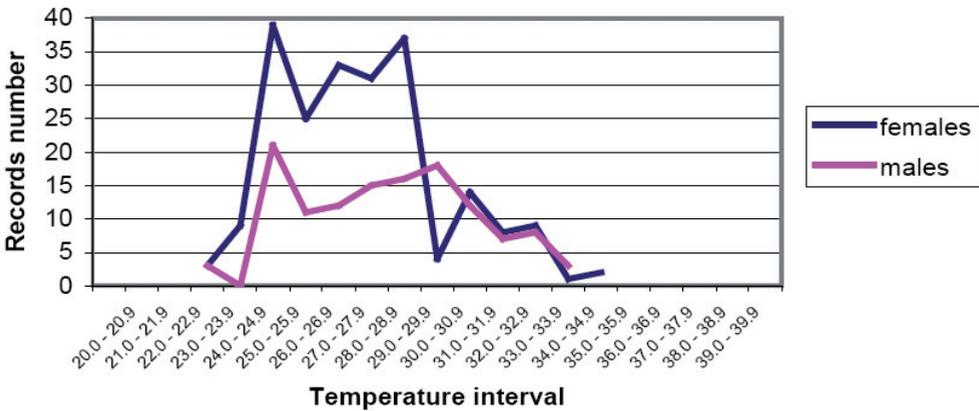


Figure 4. Distribution of feeding behaviour records according to body temperature intervals.

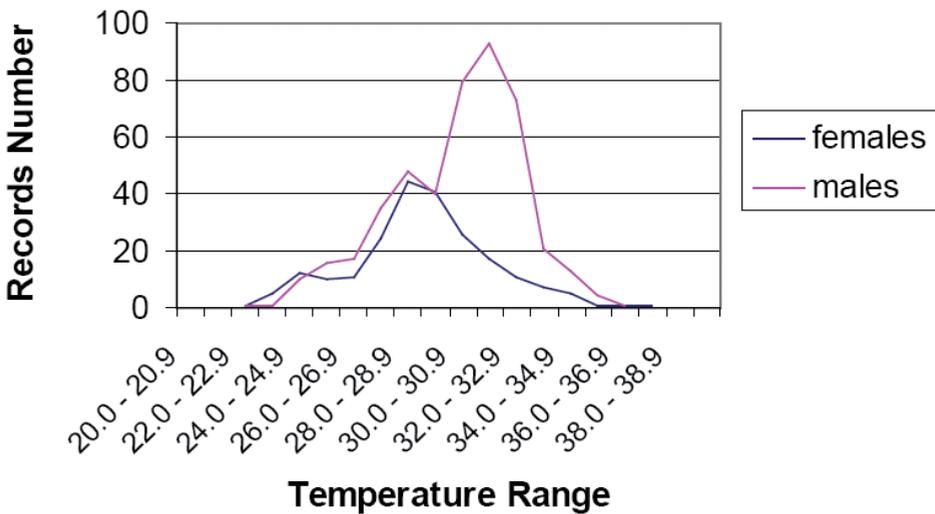


Figure 5. Distribution of walking behaviour records according to body temperature intervals.

Amphibians and reptiles in the collection of the Zoological Museum of the Tomsk State University (Western Siberia, Russia)

S. V. Moskvitin¹, V. N. Kuranova²

Abstract. In this paper we provide information about the amphibians and reptile collections in the Zoological museum of Tomsk State University (Russia), including a historical overview of the development of these collections over the past 120 years and its geographical focus.

Introduction.

Museum collections are the main source of information about biodiversity and the basis of scientific information and aesthetic knowledge of nature as was stated in the Resolution I-th International Congress on Reservation of Natural-Scientific Collections, Madrid (1992).

The Zoological Museum of the Tomsk State University presents a classical zoological collection which has been founded some 120 years ago (fig. 1, 2). The Zoological Museum has started its function with the opening of the Imperial Siberian University in Tomsk in 1888 and is connected with the activity of professor N. F. Kaschenko and the “prosector” S. M. Chugunova .

The first herpetological collections were received in 1890. They were collected with the help of employees of the university researching the territory of Western Siberia, including Altai. The herpetological material that was connected with the annual expeditions of Zoological Museum of the Tomsk State University was often published in the Scientists Reports of the Tomsk State University and the year-books of the Zoological Museum of Imperial Science Foundation (N. F. Kaschenko, A. M. Nikolsky, G. E. Ioganzen, M. D. Ruzsky, S. M. Chugunov and others).

The earliest receipt of collections is 1890. Golubeva (1923) has published the results of regional herpetofauna’s research. Then systematic research of the batracho-fauna has not been conducted due to the educational practices of students. The following stage of the museum’s growth is connected with the employment

of zoologists (herpetologists V. N. Kuranova, I. V. Laletin) and the development of ecological and population studies.

In the Zoological Museum the annotated electronic catalogue with illustrations is accessible at: http://www.inf.tsu.ru/Works/Bio/site.nsf/entry_zoo

Results

Characteristics of the collections of Amphibia and Reptilia of the Zoological Museum in the period 1891-1950.

The collections gathered between 1891-1950 and consisting of 1102 specimens (100 Caudata, 483 Anura, 13 Testudines, 1 Rhynchocephalia, 285 Sauria, 217 Serpentes, 3 Crocodylia) is least known.

This collection includes a total of 583 specimens in the class Amphibia; 34 species belonging to 17 genera, 12 families, and 2 orders.

Order Caudata: 10 species, 8 genera, 6 families (Hynobiidae, Salamandridae, Plethodontidae, Ambystomidae, Proteidae, Sirenidae).

Order Anura: 24 species, 9 genera, 6 families (Discoglossidae, Pelobatidae, Pipidae, Hylidae, Rhacophoridae, Ranidae).

The specimens originated from Europe (Italy, Germany, Sardinia, Transcarpathia, European part of Russia); Asia (Western, Central and Eastern Siberia, Kazakhstan, Mongolia, Kamchatka); Africa (Madagascar); North and Central America.

Class Reptilia: 519 specimens; 101 species, 39 genera, 22 families, 5 orders.

Order Testudines: 5 species, 5 genera, 4 families (Trionychidae, Cheloniidae, Emydidae, Testudinidae).

Order Rhynchocephalia: 1 species, 1 genus, 1 family (Sphenodontidae).

Order Squamata:

1- suborder Sauria – 47 species, 12 genera, 8 families

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(Gekkonidae, Agamidae, Scincidae, Lacertidae, Anguillidae, Varanidae, Iguanidae, Chamaeleonidae).

2- suborder Serpentes – 46 species, 19 genera, 7 families (Typhlopidae, Boidae, Acrochordidae, Colubridae, Elapidae, Viperidae, Crotalidae).

Order Crocodylia: 2 species, 2 genera, 2 families (Alligatoridae, Crocodylidae).

Geographic origin of the collections. The specimens originated from Europe (Dalmatia, Sardinia, Yugoslavia, the Caucasus, Austria, Germany, European part of Russia); Asia (Western, Central and Eastern Siberia, Altai, Kazakhstan, Ussuriysky and Primorsky Territory, Japan, Arabia, Java); New Zealand; South America (Brazil, Peru, Surinam). Overall, Amphibia and Reptilia originating from Western, Central and Eastern Siberia, Altai, Kazakhstan predominate in the collection.

Most numerous are the following species represented: *Rana arvalis* 324, *Lacerta agilis* 126, *Salamandrella keyserlingii* 61, *Vipera berus* 61 specimens.

Conservation Status. The collection includes species listed by the International Union of Protection of a Nature

(IUCN), the Washington International Convention (CITES), the Bern Convention, and the Red Book of Russian Federation.

Species included in the Red Book of Russian Federation (Danilov-Danilyan, 2001): *Salamandra salamandra*, *Pelodiscus sinensis*, *Testudo graeca*, *Eryx jaculus*, *Macrovipera lebetina*.

Species included in the Red Book of the IUCN: *Proteus aguinus*, *Chelonia mydas*, *Emys orbicularis* (Bern Convention Appendix II).

Species included in the Red Book IUCN, CITES Appendix I+II and Bern Convention Appendix I+II (Ananjeva et al., 2004): *Testudo graeca* (IUCN, CITES Appendix II, Bern Convention Appendix II), *Agryonemys horsfieldii* (IUCN, CITES Appendix II), *Sphenodon punctatus* (IUCN, CITES Appendix I), *Varanus griseus* (IUCN, CITES Appendix I), *Iguana iguana* (IUCN, CITES Appendix II), *Chamaeleo chamaeleo* (IUCN, CITES Appendix II), *Crocodylus rhombifer* (IUCN, CITES Appendix I), *Alligator mississippiensis* (IUCN, CITES Appendix I).



Figure 1. The general view of the poikilothermic animals' systematic exposition in the Zoological museum of the Tomsk State University.

Figure 2. Amphibian specimens for exposure in the Zoological museum of the Tomsk State University.



Informations of the collections of amphibians and reptiles of the Zoological Museum of the Imperial Siberian University (later Tomsk State University) are presented in works of Kaschenko (1896, 1898, 1902, 1909), Anikin (1896, 1902), Chugunov (1911, 1913), Nikolsky (1915, 1916, 1918), Ioganzhen (1923), Golubeva (1923), Vashkevich (1925), Borkin (1975 a, b), Kuranova (1989, 1998, 2000, 2002).

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Capture effectiveness of terrestrial drift fences and funnel traps for the Great Crested Newt, *Triturus cristatus*

Daniel Ortmann, Monika Hachtel, Ulrich Sander, Peter Schmidt, David N. Tarkhishvili,
Klaus Weddeling, Wolfgang Böhme

Abstract. We compared the effectiveness of permanent drift fences and submerged funnel traps for capturing Great Crested Newts (*Triturus cristatus*). The average effectiveness of the drift fence amounts to 49.3%, with a high fluctuation from 26 to 74%, with the number of adults captured decreasing from 138 individuals in 2001 to 35 in 2003, at first view indicating a strong decline in population size. The additional application of funnel traps and mark-recapture techniques, however, clearly demonstrated a nearly constant population size over this period. Therefore, the use of funnel traps combines high capturing effectiveness with undisturbed migration behaviour close to the pond.

Introduction

In the course of a long term study on amphibian populations in an agricultural landscape (see Hachtel et al. 2005) we compared the effectiveness of permanent drift fences and submerged funnel traps for capturing Great Crested Newts (*Triturus cristatus*). In addition to Arntzen et al. (1995) and Baker (1999) both systems were simultaneously used in the same breeding pond during 2002 and 2003. The annual population size was estimated using the Lincoln-Petersen method.

The goal of this analysis was to give recommendations of reliable methods for estimating population size of Great Crested Newts, a fundamental precondition for an appropriate conservation strategy especially in the context of the habitat directive of the European Union (Natura 2000).

Methods

The study focused on a natural pond surrounded by intensively used agricultural landscape. Using a permanent drift fence encircling the breeding site over three years and submerged funnel traps for two weeks per year, Great Crested Newts (*Triturus cristatus*) were registered by photo identification of the belly patterns. Consequently, we could use the Lincoln-Petersen method, which permits to compute the effectiveness of these two capture methods. Here, the capture probability is defined as the portion of captured animals of the total population size estimated with a capture-recapture model (Arntzen et al., 1995). A detailed description of capture methods and of the locality is given in Ortmann et al. (2005) and Weddeling et al. (2004)

Results

The number of adults captured at the drift fence decreased continuously from 138 individuals in 2001 to 35 in 2003 (Tab. 1). These numbers could, by uncritical evaluation, be misunderstood as a population decline of more than 70%. In contrast to such an interpretation, the total population size determined by Lincoln-Petersen-method shows clearly overlapping confidence intervals between the years, and no decline can be proved.

The average effectiveness of the drift fence amounts to 49.3%, with a high variability from 26 to 74 %, which is comparable to the findings of Arntzen et al. (1995).

Remarkably, the effectiveness of the drift fence significantly decreased within three years (Spearman correlation, $p < 0.001$), while those of the funnel traps increased in both years (fig. 2).

After 14 days already, the submerged funnel traps proved to be more efficient to determine the population size in comparison to daily drift fence census over eleven months.

Discussion

The large range of the fence effectiveness for *Triturus cristatus* (already shown by Arntzen et al., 1995 and Baker, 1999) shows the necessity to examine the accuracy of the method for each locality and to quantify the measurement error, if quantitative statements are to be met. Only in this way the comparability of results (e. g. between single water bodies, investigation years or especially different projects) can be assessed and further estimation of population parameters e.g. survival rates are reliable.

In agreement with Baker (1999) our data furthermore strongly suggest that drift fences considerably influence the migration behaviour of *T. cristatus*, leading to

Table 1. Effectiveness of drift fence with pitfall traps, and funnel traps in the study area from 2001-2003 for adult males and females of the Great Crested Newt (*Triturus cristatus*).

Year / Sex	2001		2002		2003	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Individuals in pitfall traps	54	84	36	48	18	17
Individuals in funnel traps	-	-	32	26	43	31
Lincoln-Petersen estimation	71 ± 20	115 ± 30	75 ± 25	78 ± 38	52 ± 19	84 ± 66
Effectiveness of pitfall traps %	76.1	73.1	48	61.5	34.6	20.2
Effectiveness of funnel traps %	-	-	42.7	33.3	82.7	36.9

an accumulation of newts inside the enclosure. For the closely related *T. dobrogicus*, Jehle *et al.* (1997) stated as well that the proportion of the registered individuals at the drift fence was reduced considerably in the course of several years, while the numbers of new catches in underwater funnel traps had been “relatively large”. For a number of North American amphibians and reptiles, Crosswhite *et al.* (1999) observed that the capture efficiency of drift fences over two years decreased. Trusting the drift fence data

alone, one would have assumed a strong decline in population size. The additional application of funnel traps and mark-recapture analysis, however, clearly demonstrated a nearly constant population size over this period. Therefore, the use of funnel traps combines high capturing effectiveness with undisturbed migration behaviour close to the pond. Thereby the question arises whether the fluctuations of the population size found in comparable studies are due to fluctuations in the capture effectiveness rather than in the real population size.

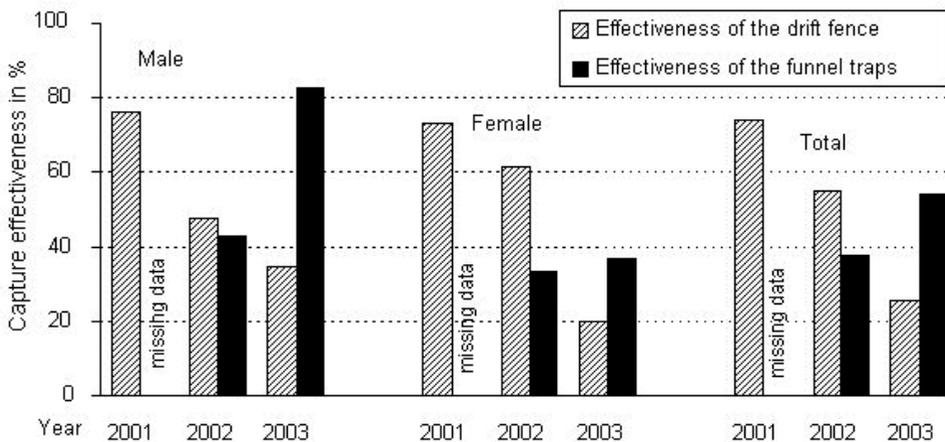
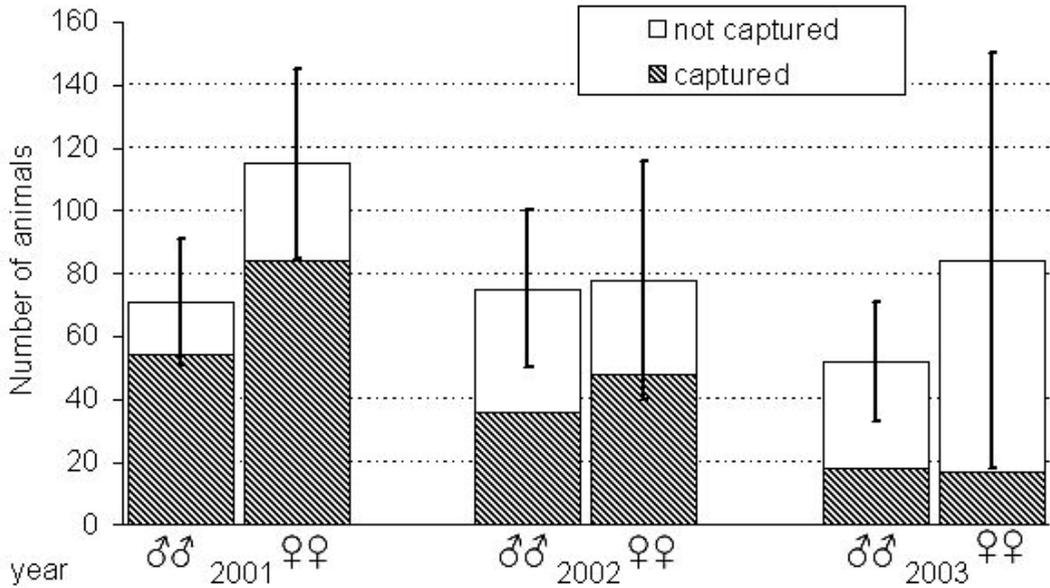
**Figure 1.** Effectiveness of drift fence and funnel traps (in %) for the Great Crested Newt (*Triturus cristatus*) from 2001-2003, for males and females. 100 % = population estimated with Lincoln-Petersen-Method.

Figure 2. Population trend of the Great Crested Newt (*Triturus cristatus*) in the study area from 2001-2003 based on drift fence data (dark bars) and corrected for mark-recapture-data (Lincoln-Petersen estimation based on recapture data by inner circle of pitfall traps (2001, emigrating individuals in autumn), and by submerged funnel traps (2002-2003, during breeding season), bars incl. 95 % confidence interval).



If the measuring error is not quantified, population size estimation and resulting trends often are strongly biased. These effects are relevant regarding the current discussion on the evaluation and conservation of populations in the EU habitat directive, in which *T. cristatus* earns special attention as a species in the appendices II and IV.

Acknowledgements. This project is financially supported as a 'testing and development project' by the Federal Agency for Nature Conservation (BfN) and the Federal Environment Ministry (BMU). Many thanks to our colleagues G. Bosbach, R. Damaschek, A. Dissanayake, R. Rottscheidt, A. Sampels, and M. Thomas for their work in the field and discussions. Permissions for catching and marking amphibians were granted by the local administration for nature conservation (Untere Landschaftsbehörde, Rhein-Sieg-Kreis, NRW).

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Amphibians and reptiles as indicators in Mediterranean agro-ecosystems: A preliminary study

Emanuele Paggetti, Marta Biaggini, Claudia Corti, Marco Lebboroni, Roberto Berti

Abstract. Agroecosystems are very vulnerable environments, due to high anthropogenic pressure that causes depletion and continuous changes. To measure the level of impact generated by agricultural practices, indicators are needed. Arthropods are commonly used as bio-indicators but their sampling requires relatively long-term investigation, consistent human resources and could even hinder the farmers' work. On the contrary investigating herpetofauna with standard methodologies can allow relatively quick surveys, easy taxonomic identification (even at species level) and avoidance of farmer disturbance. Indices of presence and richness can be well related to habitat complexity, in addition considering that Amphibians are linked to both aquatic and terrestrial habitats. Some species are particularly easy to be detected and the ratio of their occurrence can give information on the habitat status. The present study was performed in central Tuscany (Italy), in an area highly subjected to anthropic pressure.

Introduction

Agro-ecosystems are the most intensively managed environments and probably those which can change very quickly, due to periodic and chronic disturbances caused by agricultural management (Elliott and Cole, 1989). Greatest attention is thus paid to landscape and habitat conservation, including also agricultural practices (Jackson and Piper, 1989). To monitor environmental changes and determine the effects of agro-environmental measures on biodiversity different indicators are required (landscape structure, external inputs or bioindicators such as number of species, presence of key species and so on) (e.g., Bockstaller *et al.*, 1997; Büchs, 2003; Döring *et al.*, 2003). A quick monitoring system (Beattie *et al.*, 1993) or simple indicators (Büchs, 2003) are often required especially in agricultural contexts. Arthropods and birds are probably the most used bioindicators (e.g., Benton *et al.*, 2002), but indeed some problems arise when referring to agro-environments making these indicators unfitting (e.g. the employed scale, the sampling effort and the hindering of the farmer work). On the contrary, amphibian and reptile sampling causes less disturbance to the farmers' work, because they are quite simple to be recognized even at the specific level; moreover, their home ranges are better related to the average field scale size (Buys, 1995).

This work, that is part of a larger one mostly dedicated

to the arthropod diversity and to the evaluation of some agricultural measures, tries to verify the convenience of using reptiles and amphibians as indicators of the *status* of an agro-ecosystem.

Materials and Methods

Study area

The Valdera area, central Tuscany (Pisa, Italy), is a region highly subjected to anthropogenic pressure. The landscape is characterised by wide intensively managed arable lands (most of them are devoted to cereal production) while the remaining areas at field margins are covered by woodlots, riparian strips and hedgerows. The climate is mesothermal, humid, Mediterranean (mean annual temperature = 12,7 °C; average annual rainfall = 678 mm).

We identified some "environmental categories", distinguishing areas with different vegetation cover and land use characteristics. Sampling and observations were carried out mainly in four areas: an intensive culture wheat field (F), a woodlot (W) formed by an oak coppice, a pasture (P) used for sheep grazing and the experimental centre "S. Elisabetta" (Vicarello di Volterra, Pisa) belonging to the "Istituto Sperimentale per lo Studio e la Difesa del Suolo" of Florence. In this area we identified four more units:

- (1) grass strips (GS): overgrown with a continuous, thick mantle of herbaceous vegetation and inserted in the cultivated area (average size: 4.40 x 60 m);
- (2) cultivated strips (CS): separated by the grass strips (GS) and characterized by organic farming of cereals (average size = 17 x 60 m);
- (3) meadow (M): next to the strips (GS and CS). The prevalent essence is couch grass;
- (4) riparian (R): strip of vegetation along a ditch made up of herbaceous species and bramble bushes.

Sampling procedures

Arthropods were collected during a whole year (from May 2003 to May 2004) using pitfall traps. Each trap consisted of a plastic tank filled with an attractive solution of vine grade (with attractive function) and acetylsalicylic acid (with preservative function).

Traps were emptied and replaced once every fifteen days.

Reptile and amphibian data derive from: animals occasionally fallen into pitfall traps set for the arthropod fauna sampling, field observations of live animals and individuals occasionally caught in water tanks used for runoff and sediment sampling, inside the experimental centre.

Tanks checking and field observations were carried out throughout a year, contemporarily to pitfall sampling.

Statistical analyses

We used Shannon-Wiener index to assess Arthropod diversity (Shannon, 1948): for each trap we considered the total of individuals analysed at order level. We used EstimateS 5.0.1 to compute randomised accumulation curves independent from sampling temporal order.

Relative abundance of herpetofauna in the different surveyed habitats was calculated using all the available data sources. For each habitat the total number of amphibians and reptiles was divided for the number of traps/tanks. We also determined the cumulative curve relative to the number of caught species (fig.1).

In order to assess herpetofaunal diversity we used the Hierarchical Richness Index (HRI) (French, 1994): $\sum r_k * n_k$, where r represents the rank of the k -group, n is the number of specimens of the same group. This index has been chosen because it incorporates both taxonomic diversity and abundance in a single measure of richness that is less confusing than other indexes and it ranks sites according to easily definable objective criteria (Fabricius *et al.*, 2003).

To define an eventual correlation between arthropods and herpetofauna diversity HRI (fig. 2) we tested the correlation with the Spearman non parametric coefficient. A regression curve was calculated too.

Results

In fig.1 herpetofauna relative abundance (columns) and cumulative number of species (line) are shown: P and F were the “poorest” habitats and CS presented intermediate values of species richness and abundance. The other habitats, with lower anthropogenic disturbance, showed higher values. Fig. 2 shows the results relative to French index for each habitat (second column, divided for the sampling effort), together with number of species (first column) and relative abundance (third one). The riparian unit is the richest one, due to the presence of more amphibians; it is important to notice the differences in the number of species and the abundance between cultivated (organic) strips and the intensive field. Similar results have been found as far as arthropod diversity is concerned, suggesting the usefulness of uncultivated strips or grassy field margins.

Herpetofauna HRI and arthropod Shannon Wiener index resulted to be significantly correlated (Spearman's $\rho=0.964$; $P<0.01$). The obtained

regression curve (fig.3) fits well ($R: 96,76$; $V.ex: 93,62$). The curve has a particular sigmoid shape suggesting the presence of an ecological threshold.

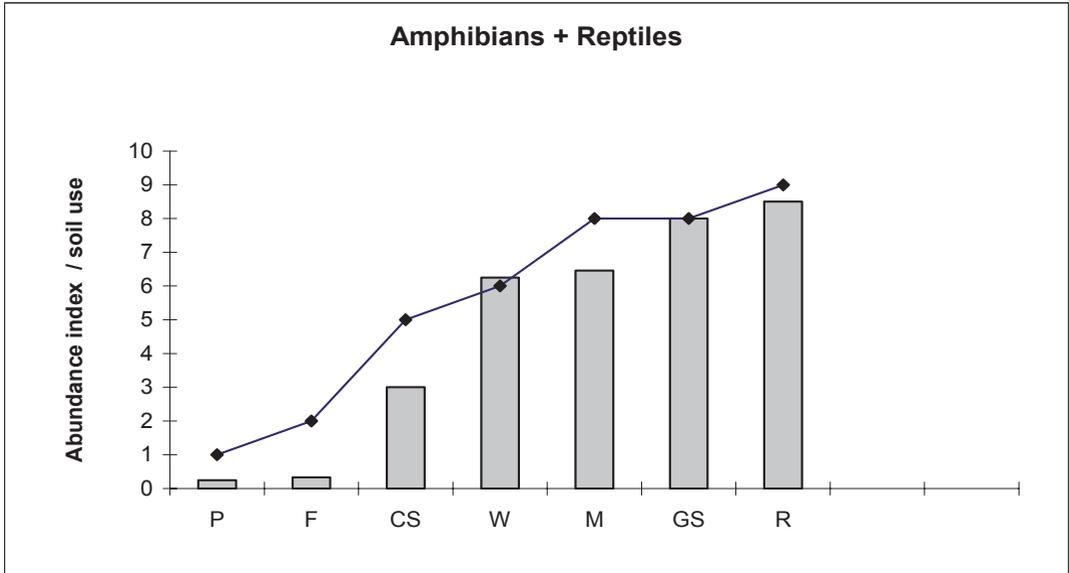
Discussion

Arthropods are commonly used as bio-indicators in agro-ecosystems and their reliability is confirmed by a large number of studies (e.g., Brown, 1991; Kremen *et al.*, 1993; Duelli, 1997; Döring *et al.*, 2003; Duelli and Obrist, 2003; Jeanneret *et al.*, 2003). However arthropod sampling can necessitate relatively long-term investigations and their taxonomic identification, particularly at the specific level, requires an enormous amount of resources in terms of time, money and specialists (Cardoso *et al.*, 2004). Using arthropods as indicators can therefore turn out to be too much expensive and time consuming when quick biodiversity surveys are required. This happens, for example, in agro-ecosystems where the possible hindering of farmers' work must be considered, too. In the present study, that is part of a wider research mainly focused on arthropods, we tried to find an alternative way, proposing herpetofauna as a possible indicator. In fact investigating amphibians and reptiles can offer some benefits: they are widespread in the Mediterranean regions, also in anthropic modified areas, they require relatively quick survey time using standard methodologies and their taxonomic identification, even at species level, is relatively simple. In particular, some species are easy to be detected and the ratio of their occurrence can give information on the habitat status (Corti *et al.*, 2001). The use of herpetofauna could provide a useful shortcut for biodiversity surveys in agro-ecosystems, making possible a great saving of financial and human resources. The results show conformity to the indication deriving from Arthropods, confirming the importance of semi-natural habitats for the preservation of biodiversity in agro-environments. As a preliminary deduction, we can affirm that herpetofauna could be used as an agro-environmental indicator, at least at a local level.

Acknowledgements

We are very grateful to the “Istituto Sperimentale per lo Studio e la Difesa del Suolo” of Florence for allowing us to work in the experimental centre of Vicarello (Pisa) and in particular Paolo Bazzoffi, director of the Section of Physics. We also thank Marco Dellacasa and Leonardo Dapporto for their help and suggestions.

Figure 1. Relative abundance of herpetofauna in each habitat (columns) and cumulative number of species (line). For soil use abbreviations see subchapter Study Area in Material and Methods.



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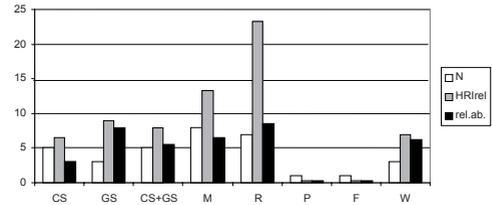


Figure 2. Diversity and abundance of herpetofauna. The first column shows the number of species found in each habitat, the second shows the HRI value (considering the sampling effort), the third the relative abundance.

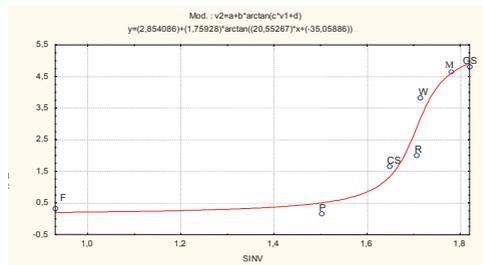


Figure 3. Correlation between Arthropod diversity (Shannon index) and herpetofauna richness (HRI, modified for the total number of species).

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Variation of moulting activity in *Lacerta agilis* and *Zootoca vivipara* (Reptilia: Sauria: Lacertidae)

S.V. Patrakov, V.N. Kuranova

Abstract. Specimens of *Lacerta agilis* and *Zootoca vivipara* were investigated at the vicinity of Tomsk, Russia, with respect to their moulting activity. There are species-specific, age-specific, sex-specific, seasonal and annual differences in the process of moulting. Seasonal dynamics of moulting had one or two peaks per season and depended on the habitat. Because of intensive growth, immature individuals had 2 or 3 moults per season. Adult females may shed their skin after pregnancy. According to changing abiotic factors the frequency of moulting increased or decreased. Individuals usually moult non-simultaneously. Differences in moulting frequency seem to be species-specific.

Introduction

It is known that hormone influence is the trigger mechanism for moulting in reptiles. When the pituitary gland affects the thyroid gland then thyroïdin defines the beginning of moulting. This process is not autonomous, but triggered by the environment and abiotic factors. Quantitative data on the moulting dynamics of Lacertilia are fragmentary. In the Asian part of Eurasia this process was mainly studied in *Eremias* and *Phrynocephalus* lizards (e.g., Semenov and Shenbrot, 1986; Kotenko, 1985). Few aspects of the moulting in *Lacerta agilis* and *Zootoca vivipara* in the Asian part of their huge natural range were studied by Garanin (1983). The purpose of the present study is the identification of species-specific, age-specific, sex-specific, seasonal and annual differences in moulting activity in the two sympatric species *L. agilis* and *Z. vivipara*.

Materials and methods

Research was carried out from May to September in the years 2002-2004 on two stationary experimental grounds in the vicinity of Tomsk, Russia. The size of the first experimental ground (investigated in 2002) is 800 m². It is located in the ecotone of overgrown cutting and a plot of mixed forest in the drainage trench. The size of the second experimental ground (investigated 2003-2004) is 2100 m². It was located within pine forest, approximately 500 m distance from the forest edge. The northern border was pine forest, whereas the southern border was a mixed forest with the prevalence of small-leaved plants. In the south, the ground is covered by dense brushwood.

Capturing of lizards was done by hands. Individual marking was done using paint from moult to moult and by phalangeal amputation (Kuranova et al., 1981). Counting of lizards was carried out on transects and by the route method. The first experimental ground was split into 464 squares, the second one into 319 squares (2 x 2 m

each), respectively. For each square the number of individuals of *L. agilis* and *Z. vivipara* was calculated. In total, 119 record excursions were conducted; 82 individuals of *L. agilis* and 151 individuals of *Z. vivipara* of both sexes and different ages were marked; 48% and 36%, respectively, of the repeatedly moulted individuals were registered. Statistical data processing was done according to traditional methods (Rokitsky, 1967; Lakin, 1980) and the use of application programs (Microsoft Excel 7.0).

Results and discussion

Specific dynamics of seasonal moulting

Lacerta agilis: Adult females and males showed the same tendency of moulting during one season. At the time of emergence from hibernation (April–May) lizards shed the skin. Due to the duration of the certain period of emergence from hibernation this process was prolonged (June–July). For this reason, most of the moulting individuals were registered in June. In July the recordings of moulting lizards was significantly lower. In August lizards moult repeatedly prior to their disappearance into hibernation. This fact explains the second seasonal peak in moulting activity (Fig. 1).

In contrast to adults, immature males and females of *L. agilis* shed their skin asynchronously. In June, moulting immature males were registered more often than immature females. This is quite different in the fall (Figs. 1-2).

Zootoca vivipara: Clear differences in the nature of moulting among individuals of different sexes were noticed: males (adults and subadults) moult relatively smoothly and synchronously from June to July, whereas intensity of moulting of females of the same groups is different in various periods of the season (Figs. 1-2). Climatic conditions have a significant influence on the seasonal nature of moulting. Under conditions of the lingering cold spring in 2003 the largest value of

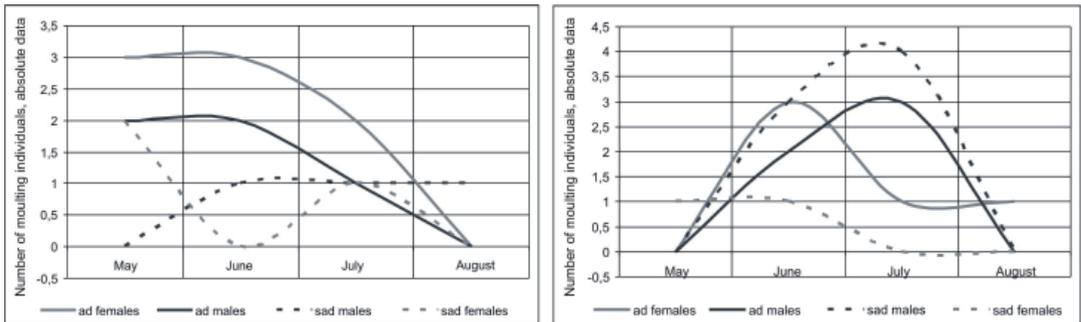


Figure 1. Seasonal dynamics of moulting of lizards in the vicinity of Tomsk in 2002. Left, *Lacerta agilis*; Right, *Zootoca vivipara*.

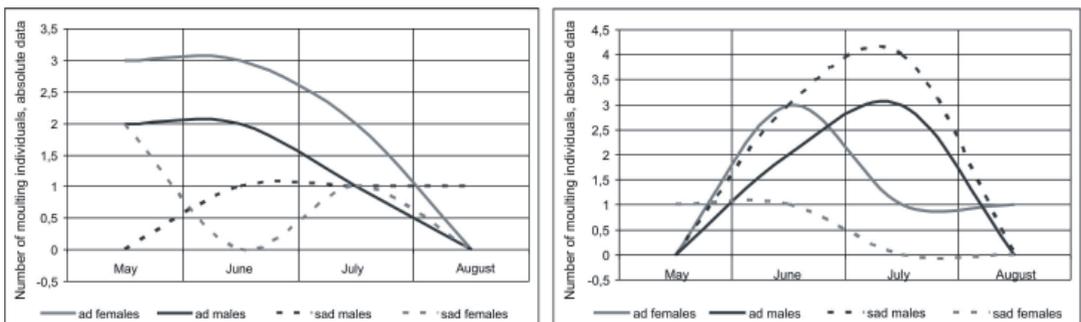


Figure 2. Seasonal dynamics of moulting of lizards in the vicinity of Tomsk in 2003. Left, *Lacerta agilis*; Right, *Zootoca vivipara*.

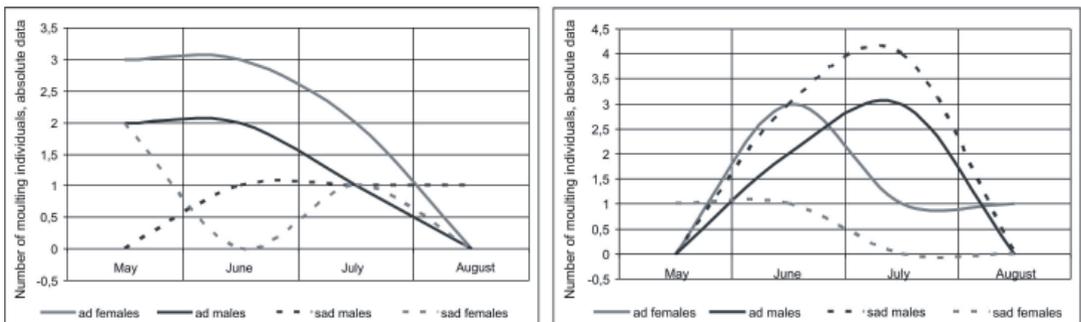


Figure 3. Seasonal dynamics of moulting of lizards in the vicinity of Tomsk in 2004. Left, *Lacerta agilis*; Right, *Zootoca vivipara*.

moulting females was noticed in July, whereas in 2002 this peak was obvious in June (Figs. 1-2).

Annual variability of moulting

Lacerta agilis: Phenology of the process changes according to climatic conditions of the specific year of observations. There is an annual variability in the duration and intensity of moulting (Figs. 1-3). In 2003, the spring began rather late. The process of moulting

was prolonged. Decrease of moulting frequency was smoother (Figs. 1-2). In 2004, the spring began quite late. The first half of the summer was cold and wet. The result was a delay of the emergence from hibernation and moulting was extended for the whole summer period. The peak of moulting was observed in August before the disappearance of the lizards into hibernation and at the same time the immature groups moulted synchronously in a considerable degree (Fig. 3). In 2003-2004, 25.5%

of 47 marked individuals were moulted, 19.1% of them repeatedly. This fact confirms the presence of annual variability in the frequency and intensity of moulting.

Zootoca vivipara: In 2003-2004, 37.5% of 104 marked individuals were moulting. Phenology of the process changes according to climatic descriptions of the specific year of observations. There is an annual variability in the duration and intensity of moulting (Figs. 1-3). Since phenology of the process changes according to climatic conditions of the specific year of observations, in 2004 the moulting of both species was characterized by considerable duration because of the late beginning of spring. Both species show the same tendency: moulting is prolonged from May to July (Fig. 3B).

Our results clearly show species-specific, age-specific and sex-specific differences in moulting activity, as well as seasonal and annual variation in the same process. The nature of the seasonal dynamics may have a single or two peaks of moulting activity, obviously related to habitat conditions. Because of intensive growth,

immature individuals have 2 or 3 moults per season. Adult females usually shed their skin after pregnancy. According to the variation of abiotic factors moulting can be prolonged or short-termed.

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Conservation and distribution of *Neureergus microspilotus* (Caudata: Salamandridae) in the Zagros Mountains, Kermanshah Province, Western Iran

Nasrullah Rastegar-Pouyani

Abstract. The mountain-dweller newts of the genus *Neureergus* Cope, 1862 are currently recognized in Iran with three species: *Neureergus crocatus* Cope, in northwestern Zagros, west Azerbaijan Province, *N. microspilotus* Nesterov, in central Zagros, Kurdistan and Kermanshah Provinces, and *N. kaiseri* Schmidt, in southwestern Zagros Mountains, Lorestan Province. While the first two species are found also in north-eastern Iraq and south-eastern Turkey, *N. kaiseri* is endemic to the Zagros mountains. Based on extensive research in almost all parts of the Zagros Mountains, the distribution and conservation status of the mountain spotted newt, *Neureergus microspilotus*, are discussed. The discovery of this species in some remote brooks and streams in highlands of the Zagros is noted. The effects of severe drought of recent years as well as man-made habitat destruction and pollution on the population decline of this vulnerable species are determined. It is concluded that if an urgent conservation plan for population stabilization of this species does not become effective, almost all populations of this sensitive taxon will go extinct in the near future.

Introduction

The mountain-dweller newts of the genus *Neureergus* Cope, 1862 are currently recognized in Iran with three species (Schmidtler and Schmidtler, 1975): *Neureergus crocatus* Cope, in northwestern Zagros, west Azerbaijan Province, *N. microspilotus* Nesterov, 1917, in central Zagros, Kurdistan and Kermanshah Provinces, and *N. kaiseri* Schmidt, 1952, in southwestern Zagros Mountains, Lorestan Province. Additionally, *N. crocatus* and *N. microspilotus* are found in some areas in northeastern Iraq as well as southeastern Turkey.

The mountain newt, *Neureergus microspilotus*, is an important faunal element in its main area of distribution in the Kermanshah Province. This province is located on the western periphery of the Iranian Plateau and a major part of it is covered by the Zagros Mountains and its western foothills (Fig. 1). *Neureergus microspilotus* is threatened due to habitat loss through divergence of streams for irrigation of cultivated lands. In contrast, habitat loss does not appear to be a major problem for *N. kaiseri* presumably because of the lack of cultivated land in the very rough mountainous terrain.

Based on long-term field research in various regions of the Zagros Mountains, conservation status and distribution of the mountain newt, *Neureergus microspilotus*, are shortly discussed and some

guidelines for protection and population stability of this vulnerable species are suggested.

Distribution

Neureergus microspilotus is mainly distributed in mountainous brooks and streams in deep valleys of the Zagros Mountains, in Kermanshah Province at elevations of 1200-1800m. A distribution map for this newt in the Kermanshah Province is given here (fig. 1). Additionally, some new habitats and localities for this species in Kermanshah Province are addressed. For example, *N. microspilotus* was recorded in some remote and mountainous localities (deep valleys) inside the Central Zagros Mountains at an elevation of about 1800 m in the northern and north-eastern regions of the Kermanshah Province (figs.1-2). Based on all the available evidence, it is proposed that the formation and uplifting of the Zagros Mountains in the late Miocene and early Pliocene have played an important role in radiation, isolation, speciation, and subsequent evolution in the genus *Neureergus*.

Conservation

The newts of the Zagros Mountains, especially the genus *Neureergus*, are very sensitive to environmental change because they live under extreme conditions. It is likely that the rate at which newt species are declining in abundance has been underestimated, and this potential problem needs immediate attention. It is also possible that effects from habitat destruction, pollution and drought affect the Zagros newts more

than any other of the local amphibians, both directly and indirectly.

My studies have revealed that the Zagros newts require relatively complex mosaic landscapes that include terrestrial elements for foraging, protection and hibernation, as well as aquatic habitats with good quality and a rich invertebrate food base. Connective habitats that enable migration between terrestrial and aquatic habitats are also important determinants of population size and abundance.

From the conservation standpoint, numerous problems overshadow the status of all Zagros newts, especially *N. microspilotus*. These conservational issues can be divided into three main categories: 1) Severe droughts of recent years; 2) Man-made habitat destruction; and 3) pollutants (e.g., chemicals such as herbicides, fertilizers, and pesticides).

The impact of the severe droughts of recent years on the population structure and breeding capacity of the Zagros newts is quite clear. Many of the local streams, brooks and ponds in different areas of the Zagros have been dried up, leaving no chance for reproduction of the newt species that are dependent on this kind of water sources. The effect is so evident that in some areas local newt populations went extinct. This, in turn, has led to a decreasing chance of gene exchange, and hence to an increasing vulnerability.

The effects of man-made habitat destruction and chemical pollutants on population structure and range contraction of *N. microspilotus* in central Zagros Mountains in Kermanshah Province are also important. Habitat destruction, such as the elimination of wetlands, ponds, brooks and streams acts to increase the distance between various populations, and hence to decrease gene exchange. Furthermore, *N. microspilotus* is threatened due to habitat loss through the divergence of streams for irrigation of cultivated lands. Additionally, chemical pollutants (fertilizers, insecticides and herbicides)

have strong effects on population decline mainly by increasing mortality in various life stages (Malmgren, 2001).

Though the native people and villagers have been warned about the consequences of unlimited use of various chemicals, it seems that their use is still increasing. This certainly has a strong effect on the reproductive success and on various life stages of the mountain newt (Rastegar-Pouyani, 2003).

These man-made interferences, leading to extreme environmental stress and dramatic population declines, led to the consequence that all Zagros newts (*Neurergus microspilotus*, *N. crocatus*, and *N. kaiseri*) are now regarded as threatened.

Therefore, it is of paramount importance to take necessary steps for the conservation of the declining and threatened newt taxa of the Zagros, especially *N. microspilotus*.

Acknowledgements. I wish to thank the Iranian Department of the Environment in Kermanshah as well as Razi University Authorities for financial support during field work in the Zagros Mountains, western Iran.

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Systematics of the genus *Asaccus* (Sauria: Gekkonidae) on the Zagros Mountains, Iran

Nasrullah Rastegar-Pouyani

Abstract. The gekkonid lizards of the genus *Asaccus*, Dixon and Anderson 1973, are currently recognized with at least nine known species (*Asaccus elisae*, *A. griseonotus*, *A. kermanshahensis*, *A. kurdistanensis*, *A. nasrullahi*, *A. gallagheri*, *A. montanus*, *A. platyrhynchus*, and *A. caudivolvulus*). Of these, the first taxon, *A. elisae*, occurs in western Iran, Iraq, Turkey, and Syria; and the next four species occur in the Zagros Mountains and its western foothills. On the other hand, the last four species of *Asaccus* are mostly restricted in distribution to the mountains of northern Oman in southeastern regions of the Arabian Peninsula. Based on long-lasting field work and research in most parts of the Zagros Mountains, systematics and distribution of the genus *Asaccus*, with especial reference to the Iranian species, are discussed. Some new records and new localities as well as specimens belonging to undescribed taxa of this genus are presented. The role of the Zagros Mountains in radiation, isolation, speciation and subsequent evolution of *Asaccus* is explained. It is shown that *Asaccus* is a vicariant taxon and that the evolution and progression of the Persian Gulf, as a huge vicariant event, has played the most important role in separation and isolation of the northern (Zagros) populations from the southern (Arabian Peninsula) populations of this vicariant taxon. Based on new evidence, the Zagros Mountains is regarded as the center of diversification for *Asaccus*.

Introduction

One of the least known genera of Gekkonidae is the genus *Asaccus* Dixon and Anderson, 1973 (formerly *Phyllodactylus* Gray, 1828) which is distributed in some parts of the Middle East region. In 1973, Dixon and Anderson described a new species and genus of gecko from an unidentified locality around Islamabad, Kermanshah Province, western Iran. These workers suggested the generic name *Asaccus*, based on the absence of cloacal sacs, and named their new taxon as *Asaccus griseonotus*. About one month later (in December 1973), Eiselt described a new species belonging to the genus *Asaccus* and named it as *Phyllodactylus ingae*, based on only one specimen from about 110km southwest of Khorram Abad city, Lorestan Province, southwestern Iran (Eiselt, 1973). Later on, and due to time priority role, it was shown that Eiselt's new species is just a junior synonym of *Asaccus griseonotus* (Anderson, 1999: 136). Prior to suggestion of *Asaccus* as a new generic name, all the gekkonid lizards with the same generic characters were regarded as belonging to the genus *Phyllodactylus* Gray, 1828 with just one described species in Iran (e.g., *Phyllodactylus elisae* Werner 1895, from western and southwestern regions of the Iranian Plateau) (Kluge, 1991, 1993, 2001; Bauer et al., 1997). Since then, several new species of *Asaccus* have been described based on distinctive species-specific characters: *Asaccus montanus* Gardner,

1994 (from the mountainous regions of United Arab Emirates); *A. platyrhynchus* Arnold and Gardner, 1994 (from Tanuf, Oman); *A. caudivolvulus* Arnold and Gardner, 1994 (from Jebel Ras, United Arab Emirates); *A. kermanshahensis* Rastegar-Pouyani 1996 (from 45km north of Kermanshah city, Kermanshah Province, western Iran); *A. kurdistanensis* Rastegar-Pouyani, Nilson & Faizi, 2006 (from Kurdistan Province western Iran) and *A. nasrullahi* Werner, 2006 (from Lorestan Province, southwestern Iran). Among all species of *Asaccus*, it seems that *A. elisae* is the most widespread taxon being distributed in Iran, Iraq, Turkey and Syria (Dixon and Anderson, 1973; Martens and Kock, 1991; Leviton et al, 1992; Arnold and Gardner, 1994; Rastegar-Pouyani, 1996; Varol et al., 1997, 2002). The most distinctive morphological characters of four of the Iranian species of *Asaccus* are represented in table 1.

Taxonomy

The distinctive and vicariant lizards of the genus *Asaccus*, Dixon and Anderson 1973, (formerly known as *Phyllodactylus* Gray, 1828) encompass at least nine known species (*Asaccus kurdistanensis*, *A. elisae*, *A. griseonotus*, *A. kermanshahensis*, *A. gallagheri*, *A. montanus*, *A. platyrhynchus*, *A. caudivolvulus* and a newly described species *Asaccus nasrullahi* based on material deposited in the Copenhagen Museum, Denmark previously misidentified as *Ptyodactylus hasselquistii* (Y. Werner, pers. commun.). Of these, *A. griseonotus* occurs in the western foothills of the Zagros Mountains, western Iran, and northeastern Iraq, and the recently described species, *A. kermanshahensis*,

is restricted in distribution to the type locality in the central Zagros Mountains, Kermanshah Province, western Iran. On the other hand, *Asaccus nasrullahi* Werner is restricted in distribution to Lorestan Province, southwestern Iran (Y. Werner, pers. comm.). Arnold and Gardner (1994) revised *Asaccus* based mainly on material from the southern parts of the range in Oman and United Arab Emirates and described two new species (*Asaccus platyrhynchus* and *A. caudivolvulus*). In their morphology-based phylogenetic analysis, and based on a matrix of 16 characters, Arnold and Gardner (1994) divided *Asaccus* species into four monophyletic groups. In their tree, *Asaccus griseonotus* represents a separate taxonomic and phylogenetic entity and the two species, *A. elisae* and *A. montanus*, comprise a monophyletic group (Arnold and Gardner, 1994: 439).

With regards to the Arnold and Gardner phylogenetic tree, the positions of the two new species, *Asaccus nasrullahi* Werner and *A. kurdistanensis* Rastegar-Pouyani et al. remain to be determined, though Werner (pers. comm.) regards his new species of *Asaccus* (= *A. nasrullahi*) as being, phylogenetically, closest to *A. griseonotus*.

Further field work and collecting more material in different parts of the Zagros Mountains and neighboring regions (which is now being carrying out by us through collecting some more specimens of undescribed taxa of *Asaccus* from the central Zagros Mountains in Kermanshah and Lorestan Provinces that are awaiting taxonomic descriptions) will shed more light on taxonomic, biogeographic, and phylogenetic status of this disjunct and vicariant taxon.

Biogeography

The distribution patterns of the Iranian Plateau lizards (including the genus *Asaccus*), to a great extent, have been affected by the occurrence of some important and drastic vicariant events, especially the uplifting and evolution of the Zagros and Elburz Mountains in the

late Tertiary, about 15-9 MYBP (million years before present). These two mountain systems have played the most important role in shaping up the past and present distribution patterns of various taxa (Macey et al., 1998, 2000; Rastegar-Pouyani, 1999a, b, c, Rastegar-Pouyani & Nilson 2002).

As to the study of historical biogeography of the vicariant and disjunct lizards of the genus *Asaccus*, so far, very few scenarios have been proposed regarding the center of origin and diversification of this mainly rock-dwelling genus (Anderson, 1968; Rastegar-Pouyani, 2003). For instance, Rastegar-Pouyani (2003) suggested that either the Zagros Mountains or the mountains of Oman and United Arab Emirates can be regarded as the center of origin and diversification for *Asaccus*. As mentioned earlier, the genus *Asaccus*, as a vicariant taxon, has now been divided into two distinct branches: 1) a northern branch, encompassing at least 5 known species, which are mainly distributed on the Zagros Mountains and its neighboring regions (see above).

2) a southern branch, consisting of 4 described species which are distributed in the eastern and southeastern regions of Arabian peninsula, mostly in the mountains of northern Oman and United Arab Emirates.

Since most described species of *Asaccus* are now occurring in the Zagros Mountains and neighboring areas, I am now inclined to consider the Zagros Mountains as the center of origin and diversification for this taxon.

The ancestor of this taxon was probably distributed in the mountains and small caves. Then firstly via one or more dispersal waves, this ancestral taxon expanded its range towards the high mountains of the south. Due to the occurrence of geomorphic events and climatic fluctuations which led to increasing progression of the Persian Gulf and the Oman Sea, the previously contiguous ancestral populations became disjunct in distribution: one branch was restricted to the southern mountains, south of the Persian Gulf and Oman Sea,

Characters	<i>A. elisae</i>	<i>A. griseonotus</i>	<i>A. kermanshahensis</i>	<i>A. kurdistanensis</i>
Postmentals	Two pairs	Two pairs	Four pairs	Three pairs
Tubercles on head	Present	Absent	Present	Present
Dorsal tubercles	Strongly keeled	Weakly keeled	Smooth	Weakly pointed
Dark rings on tail	Present	Present	Absent	Present
Diameter of individual back tubercle	>2/3 of ear diameter	<half of ear diameter	>3/4 of ear diameter	>half of ear diameter
Ear diameter	>1/3 of eye diameter	>1/2 of eye diameter	<1/3 of eye diameter	<1/2 of eye diameter
Granules bordering postmentals	18-20	15-18	21-24	16-20
Upper labials	9-11	9-10	9-12	9-10
Lower labials	9-10	7-9	8-10	8-9
Dorsal tubercles in a transverse row	9-13	10-12	8-10	8-11
Maximum SVL (mm)	57.9	70.5	55.7	63.5
Scales across midorbital region (in a single row)	23-28	20-25	22-26	18-20

Table 2. Comparison of distinctive morphological characters in four Iranian species of *Asaccus* (Rastegar-Pouyani 1996; Rastegar-Pouyani et al. 2006; present paper).

and the other branch confined to the northern mountains (the Zagros Mountains and its western foothills).

With regards to the above-mentioned account, it is obvious that the present distribution patterns of *Asaccus*, as a disjunct genus, is the result of both dispersal waves from the center of origin in the northern parts of the range (e.g., the Zagros Mountains) towards the south (e.g., Oman and United Arab Emirates mountains) as well as vicariant events (e.g., plate tectonics, and evolution and increasing progression of the Persian Gulf and Oman Sea), in the Late Tertiary (about 15-9 MYBP).

In short, based on all the available evidence, in the history of *Asaccus* separation of the high mountain ranges of the Iranian Plateau and the mountains of eastern and southeastern Arabian peninsula via evolution and progression of the Persian Gulf and Oman Sea, as huge vicariant events, has had a drastic effect in isolation, speciation, and subsequent evolution of this taxon.

Further field work and collecting more material in various parts of the whole range as well as morphological and molecular analysis of relationships among different taxa of *Asaccus* (which is now at hand) may help to shed more light on determination of history and evolution of this south Palearctic primitive genus of gekkonids.

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Peculiarities of the hemipenes of the gekkonid lizard genera *Aristelliger* Cope, 1861 and *Uroplatus* Duméril, 1806

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Abstract. We report on calcified internal hemipenial structures (hemibacula) in the gekkonid lizards *Aristelliger georgeensis* and *Uroplatus lineatus*. Whereas such structures were already known from other *Aristelliger* species, they are first described here for a member of *Uroplatus*. The complex hemipenial structure gives *U. lineatus* a unique position within its genus, and both genera although not closely related to each other, have a special position within gekkonid lizards in general due to their complex hemipenial ornamentation.

Introduction

Gekkonid hemipenes are generally characterized by a more or less club-shaped trunk and pedicel portion as opposed to a voluminous apex. In many geckos, the apex is subdivided into two lobes. Moreover, a calyculate surface ornamentation on various parts of the three portions of the organs are characteristic for gekkonid hemipenes (Böhme, 1988).

A surprising peculiarity found in gekkonid hemipenes was the presence of internal, ossified structures or hemibacula which were first described for two *Aristelliger* species (*A. cochranae*, and *A. praesignis*: Kluge, 1982). A third species of this genus, *A. georgeensis*, has likewise well differentiated hemibacula which we describe and discuss in the present paper. Surprisingly, we found in the course of our joint work on the genital morphology of geckos remarkably ossified internal supportive structures also in one species of the genus *Uroplatus* (*U. lineatus*), which are also described and discussed below.

Results

1. *Aristelliger georgeensis*

(Duméril & Bocourt, 1873)

The hemipenis of *A. georgeensis* is relatively stout (Fig. 1). Pedicel and truncus (terminology according to Böhme, 1988) are short and not visibly subdivided on the asulcal side; on the sulcal side, however, a basal, thin but distinct oblique fold marks the borderline between

the two portions of the organ. The voluminous apex consists of two equal-sized lobes. The sperm groove or sulcus spermaticus is basally narrow and deep, bordered by well developed sulcal lips. Above the truncus, the sperm groove broadens, but before its bifurcation, it narrows again on the central apex. From there, the two sulcal branches run towards the two lobes where they pass the terminal, projecting parts of the hemibacula. On the asulcal side of the apex, the sulcal branches are (only proximally) bordered by strong tissue wells.

The ornamentation of the lobes of the *A. georgeensis* hemipenis is extraordinarily complex. At their outer margins they have five soft-tissue papillae which are sulcally arranged in two pairs, the fifth being a single, horn-like asulcal papilla.

Calyces can be found only on the apex where they extend asulcally to its central part (Fig. 2).

The two bones in each lobe of *A. georgeensis* have a similar form as the hemibacula of *A. praesignis*. The first ossified element is 2.2 mm long and has a lanceolate shape. Its terminal part, i.e. the part projecting out of the tissue of the everted hemipenis, is symmetrically toothed. The second bone is fork-shaped, the two branches being unequal in length. The shorter branch is 1 mm, the longer 2.2 mm long. Also the forked bone has teeth of unequal size. Only the longer branch could be found to project out of the tissue in a fully turgid hemipenis. Both bony elements are connected with the portion of the retractor muscle running to the lobe tip; the forked-shape bone has this connection in its arched lower part (Fig. 3).

The retractor muscle itself is apically deeply forked, in the middle portion roundish in cross section, but depressed in its basal portion. It runs ventrally in longitudinal direction, parallel to the body axis, but shifts terminally to the dorsal side. The musculus retractor penis extends over the entire tail root.

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Figure 1. Hemipenis of *Aristelliger georgeensis*, asulcal view.



Figure 2. Apical region of the hemipenis of *A. georgeensis* viewed from above.



Figure 3. Hemibacula with retractor muscle in *A. georgeensis*.

2. *Uroplatus lineatus* Duméril & Bibron, 1836

The hemipenis of *U. lineatus* is well divided in three parts: pedicel, truncus and apex (Figs. 4 and 5). The pedicel is sulcally differentiated from the truncus by a skinfold, asulcally by a constriction. The truncus is flax-combed like a wash-board. The cup-shaped apex has two short, column-shaped lobes each of them emerging sulcally from a shallow concavity. The sperm groove is very narrow and bordered by swollen sulcal lips. The sulcus bifurcates on the apex into two flat areas from where on the one hand two narrow grooves run to the lobe centers, but on the other hand, there is a free passage over smooth tissue to the apical side of the organ. The apex is covered with calyces on both sides, the margins of which are denticulated. The calyculation is very distinct on both lobes and reaches terminally the hemibacula.

The lobes of *U. lineatus* contain surprisingly hard, but obviously not yet fully ossified hemibacula. Each lobe has a fork-shaped hemibaculum of the same kind. The basal part of the investigated hemibaculum is arched and very massive. The two diverging long branches are of unequal size (4 and 3 mm respectively) and apically bent inwards. Of these two branches, only the longer, hook-shaped one projects out of the tissue of the lobe

(Fig. 6). The shorter branch of the hemibaculum does not perforate the apical part of the lobe. Basally, the hemibacula are tightly connected with the respective branch of the retractor muscle (Fig. 7). For the retractor muscle itself the same as said above for *A. georgeensis* is true.

Discussion

The most striking feature of the *A. georgeensis* hemipenis is the complex apical structure. The apical regions of the hemipenes of *A. cochranæ* and *A. praesignis* also seem to be complexly structured (see Kluge 1982 and the drawings therein). Ossified structures in the tail root region of *Aristelliger* (*A. lar* and *A. praesignis*) had already been mentioned by Russell (1977) who classified them, however, as cloacal bones, a structure common in many geckos but not homologous with hemibacula.

Gekkonid hemibacula as internal supportive structures of the hemipenes of *A. cochranæ* and *A. praesignis* and their possible function have first been described and discussed by Kluge (1982). Their presence in *A. georgeensis* and *A. lar* make it likely that they occur in all species of the genus. Specifically distinct, however, is their number (paired or single) per lobe.

The descriptions of the hemipenes of *U. ebenai*, *U. fimbriatus*, *U. henkeli*, and *U. sikorae* (Böhme and Ibsch, 1990, Böhme and Henkel, 1995) as well as of *U. alluaudi*, *U. malama*, *U. malahelo* (Nussbaum and Raxworthy, 1994, 1995) and *U. guentheri* (Rösler, 2005) give no hints on the presence of hemibacula. However, in some of these species some hardened apical structures do occur (Böhme, unpubl. data) although they are not at all comparable with the calcified hemibacula found in *U. lineatus*; moreover, they do not perforate cutis and epidermis of the apical lobes.

Such a situation can also not be expected in *U. pietschmanni*, because this species, the hemipenis morphology of which is still unknown, is unlikely to be a close relative of *U. lineatus* (Böhle and Schönecker, 2003). The apical lobes of the other *Uroplatus* species (see the references cited above) do have complex apical structures, however, in contrast to *U. lineatus*, these have been interpreted as epidermal structures without participation of an internal calcified supportive system deriving from the retractor muscle. The hemipenis morphology of *U. lineatus* supports the view of Glaw and Vences (1994) that this species has an isolated position within its genus.



Figure 4. Hemipenis of *Uroplatus lineatus*, asulcal view.



Figure 5. Hemipenis of *U. lineatus*, sulcal view.

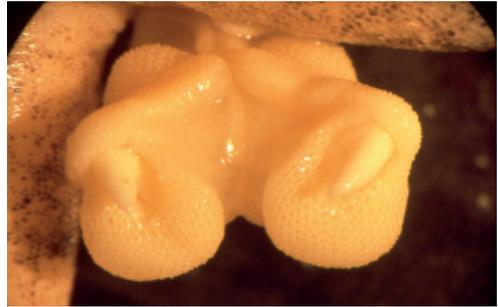


Figure 6. The hook-shaped hemibacula protruding from the hemipenis of *U. lineatus*.



Figure 7. Hemibacula with retractor muscle in *U. lineatus*.

Hemipenial structures in many geckos are relatively simple. A club-shaped basal part (consisting of pedicel and truncus) is followed by a voluminous apex with paired lobes which are mostly spherical. The center of each lobe may bear a single, soft epidermal processus. By their complex apical structures, the hemipenes of *Aristelliger* and *Uroplatus* differ drastically from this general gekkonid bauplan, although their apical gross morphology is not homomorphic. This is also true for the internal supportive, calcified structures present in representatives of both genera, being neatly denticulated in *Aristelliger* as opposed to massive and smooth in *Uroplatus*. In any case, due to these genital morphological differences, both genera hold a special position among the Gekkonidae.

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The reptiles of the Socotra archipelago with special remarks on the slender blind snakes (Leptotyphlopidae: *Leptotyphlops*)

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Abstract. We provide a list of the reptiles of the Socotra archipelago with comments on the respective number of taxa and the percentage of endemism. Special attention is laid on the Socotran species of *Leptotyphlops*, the morphological variability of which is addressed in some detail.

Introduction

The Socotra archipelago is part of the Republic of Yemen and situated in the north-western part of the Indian Ocean. It covers a land area of about 3800 km² and comprises the main island Socotra (3600 km²), Abd al Kuri (162 km²), Samha (45 km²), Darsa (10 km²) and some guano-covered rocks (Jazirat Sabuniyah, Ka'1 Fir'awn).

Though the data are based on fifteen visits to the archipelago between 1982 and 2005, two expeditions on which the authors went together (3.11.1997-12.11.1997 and 13.02.1999-5.03.1999) proved particularly informative.

The herpetofauna of the Socotra archipelago

So far no traces of amphibians have been found on the archipelago. There have been records or sightings in the sea of four species of marine turtles (Cheloniidae), and three of them (*Chelonia mydas*, *Eretmochelys imbricata* *bissa*, *Caretta caretta*) are supposed to use sandy beaches on the islands for egg laying (Schätti and Desvoignes 1999, Rösler and Wranik 2003).

Twelve genera of terrestrial reptiles occur on the islands of the archipelago. At the generic level endemism is 42%. Some 30 terrestrial species and forms have been reported and 86,7 % of them are thought to be endemic.

The taxonomy of several species (such as *Haemodracon riebeckii*, *Hemidactylus homoeolepis*, *Mesalina balfouri*, *Hemerophis socotrae*) requires further research. Most

diverse are the reptiles on the main island Socotra (26 species and forms). Endemism is 65,4%. Six species occur on Samha and four species on Darsa. Five of the recorded species on Samha from the genera *Haemodracon*, *Hemidactylus*, *Trachylepis*, *Mesalina* and *Hemerophis* are also known from Socotra. Endemic to Samha and Darsa is only a species of the genus *Pristurus*. However, the knowledge on the reptiles of these two smaller islands is still incomplete, and in case of Darsa it is unknown, if the genus *Hemidactylus* is present or not. Six species have been reported from Abd al Kuri. Endemism is 50 %. Recorded from Abd al-Kuri and Socotra are only *Pristurus abdelkuri* and *Hemidactylus homoeolepis*. The latter is also known from Samha. No traces of snakes have yet been found on Abd al Kuri.

The only species recorded on all four islands is *Trachylepis socotrana* (Table 1).

Lizards (Sauria)

The lizards of the archipelago belong to the families Chamaeleonidae, Gekkonidae, Scincidae and Lacertidae.

Chamaeleonidae

There is only one endemic species, *C. monachus* (Subfamily Chamaeleoninae) known from the main island Socotra.

Gekkonidae

The geckos comprise about 16 species and two forms of uncertain status in three genera (*Pristurus*, *Hemidactylus*, *Haemodracon*). All belong to the subfamily Gekkoninae. Endemic are the genus *Haemodracon* and all species of the genus *Pristurus* recorded on the islands of the archipelago. The genus *Pristurus* is distributed in East-West direction from Iran to Ethiopia and in North-South direction from Jordan to Kenya. The genus *Hemidactylus* has a worldwide distribution and comprises 9 species on the archipelago, 5 of them considered endemic. Further

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Taxon	Sokotra	Samha	Darsa	Abd al Kuri
Chamaeleonidae				
<i>Chamaeleo monachus</i>	yes	no	no	no
Gekkonidae				
<i>Haemodracon riebeckii</i>	yes	yes*	no	no
<i>Haemodracon trachyrhinus</i>	yes	no	no	no
<i>Hemidactylus dracaenacolus</i>	yes	no	no	no
<i>Hemidactylus flaviviridis</i>	yes	no	no	no
<i>Hemidactylus forbesii</i>	no	no	no	yes
<i>Hemidactylus granti</i>	yes	no	no	no
<i>Hemidactylus homoeolepis</i>	yes	yes	no	yes
<i>Hemidactylus oxyrhinus</i>	no	no	no	yes
<i>Hemidactylus pumilio</i>	yes	no	no	no
<i>Hemidactylus</i> form A	yes*	no	no	no
<i>Hemidactylus</i> form B	yes*	no	no	no
<i>Pristurus abdelkuri</i>	yes	no	no	yes
<i>Pristurus insignis</i>	yes	no	no	no
<i>Pristurus insignoides</i>	yes	no	no	no
<i>Pristurus guichardi</i>	yes	no	no	no
<i>Pristurus obsti</i>	yes	no	no	no
<i>Pristurus samhaensis</i>	no	yes	yes	no
<i>Pristurus sokotranus</i>	yes	no	no	no
Scincidae				
<i>Hakaria simonyi</i>	yes	no	no	no
<i>Trachylepis socotrana</i>	yes	yes	yes	yes
Lacertidae				
<i>Mesalina balfouri</i>	yes	yes	yes	no
<i>Mesalina kuri</i>	no	no	no	yes
Typhlopidae				
<i>Typhlops socotranus</i>	yes	no	no	no
Leptotyphlopidae				
<i>Leptotyphlops filiformis</i>	yes	no	no	no
<i>Leptotyphlops macrurus</i>	yes	no	no	no
<i>Leptotyphlops wilsoni</i>	yes	no	no	no
Colubridae				
<i>Hemerophis socotrae</i>	yes	yes	yes	no
<i>Dityophis vivax</i>	yes	no	no	no
Trogonophidae				
<i>Pachycalamus brevis</i>	yes	no	no	No

* Taxa could not clearly be attributed to any known species.

Table 1. The terrestrial reptiles of the Socotra archipelago (**fat** marks - endemic species).

research is required in order to establish the status of two forms (*Hemidactylus* Form A und B).

Scincidae

Only two species of skinks inhabit the archipelago; both are endemic. One belongs to the genus *Hakaria* (Subfamily Scincinae) and the other to the genus *Trachylepis* (Subfamily Lygosominae). The genus *Hakaria* is monotypic and endemic to Socotra, while species of the genus *Trachylepis* are widespread in Africa and Madagascar.

Lacertidae

The Lacertidae are also represented by two endemics, closely related species of *Mesalina*, a genus with a Saharo-Sindian distribution.

Snakes (Serpentes)

The snakes of the archipelago belong to the families Colubridae, Typhlopidae and Leptotyphlopidae.

Colubridae

The colubrid snakes are represented by two endemic species. They belong to the genera *Hemerophis* (Subfamily Colubrinae) and *Ditytophis* (Subfamily Pseudoxyrhophiinae), which are both endemic to the Socotra archipelago.

Typhlopidae

Typhlops socotranus, the only known species from the main island Socotra, is endemic. The genus *Typhlops* occurs worldwide, except Australia.

Leptotyphlopidae

Three endemic Socotran species are reported of the genus *Leptotyphlops*. However, in order to establish their status, further research will be necessary (see below). Species of the genus *Leptotyphlops* are known from America, Africa and in Asia up to India.

Worm Lizards (Amphisbaenia)

The worm lizards of the archipelago belong to the Family Trogonophidae.

Trogonophidae

The monotypic genus *Pachycalamus* is endemic to Socotra.

The Socotran species of the genus *Leptotyphlops* Fitzinger, 1843

Three endemic species of the genus *Leptotyphlops* are reported from Socotra. These are *L. filiformis* Boulenger, 1899, *L. macrurus* Boulenger, 1899 and *L. wilsoni* Hahn, 1978.

On the basis of some new samples [collections W.

Wranik (CWWR), Museum für Tierkunde (MTD D), Naturhistorisches Museum Wien (NMW), Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK)] a preliminary, short view on some morphological peculiarities is given. A detailed description of the new material, including a discussion of taxonomical questions, is under preparation.

Leptotyphlops cf. *filiformis* Boulenger, 1899

Material: n = 1

ZFMK 82508 — Wadi Zeeriq, 12°31'08" N, 53°59'09" E, Dixam plateau, Socotra.

Glauconia filiformis (= *Leptotyphlops filiformis*) was described on the basis of four specimens by Boulenger (1899). Two specimens of the type series are destroyed (see Schätti and Desvoignes 1999).

Characters of specimen ZFMK 82508: total length 182 mm; tail length 15 mm; diameter at midbody 1,5 mm; diameter at tail 1,1 mm; index total length/tail length 12,13; total length/midbody diameter 121,33; total length/tail diameter 13,64. Rostral hooked; 529 dorsals and 45 subcaudals; caudal spine small, not curved.

Remarks: ZFMK 82508 matches the lectotype of *L. filiformis* BMNH 1946.1.11.1 in the following characters: form of rostral, tip of tail, form and arrangement of head scales, number of scales at midbody and at the middle of tail. ZFMK 82508 differs from *L. filiformis* in a substantial bigger total length, a smaller diameter at midbody and more dorsals (own findings).

Leptotyphlops macrurus (Boulenger, 1899)

Material: n = 4

ZFMK 82533 — Qaareh, Noged plain, 12°20'10" N, 53°37'56" E, Socotra; ZFMK 82551-3 - Khayrha mountains, 12°38'50" N, 53°27'45" E, Socotra.

Glauconia longicauda was described on the basis of four specimens by Boulenger (1899). *G. longicauda* is a younger homonym of *Stenostoma longicaudum* (Peters, 1854) (= *Leptotyphlops longicaudus*). Boulenger (1903) replaced the name *Glauconia longicauda*, in an almost identical description, by *Glauconia macrura* (= *Leptotyphlops macrurus*).

L. macrurus differs from *L. filiformis* in the proportional bigger diameter at midbody and the strong caudal spine. According to Hahn (1978) *L. wilsoni* differs from *L. macrurus* in the following features: a hooked snout, divided occipitals (?).

ZFMK 82533 and ZFMK 82551-3 show the following width of variation in size, body proportions and scales: total length 105-153 mm; tail length 18-25 mm; head length (= tip of snout to end of mouth) 1,5-2,1 mm; head width 1,7-2,6 mm; rostral length 0,9-1,3 mm; rostral width 0,6-0,8 mm; diameter at midbody 2,0-3,5 mm; diameter at tail 1,6-2,7 mm. Total length/tail length 5,83-6,59; head length/head width 0,77-0,88; rostral

length/rostral width 1,25-1,63; total length/diameter at midbody 41,43-55,00; tail length/tail diameter 8,15-12,50. Dorsalia 256-274, subcaudalia 46-54, scales at midbody 14, scales at middle of tail 10, number of dark dorsals 5-7.

Remarks: Snout not hooked in ZFMK 82533 and ZFMK 82553. Occipitals usually $\frac{3}{4}$ of the size of parietals, but in ZFMK 82552 on the right occipital same size as parietal, on the left occipital divided. Usually eyespot, but in ZFMK 82551 pupil and iris.

Leptotyphlops wilsoni Hahn, 1978

Leptotyphlops wilsoni was described on the basis of two specimens by Hahn (1978) (see also Corkill and Chochrane 1966). *L. wilsoni* differs from *L. filiformis* and *L. macrurus* by a short, rounded, not hooked snout and a proportional smaller rostral; from *L. filiformis* it differs additionally in a lower number of dorsals. According to Hahn (1978) the occipitals in *L. wilsoni* are undivided, but divided in *L. filiformis* and *L. macrurus*, which was not confirmed by Schätti and Desvoignes (1999).

Remarks: The nostril in the paratype (BMNH 1957.1.10.27) of *L. wilsoni* is near to the anterior supralabial, the frontal is bigger than the supraoculars, frontal and postfrontal are equally in size, the interparietal is about 1 $\frac{1}{2}$ times bigger than the frontal. The rostral is proportionally clearly smaller (0,4x0,25 mm) than in *L. filiformis* and *L. macrurus*. Caudal spine acute, not tapering, twice as long as width. Six rows of dorsalia olive green (own findings). For further characters of paratype see Hahn (1978).

Leptotyphlops sp.

Material: n= 6

CWWR (two specimens, no number) - Hasaant, 12°29'N 54°08'E, 170 m., Socotra; CWWR (no number) - Farmihin, 12°32'N 53°59'E, Socotra; CWWR (no number) - Wadi Ayhaft, 12°36'N 53°57'E, Socotra; MTD D 41253 - Hasaant, 12°29'N 54°08'E, 170 m., Socotra; NMW 13305 - Hakari, Socotra.

These six specimens, characterized by smaller diameter

at midbody and some peculiarities in body proportions (total length/diameter at midbody, number of dorsals) were provisionally designated *Leptotyphlops* cf. *wilsoni* (Rösler and Wranik 2004). In some of the compared specimens of *Leptotyphlops* single characters are beyond the variation in *L. wilsoni* given by Hahn (1978). The differences are individual and involve the following characters: body proportionally smaller, tail proportionally slender, tail proportionally shorter, tip of snout not rounded and short, pupil and iris present, lower number of dorsals, higher or lower number of subcaudals, lower number of dark coloured dorsals. From our investigations we can state a great variation in the compared specimens (see Rösler and Wranik 2004).

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Demographic variability in two populations of the European plethodontid salamander *Speleomantes strinatii*

Sebastiano Salvidio

Abstract. The demographic variability of two populations of the European plethodontid salamander *Speleomantes strinatii* living in contrasting habitats was studied in NW Italy. The first population was sampled from 1993 to 2004 on humid rock faces and the second from 1996 to 2004 inside an artificial underground cavity. The temporal variability in mean SVL, number of individuals removed during the first sampling (i.e. a population count index), abundance and capture probabilities were expressed through the coefficient of variation ($CV = SD/\text{mean}$) for each body size and sex subgroup. The two populations showed similar overall population structure but they differed in the level of variability in counts, abundance and capture probabilities. These results may have implications in amphibian ecological and monitoring studies.

Introduction

Within amphibians, terrestrial plethodontid salamanders are considered as relatively stable in time (Hairston, 1987). Indeed, these species are characterised by low variability in several demographic parameters, such as intrinsic rate of growth (r) and population turnover (Green, 2000). However, little is known about demographic variability among conspecific plethodontid populations living in different environments.

In this paper two conspecific populations of the European plethodontid *Speleomantes strinatii* living in contrasted ecological conditions were studied during several consecutive years and compared to assess their level of variability.

Materials and methods

Study populations and sites

Two *Speleomantes strinatii* populations were studied: one living inside an artificial underground cavity and the other on humid rock-faces. The cave population was sampled inside an artificial cavity located near Busalla (Genova, Liguria) at 390 m a.s.l. The environmental parameters of this habitat, excavated during World War II as an air-raid shelter, are described by Salvidio et al. (1994). The rock-face population was sampled on humid rock-faces and under the talus along a first order stream at about 380 m a.s.l. near Davagna, Genova (Salvidio, 1998). The two sites are about 10 km apart, but differ in several environmental parameters such as illumination, relative humidity and temperature: the cave being much less variable than the rock-face environment.

Population structure

Both populations were sampled by means of removal sampling (Salvidio, 1998, 2001) during their peak activity season (i.e., July in the cave and October in the rock-face habitat). Salamanders were measured with a transparent plastic ruler, from the snout to the posterior end of the vent (SVL) and mature males were recognised by the presence of a swollen chin gland. Salamanders larger than 58 mm in SVL and lacking the chin gland were considered reproductive females, according to a previous study (Salvidio, 1993). The SVL polymodal frequency-distribution histograms were analysed with the FAO-ICLARM Stock Assessment Tools (FiSAT) computer programme (Gayanilo et al., 1996). Population abundance (N_e) and capture probabilities (p) were estimated for each subgroup using CAPTURE software (White et al., 1982). However, for some subgroups of the rock-face population, the number of individuals that were removed in successive occasions did not decline and the abundance estimation failed (see Table 1).

Data analysis

Four parameters for each body-size group were analysed: mean SVL, number of individuals captured during the first capture occasion (C , a count index), N_e and p . For each parameter the coefficient of variation ($CV = \text{standard deviation}/\text{mean}$) was calculated. CV varies between 0 and 1, is scale insensitive (Allison, 1978), and allows the comparison of quantities with different measurement units; a smaller CV indicates a relative lower variability. Since *S. strinatii* individuals are long-lived and may be recaptured in successive years, the data sets were not independent. Thus, a repeated measure analysis of variance (RM ANOVA) with the restricted model (Kutner et al., 2005) treating habitats and parameters as fixed factors and subgroups as random factor was used. The CV values were arcsine square root transformed before analyses. Normal distributions of fits and error terms were analysed by means of Kolmogorov-Smirnov (K-S) goodness-of-fit test with $\alpha = 0.05$.

Results

Population structure

In both populations the body-size distributions were successfully subdivided into three components ($\chi^2 >$

	Parameter	Sampling years	Mean	SD	CV
Cave habitat					
First year juveniles	SVL	9	23.700	2.193	0.093
Second year juveniles	SVL	9	35.300	2.120	0.060
Sub adults	SVL	9	47.822	1.989	0.042
Males	SVL	9	58.956	1.173	0.020
Females	SVL	9	63.967	0.620	0.010
First year juveniles	count	9	18.000	9.487	0.527
Second year juveniles	count	9	10.556	5.790	0.549
Sub adults	count	9	14.667	6.042	0.412
Males	count	9	18.333	4.093	0.223
Females	count	9	12.778	4.685	0.367
First year juveniles	Ne	9	29.556	10.772	0.364
Second year juveniles	Ne	9	16.889	8.565	0.507
Sub adults	Ne	9	22.000	6.225	0.283
Males	Ne	9	24.778	5.118	0.207
Females	Ne	9	17.333	5.385	0.311
First year juveniles	p	9	0.600	0.175	0.292
Second year juveniles	p	9	0.663	0.145	0.218
Sub adults	p	9	0.646	0.151	0.234
Males	p	9	0.733	0.063	0.087
Females	p	9	0.738	0.066	0.090
Rock-face habitat					
First year juveniles	SVL	12	30.050	2.174	0.072
Second year juveniles	SVL	12	41.850	2.568	0.061
Sub adults	SVL	12	52.108	1.408	0.027
Males	SVL	12	58.000	2.015	0.035
Females	SVL	12	63.208	1.369	0.022
First year juveniles	count	12	12.167	4.933	0.405
Second year juveniles	count	12	6.667	3.200	0.480
Sub adults	count	12	2.917	1.564	0.536
Males	count	12	5.833	2.125	0.364
Females	count	12	5.667	2.146	0.379
First year juveniles	Ne	12	32.250	13.586	0.421
Second year juveniles	Ne	12	21.500	15.211	0.707
Sub adults	Ne	7	9.143	4.879	0.534
Males	Ne	9	22.889	10.240	0.447
Females	Ne	11	14.727	6.798	0.462
First year juveniles	p	12	0.428	0.146	0.341
Second year juveniles	p	12	0.383	0.120	0.314
Sub adults	p	7	0.545	0.182	0.334
Males	p	9	0.365	0.120	0.329
Females	p	11	0.508	0.153	0.301

Table 1. Demographic variability in two *Speleomantes strinatii* populations. Count = salamanders captured during the first removal occasion, p = capture probability, Ne = abundance estimated, SD = standard deviation, SVL = snout-vent length (mm), CV = coefficient of variation.

Table 2. RM ANOVA (restricted model) of the variability in counts, estimated abundance and capture probabilities in two *Speleomantes strinatii* populations.

Source	Degree of Freedom	SS	MS	F	P
Subgroup	4	497.27	124.32	17.15	0.001
Habitat	1	396.43	396.43	9.36	0.038
Parameter	2	774.17	387.08	17.87	0.001
Subgroup * Habitat	4	169.39	42.35	5.84	0.017
Subgroup * Parameter	8	173.33	21.67	2.99	0.071
Habitat * Parameter	2	140.47	70.23	9.69	0.007
Error	8	57.98	7.25		
Total	29	2209.04			

0.05, in all cases) in agreement with previous studies (Salvidio, 1998, 2001). The two smallest components comprised immature salamanders in their first and second year, while the third was a mixture of larger animals of different sizes and ages. Within this subgroup, in addition to reproductive males and females, large immatures lacking the chin gland and smaller than 58 mm in SVL were considered subadults as in Salvidio and Pastorino (2002). Mean values of the demographic parameters are given in table 1. Mean SVL values of the two immature and the subadult subgroups were smaller in the cave population in comparison to the rock-face one, as the former population was sampled earlier in the season (July vs. October). On the other hand, SVL of both mature males and females did not show significant differences (RM ANOVA, $F = 1.12$, $DF = 1$, $P = 0.298$; K-S test for error terms $D = 0.067$, $n = 36$, $P > 0.10$).

Analysis of population variability

The different parameters studied showed different levels of variability (Table 1). The lowest and more constant CV values were for mean SVL ($0.093 < CV < 0.100$) in both populations, while the highest ones were those observed for census indexes (C) and estimated abundances, in particular in the rock-face population ($0.420 < CV < 0.707$).

The RM ANOVA on the CV values of the three further parameters (i.e., excluding SVL) showed that the overall variability was significantly different between populations and also among age groups within populations (Table 2). Since RM ANOVA fits and error terms were normally distributed (K-S test $P > 0.15$), the model seemed appropriate for the data set analysed.

Discussion

In this study the mean number of years used to calculate the CV values was 10.05 (range 7-12), a time period roughly corresponding to life expectancy for terrestrial plethodontid species similar in body size to *Speleomantes strinatii* (Hairston, 1987; Castanet et al., 1996; Bruce et al., 2002). Thus, the period used to calculate mean CV values was appropriate. In the two populations, the overall body-size structure and the mean size of both males and females were similar and showed low interannual variations. These data suggest that habitat differences had little influence on growth rates and by consequence, age structure. On the other hand, the overall population variability was different between the populations: it was lower in the cave than in the rock-face habitat, in particular concerning estimated abundance (N_e) and capture probabilities (p). These parameters, which may be considerably influenced by local ecological conditions, displayed significant inter- and intrapopulation variation. These results are therefore consistent with a study showing that *Speleomantes* populations living in different environments may show a different level of variation in capture probabilities (Salvidio, 2001). In addition, this study shows that different populations and even different age groups within populations may react differently to local ecological conditions.

The results of this study are of interest when planning monitoring programmes, since conspecific populations and different age groups within the same population may show different levels and patterns of variation, in particular in counts and estimated density. Thus the importance of standardisation in long term monitoring studies and in the validation of count indexes by

calculating the capture probabilities for each population and even each different age or sex group is stressed (see Schmidt, 2003, 2004).

Finally, these results show that short time periods may be adequate in assessing some demographic parameters such as sex ratio and overall population structure, but could be misleading in calculating parameters that show intrinsically high levels of interannual variability (i.e. count indexes and abundances).

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Reproductive activity of *Lacerta agilis* and *Zootoca vivipara* (Reptilia: Sauria: Lacertidae) in western Siberia

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Abstract. From 2001 to 2005 the main reproductive characteristics of 180 specimens of *Lacerta agilis* and 282 specimens of *Zootoca vivipara* were studied using common zoological, histological, cytological and immunohistochemical methods. The maximum duration of the activity period of both species is about 4.5 months per year. Emergency from hibernation depends on the course of spring, biotope, age and sex of the lizards. Males of both species emerge from hibernation 10-15 days earlier than females. The emergency from hibernation of the first breeding males and females of *L. agilis* and *Z. vivipara* occur simultaneous with the older individuals of the same sex. However, spermatogenesis, vitellogenesis, laying of eggs in *L. agilis* or birth of young in *Z. vivipara* occur in 1-3 weeks later than at older specimens. In both species, the reproductively active part of the populations mainly included specimens after their third time of overwintering, whereas only a small portion reproduced after a second time of hibernation. Spermatogenesis in both species could be observed from beginning of May until mid of June. Testicles of both species reach maximal sizes at the moment of the appearance of the spermatozoa (beginning of May) and have minimum size in mid of summer. In both species, all mature females of a population took part in reproduction. Fecundity of females is correlated with snout-vent length. The phenomenon of long-term spermatozoid deposition in female oviducts is confirmed.

Introduction

Lacerta agilis and *Zootoca vivipara* are among the most widespread species of lizards in the northern parts of Eurasia. The southeastern part of West Siberia is at the northern limits of the range of *L. agilis* and at the centre of the *Z. vivipara* range. The reproductive biology of both species has been studied comprehensively in European regions of their areals (e.g., Jablovkov, 1976; Bischoff, 1984; Saint-Girons, 1984; Amat et al., 2000; Roig et al., 2000). In contrast, there are only few data about features of sexual cycles, linear sizes of sexually mature species, their fecundity and age of sexual maturity in Asian parts of the species' range (e.g., Popoudina, 1976; Kuranova, 1983; Orlova et al., 2005).

Materials and methods

During the years 2001-05 research was conducted in the southern part of the taiga zone (53°30' N, 84°50' E) where both species occur. 180 specimens of *L. agilis* (84 females, 96 males) and 282 specimens of *Z.*

vivipara (137 females, 145 males) were studied. The age of lizards was determined on behalf of longitudinal (thin) sections of tubular bones in polarized light of a microscope (see Kornilova et al., 1996). Sexual maturity was determined by the size of lizard (snout-vent length = SVL), its colour, and the state of gonads. Age and seasonal dynamics of testicles is described by index L_{tes}/SVL and D_{tes}/SVL (L_{tes} = length of testicle, D_{tes} = diameter of testicle). Using a standard histological technique 205 preparations of reproductive system of *L. agilis* and *Z. vivipara* were made. The 3-chromatic method of preparations of Mallori was used for dyeing.

The fecundity was defined based on number of eggs present in oviducts, an egg clutch or size of spawn. Spermatozooids in urogenital organs of females were revealed by means of marked antibodies (Ackerman et al., 1988; Kremer and Jagers, 1992). Mathematical processing was carried out by means of the software package STATISTICA 6.0 and Microsoft Excel 2000. Degree of correlation of parameters is defined by Spirman's coefficient (r_s).

Results and discussion

Sexual maturity of Lacerta agilis

Males: In the study area most of the males became sexually mature after the third hibernation period and at the minimum SVL of 66.8 mm (established by histological methods). Some males already took part in reproduction after the second emergency of hibernation ($SVL_{min} = 57.5$ mm, 8.3%). Growth of testicles of sexually mature specimens is slowed down but not

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stopped with older age. The increase of gonad length until the end of an individual life is equal to 12% and 4% concerning the diameter ($n = 66$, sad-1: $L.tes./L. = 0.09 \pm 0.01$, $D.tes./L. = 0.04 \pm 0$; ad: $L.tes./L. = 0.10 \pm 0 - 0.12 \pm 0$, $D.tes./L. = 0.05 \pm 0$). The increase of SVL is accompanied by proportional increase of testicle length ($r_s = 0.35$, $p < 0.01$).

Females: All sexually mature females took part in reproduction after the third emergency of hibernation ($n = 11$), and most of them (56.5%) have vitellogenesis only in the spring after the third emergency of hibernation. The minimum SVL of fertilized females of this age ($n = 27$) is 67.2 mm.

Sexual maturity of Zootoca vivipara

Males: Sexual maturity was noted after the third emergency of hibernation ($n = 98$, $SVL_{min} = 48.9$ mm). From birth till sexual maturity (after the third emergency of hibernation) there is an intensive increase of testicles length. Already after the second emergency of hibernation the diameter of testicles increase proportionally to the SVL ($n = 62$, sad-1: $L.tes./L. = 0.09 \pm 0$, $D.tes./L. = 0.04 \pm 0$; ad: $L.tes./L. = 0.10 \pm 0 - 0.11 \pm 0$, $D.tes./L. = 0.05 \pm 0$).

Females: Sexual maturity of most females ($n = 28$, 87.5%) was noticed after the third emergency of hibernation, and in 12.5% of individuals after the second one.

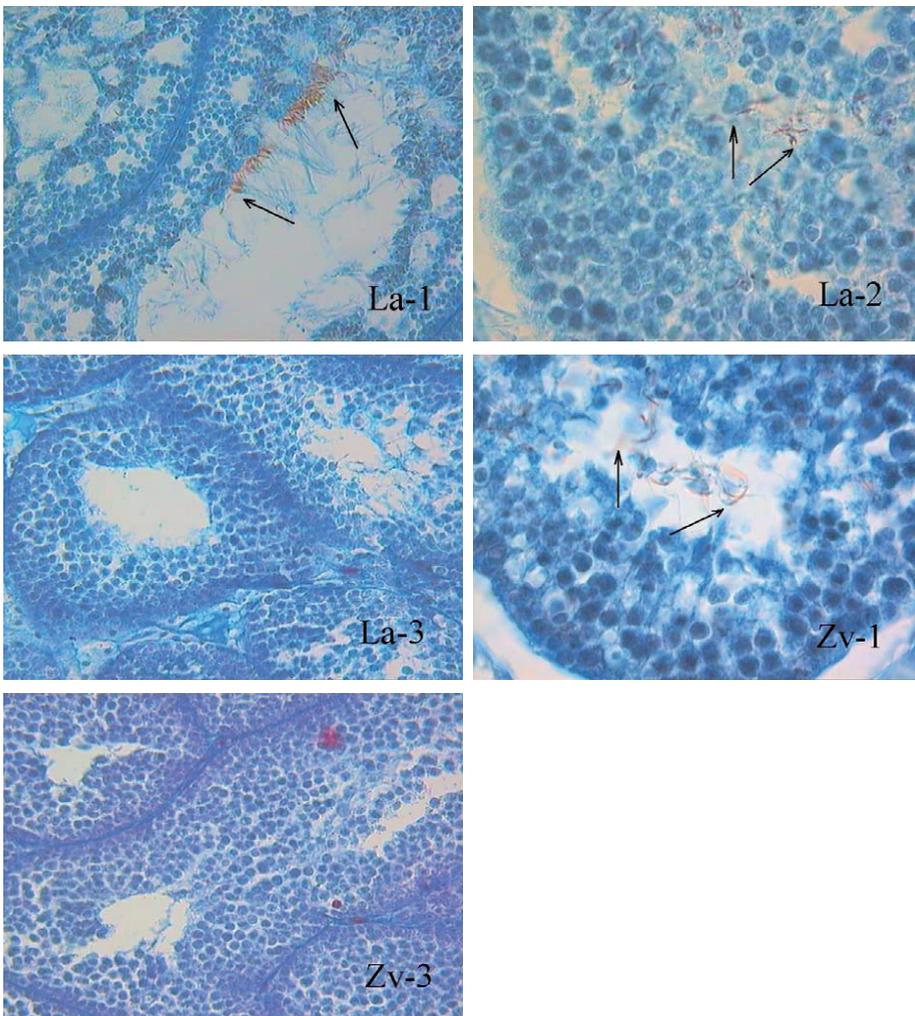


Figure 1. Testicles of *Lacerta agilis* (La) and *Zootoca vivipara* (Zv) at the beginning (the end of May: 1 mature male, 2 - male participating in reproduction for the first time) and at the end (3 - first half of August) of the activity period (magnification 40x/0.65 (La-1, La-3, Zv-3), 100x/1.25 (La-2, Zv-1)). Arrows indicate spermatozooids.

Thus, in the study area *L. agilis* got sexually mature slightly later compared to regions in the center of its areal, and simultaneously when compared to populations from the western and northwestern periphery of its range (see Jablokov, 1976; Glandt, 1988; Amat et al., 2000). In *Z. vivipara* maturity during the first 2-3 years was documented in most populations from its range (Roig et al., 2000; Orlova et al., 2005).

Phenology of reproduction

The emergency from hibernation and the dates of the main stages of reproductive cycles depend on the spring

weather conditions and the biotope. The duration of the activity period of both species is about 4.5 months per year. Sexually mature males of both species are the first ones who emerge from hibernation from end of April to beginning of May. The emergency from hibernation of females of both species is 10-15 days delayed compared to males. Mass copulations takes place in the middle of May. The emergency from hibernation of the first breeding males and females of *L. agilis* and *Z. vivipara* occur simultaneous with the older individuals of the same sex. However, spermatogenesis, vitellogenesis and egg laying in *L. agilis* or birth of young in *Z. vivipara* occur within 1-3 weeks.

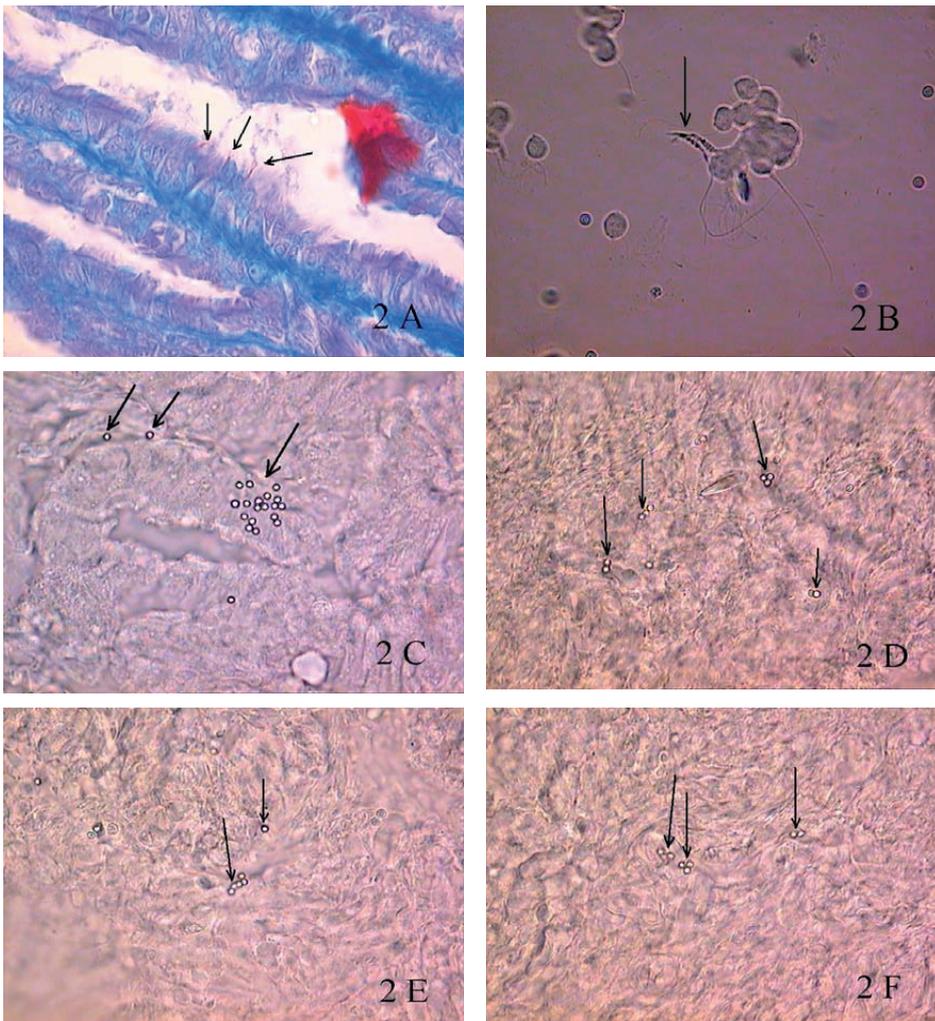


Figure 2. Depositing of spermatozoids in the genital tracts of female *Lacerta agilis* and *Zootoca vivipara*: 2A – few spermatozoids in the lower parts of the oviduct of female *L. agilis* few hours after copulation (magnification 40x/0.65); 2B - cytologic preparation of testicles of *L. agilis* with spermatozoids marked by antibodies (magnification 100x/1.25) (magnification 100x/1.25). Location of deposition of spermatozoids in female’s oviducts marked with antibodies: 2C – *L. agilis*, before copulation, middle of May; 2D - *L. agilis*, after egg laying , middle of June; 2E - *Z. vivipara*, after copulation, end of May; 2F - *Z. vivipara*, middle of August (polarized light, magnification 40x/0.65).

Sexual cycles

Males: Both species have spermatogenesis of the mixed type which has a similar character all over the species' geographical range. Summer spermatogenesis and spring spermiogenesis was accompanied by copulations and ovulations at the end of spring (Saint-Girons, 1984; Amat et al., 2000). Spermiogenesis is very short and was observed only during spring time. The spermiogenesis of both species was documented from the beginning of May till the middle of June (Fig. 1). Its frequency was unstable in spring, increased from the beginning till the end of May (reaching a maximum) and gradually decreased from the beginning to the middle of June. The maximum number of spermatozooids in the testicles was detected in the second half of May. During other times of the active period spermatozooids were not found in prepared testicles (Fig. 1). The spermiogenesis of specimens taking part in reproduction for the first time is shorter and less intensive than that of older individuals (Fig. 1).

The length of testicles in both species is at a maximum when mature spermatozooids will appear (in the beginning of May) and is minimum in the middle of the summer. Reduction of testicle length was observed within 10 days after emergency from hibernation (*L. agilis*: $L.tes_{max}/SVL = 0.12 \pm 0.001$; *Z. vivipara*: $L.tes_{max}/SVL = 0.12 \pm 0.008$), in 10 days - diameter (*L. agilis*: $D.tes_{max}/SVL = 0.06 \pm 0.001$; *Z. vivipara*: $D.tes_{max}/SVL = 0.07 \pm 0.015$). Reduction was observed till the middle of June (*L. agilis*: $L.tes_{min}/SVL = 0.08 \pm 0.002$, $D.tes_{min}/SVL = 0.03 \pm 0.001$; *Z. vivipara*: $L.tes_{min}/SVL = 0.08 \pm 0.001$, $D.tes_{min}/SVL = 0.04 \pm 0.001$). The increase of testicle size was observed from the end of June till the beginning of August (*L. agilis*: $L.tes/SVL = 0.01 \pm 0.002$; $D.tes/SVL = 0.04 \pm 0.005$; *Z. vivipara*: $L.tes/SVL = 0.11 \pm 0.003$; $D.tes/SVL = 0.05 \pm 0.002$).

Females: In the middle of May oocytes in different stages of development were present in the ovaries of females (in *L. agilis*, oocyte diameter 0.8-7.5 mm; in *Z. vivipara*, oocyte diameter 0.6-4.7 mm). From the middle of May till the beginning of June mass copulations of lizards were observed. Efficiency of copulation is very high as all investigated mature females in both species took part in the reproduction.

The fecundity of females of both species depends on length of a body. Total number of *L. agilis* females ($n = 27$) contained an average number of eggs of 6.9 ± 0.5 (3-13) ($r_s = 0.64$, $p < 0.001$). Of them, young females ($n=11$) with SVL of 71.4 ± 0.8 (67.2-74.6) mm contained 4.9 ± 0.3 (3-7) eggs; elder individuals ($n=16$) with SVL

of 88.3 ± 1.8 (75.0-104.9) mm contained 8.1 ± 0.6 (3-13) eggs. Females of *Z. vivipara* ($n=77$) with SVL of 61.0 ± 0.6 (46.2-81.0) mm contained 6.5 ± 0.2 eggs (1-14) ($r_s = 0.62$, $p < 0.001$).

Deposition of spermatozooids

Investigation of the reproductive system of one female of *L. agilis* revealed the presence of follicles with a diameter of 4.1-8.8 mm in the ovaries as well as the presence of spermatozooids in the lower parts of its oviducts. Few hours after copulation an insignificant number of spermatozooids freely settled in gleams of the lower parts of the oviducts; most of the spermatozooids took root by means of their heads in folds of a mucous membrane and of the secretory epithelium (Fig. 2A).

Deposited spermatozooids were changed with the head being shortened and the terminal filament being absent. In the middle and upper parts of oviducts no spermatozooids were present. The modified spermatozooids were preserved in the epithelium and were not visible upon histological preparations, but were detected using marked antibodies. Immunohistochemical reactions showed that the used antibodies were connected with the head and the cervix of spermatozooids (Fig. 2B).

During investigation of females of both species during all periods of their activity the phenomenon of deposition of spermatozooids was always observed in the epithelium and in the basal membrane of the lower and the upper parts of the oviducts, whereas they were lacking in middle parts of oviducts. Spermatozoid deposition of female *L. agilis* and *Z. vivipara* were evident directly after copulation, laying of eggs or birth of young, before emergency from hibernation and in spring prior to copulations (fig. 2C, D, E, F), namely throughout the year. These observations confirm the phenomenon of earlier penetrations and spermatozoid deposition in females of *L. agilis* as described by Genin (1955).

The phenomenon of long-term storage of sperm in female oviducts is known in various groups of reptiles. Preservation of capable spermatozooids provides sperm competition while plural copulation occur, or fertilization of repeated laying and is an obligatory component of reproduction of the species having asynchronous reproductive cycles (e.g., Olsson et al., 1994; Blackburn, 1998; Gist and Congdon, 1998).

In conclusion, in the study area sexually mature individuals of *L. agilis* start being active simultaneously with peripheral populations from European regions of its range; *Z. vivipara* reaches sexually maturity at the same time as any known populations within its geographical

range. To a great extent, beginning of sexual maturity and basic reproductive characteristics are determined by climate conditions of previous years. The duration of the activity period, namely the time of reproduction of both species and its intensity depends on the biotope and climate conditions of the respective year.

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Dispersal of *Triturus alpestris* and *T. vulgaris* in agricultural landscapes – comparing estimates from allozyme markers and capture-mark-recapture analysis

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Abstract. Demes of the two newt species *Triturus alpestris* and *T. vulgaris* occupying five water bodies in an agricultural landscape in western Germany were investigated with respect to their dispersal behaviour and population structure. We applied two different approaches to investigate dispersal and population structure: (1) mark-recapture methods for breeding adults and (2) F-statistics estimated from the outcome of allozyme electrophoresis. Both approaches yield similar results for the two species indicating low differentiation between the demes, suggesting either high level of gene flow caused by migration or a short time passed after deme separation. Mark-recapture-methods revealed a considerable number of migrants, indicating that low genetic differentiation probably is due to recent gene flow. The problems of data interpretation in cases of demes founded only a few years ago are discussed.

Introduction

Dispersal is a key factor for the survival of amphibian populations in agricultural landscapes (e.g. Cushman 2006). It strongly affects two crucial aspects of population ecology. First, dispersing animals usually contribute to gene flow between demes, preventing them from potential negative effects of inbreeding in isolated populations (overview in Whitlock, 2001). Second, migrating individuals may significantly affect the demography of populations and prevent the extinction of small demes (rescue effect, e.g. Stacey et al., 1997; Hanski, 2001; Trenham et al., 2001). Thus, estimates of the dispersal potential of organisms are of special interest for conservation biology because dispersal strongly affects survival probability of populations on different spatial scales.

Methodically, estimates of animal dispersal in a spatially limited study area can be obtained with two different approaches: So called direct methods use capture-mark-recapture-techniques (CMR) in the field to measure the portion of animals that disperse at a certain distance from the marking location (Gill, 1978, Perret et al., 2003). The biggest problem of this technique is, that recaptures are limited to a predefined study area, which may lead to biased results. Despite this approach is time and

cost expensive, it usually cannot distinguish between mortality and the proportion of animals that leave the study area (Fletcher et al., 2002, Koenig et al, 1997). Thus, this procedure systematically underestimates the proportion of dispersing animals, although recent developments of CMR-Statistics partly overcome such problems (Perret et al., 2003). On the other hand, not all dispersing animals contribute to gene flow, due to failure in successful breeding in the colonized demes (Jehle et al., 2001), which would lead to an overestimation of gene flow when using CMR data. One advantage of direct approaches is the possibility to identify the relevant stages in a life cycle, that contribute to dispersal (MacDonald and Smith, 1990). Nowadays, so called indirect methods are becoming more important for dispersal estimates. These approaches use genetic markers to estimate genetic differentiation between subpopulations (F_{st} and related measures) and are usually not as time and cost expensive as the direct methods. On the one hand the estimates usually are not biased by spatial limitations of study areas since they are not affected by non detected migrants or deme specific mortality (Fletcher et al., 2002). On the other hand these methods often cannot distinguish between the effects of recent and historical gene flow due to the low drift effects over short separation time between demes. Moreover, they usually cannot identify the dispersal relevant stage in the life cycle of an animal.

During a long-term monitoring of amphibian populations in an agricultural landscape in western Germany we compared the direct and indirect estimates of newt dispersal to get insights into the population structure and dispersal of two common pond breeding newt

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Table 1. Number of migrants, number of marked and recaptured newts differentiated by species and sex, sum of all ponds and years of survey.

species	sex	marked individuals	recaptures	migrants	Dispersal rate
<i>T. vulgaris</i>	male	5775	909	48	0.053
	female	7022	1229	56	0.046
<i>T. alpestris</i>	male	4868	3115	124	0.040
	female	4546	3457	169	0.049

species, *Triturus vulgaris* and *T. alpestris*. The study area comprised five ponds, two natural waters and three of artificial origin. The three artificial ponds have been created 12 years before genetic samples were taken. Newts represent a suitable model for comparing these approaches, because their local demes are spatially well defined in pond breeding communities.

Study area and methods

The study was carried out at five ponds situated in a typical agricultural landscape near Bonn in the western part of Germany (Fig. 1) situated at a edge of a forest. Ponds 3 and 5 are natural waterbodies, whereas ponds 1, 2 and 4 were artificially created in 1988. According to skeletochronological data, the average age of adult newts from the study area varies between 3–4 years (Rottscheidt 2002, Thomas 2002). The natural ponds represent ancestral breeding habitats, whereas in artificial ponds not more than four generations could pass after the first colonization events. Newts were captured in pitfalls at permanent drift fences encircling the breeding ponds from 2001 until 2003. Estimated census population sizes within study time in the ponds ranged from 126 to 1,874 individuals in alpine newt and 132 to 4,342 in smooth newt (Weddeling *et al.* 2004). Ponds were surrounded mainly by acres,

pastures and woodland (Fig. 1). The distances between the ponds ranged from 270 m to 1,800 m.

Adult newts were marked using toe-clipping with year specific group marking. We calculated the proportion of dispersing adult newts based on the total number of recaptures made as requested in Bowne and Bowers (2004) to prevent bias due to different capture effort and mortality. Dispersal of juveniles could not be tracked since it is not possible to mark them permanently due to tissue regeneration (Glandt, 1980).

In 2001, the tissue samples from toes and tail were taken in order to analyze allele frequencies in polymorphic allozyme loci as genetic marker. We sampled 250 adults and 182 juveniles of *T. vulgaris* as well as 300 adults and 184 juveniles of *T. alpestris*.

Tissues were kept in test tubes on ice during field work and afterwards stored at -80°C . We used vertical starch gel electrophoresis (STAGE) with Tris-Citrate TC 2 or Aminomorpholine-Citrat buffer (Hillis *et al.*, 1996). The staining protocols followed Hillis *et al.* (1996) with PMS and MLB as electron transmitter and MTT as dye. In both species, 18 allozyme loci were tested for polymorphism. 4 out of 18 tested allozyme loci (MDH, MPI, 6-PGDH, PGM) showed polymorphisms between the individuals in *T. vulgaris* whereas six loci in *T. alpestris* exhibited considerable variability (LDH, MDH, ME, MPI, 6-PGD, PGM). For both approaches, F_{st} was calculated and compared with the software ARLEQUIN3 (Excoffier *et al.*, 2005) and GENEPOP (Raymond and Rousset, 1995). We tested for isolation by distance using Mantel test software ISOLDE in GENEPOP.

Results

Direct approach

The analysis of deme specific marking showed an averaged dispersal rate of adult newts of 4.6 % for

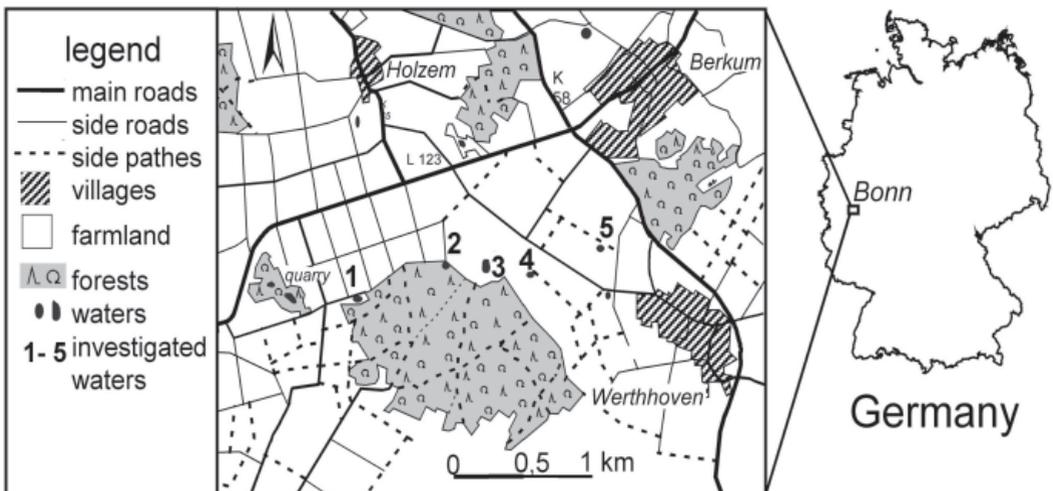
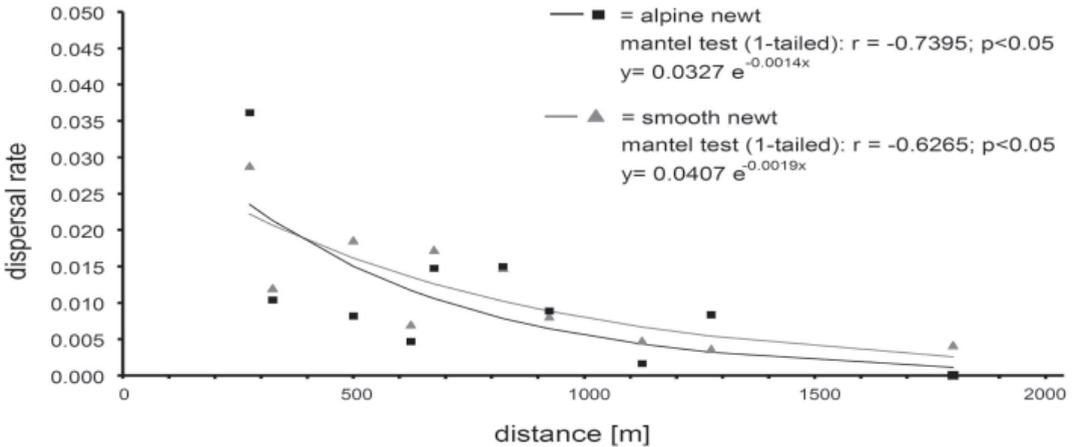


Figure 1. Map of the study area south of Bonn. The numbers mark the five investigated breeding ponds 1 to 5. Pond 3 and pond 5 are natural waterbodies, whereas the three remaining ones are artificially created in 1988.

Figure 2. Isolation by distance effect of dispersal rate in alpine newt and smooth newt within the study area (exponential function fitted), obtained from CMR data. Dispersal rate calculated as follows: no° of recaptures at different pond / no° of all recaptures with pond specific marking; sum of recaptures for both directions of migration (pond a towards b + pond b towards a)



all recaptures (Table 1), with no apparent differences between the species. Estimated pairwise adult migration rates strongly differ between ponds (Table 2). The local population size and geographical distance influences the number of migrants between demes. Mantel tests showed significant isolation by distance effects for adult newt dispersal (Fig. 2) with declining dispersal rates for increasing distances between the demes.

Wright's F_{st} was calculated from CMR data for the whole pond system (Berven and Grudzien, 1990) to correlate field data with results from the indirect approach. F_{st} values were calculated using Wright's equation $F_{st} \approx 1/(4Nm+1)$ and the harmonic mean of deme sizes (taken from Weddelling et al. 2004). Overall F_{st} estimates are low (*T. vulgaris*: $F_{st}=0.013$, *T. alpestris*: $F_{st}=0.016$), indicating low genetic differentiation between demes.

Indirect approach

Overall F_{st} values calculated from allozymes for the whole pond system were low (alpine newt $F_{st}=0.02$, smooth newt $F_{st}=0.014$). Pairwise estimates among the demes varied between 0.0006 and 0.0463 in *T. alpestris* and 0.0003 and 0.0613 in *T. vulgaris*. Most pairwise comparisons did not yield in F_{st} estimates significantly different from zero (Table 3), correspondingly the Mantel tests for isolation by distance were not performed. Remarkably, for both species the comparisons between the ponds 1 and 5 on the one hand and between ponds 3 and 5 on the other hand showed F_{st} values significantly different from zero, which represent the most distant

positioned respectively the natural ponds. Additionally, *T. alpestris* showed pairwise F_{st} values significantly different from zero between pond 2 and 4 on the one hand and between pond 4 and 5 on the other hand.

Discussion

The simultaneous application of the two approaches allows some conservative conclusions about recent and historical processes in the observed populations. The genetic analysis of the two newt species showed low differentiation between the five demes (overall F_{st} values for alpine newt $F_{st}=0.02$ and for smooth newt $F_{st}=0.014$). This result is consistent with our expectation given the small geographical distances between the demes and the recent origin of the artificial ponds. They have most likely been colonized by individuals from two natural ponds (pond 3 and 5) (Schaefer 1993). Via allele frequency based F_{st} values we are not able to discriminate between the influence of the recent colonization and actual gene flow between demes. As the separation time between the ponds is not longer than three to four generations we would not expect the subpopulations to show strong genetical differentiation even if no gene flow would have existed in the last 12 years. Thus, low differentiation between demes could be either a result of the short-time colonization history of the ponds or of recent gene flow. Whitlock and McCauley (1999) point out, that precondition for the usage of F_{st} estimates as a measure of dispersal is an equilibrium state of dispersal and drift.

Table 2. Migration rate of adult alpine newts and smooth newts (sum of migrating males and females from 2000-2003 calculated as proportion of all recaptures at the outer sides of the drift fences).

Species	destination pond	source pond				
		1	2	3	4	5
alpine newt	1	3395*	0.0199	0.0253	0.0243	0.0944
	2	0.0157	1990*	0.0572	0.0219	0.0708
	3	0.0046	0.0190	692*	0.0195	0.0708
	4	0.0014	0.0042	0.0080	378*	0.0708
	5	0.0011	0.0009	0.0053	0.0024	117*
smooth newt	1	344*	0.0069	0.0016	0.0043	0.0000
	2	0.0267	531*	0.0281	0.0028	0.0368
	3	0.0214	0.0450	589*	0.0128	0.2944
	4	0.0160	0.0069	0.0078	649*	0.1472
	5	0.0000	0.0000	0.0031	0.0028	25*

* sum of all recaptures at the outer sides of the drift fences of the source pond

The capture-mark-recapture data of adult newts detected migrants between all of the demes. A significant isolation by distance effect for these migrants could be shown, indicating that migration of adult newts in agricultural areas is possible, but even on small landscape scales (up to 2000 m between ponds) dispersal is limited due to landscape characteristics. But, since we were not able to measure natal dispersal of first breeding newts, we assume that this number might underestimate overall dispersal ability of the species (Gill 1978, Berven and Grudzien 1990).

The considerable number of migrants observed in the field supports the idea of gene flow, although since

nothing is known about breeding success of these individuals, the final proof remains open.

To our knowledge, there are only very few studies that use direct and indirect estimates of dispersal simultaneously for a single study area (e.g. Sumner *et al.*, 2001) and no such measures for amphibians. In situations where deme separation time lasts long enough, combination of genetic analysis and capture-mark-recapture investigations might be an interesting approach for further understanding of processes in amphibian population structures and dynamics since dispersal ability of these animals often might be underestimated (Smith and Green, 2005). Care should

	species	pond 1	pond 2	pond 3	pond 4
pond 2	<i>T.a.</i>	0.0037			
	<i>T.v.</i>	0.0042			
pond 3	<i>T.a.</i>	0.0006	- 0.0075		
	<i>T.v.</i>	0.0003	0.0028		
pond 4	<i>T.a.</i>	- 0.0022	0.0090*	0.0044	
	<i>T.v.</i>	0.0061	0.0047	0.0015	
pond 5	<i>T.a.</i>	0.0210*	0.0463	0.0427*	0.0284*
	<i>T.v.</i>	0.0613*	0.0201	0.0360*	0.0374

* sign. different from 0

Table 2. Pairwise F_{st} values for alpine newt (*T.a.*) and smooth newt (*T.v.*) between the five investigated ponds based on allele frequencies of investigated polymorphic allozyme loci.

be taken in cases of demes founded only a short time ago, since in case of low genetic differentiation it might be difficult, to draw conclusions on gene flow, even in cases where migrants can be directly tracked (Whitlock and McCauley 1999).

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Advertisement calls of hemiphractine marsupial frogs: I. *Gastrotheca marsupiata* group

Ulrich Sinsch, Norbert Juraske

Abstract. The advertisement calls of six taxa pertaining to the Andean *G. marsupiata* group were bioacoustically analysed for taxon-specific differences. Multivariate analyses supported the specific status of five taxa (*G. griswoldi*, *G. marsupiata*, *G. peruana*, *G. piperata*, *G. pseustes*) with each taxon showing a unique combination of advertisement call features. In contrast, the remaining taxon obtained by a commercial supplier was indistinguishable from *G. pseustes*.

Introduction

Neotropical marsupial frogs (Hemiphractinae) have been considered a hyloid clade until recently Haas (2003) and Faivovich et al. (2005) demonstrated that they are probably closer related to the Leptodactylidae. Molecular data do not support unambiguously monophyly of Hemiphractinae, whereas the sister group relationship of *Gastrotheca* and *Flectonotus* which has been suggested based on morphological evidence was confirmed (Mendelson et al., 2000; Faivovich et al., 2005). The genus *Gastrotheca* has been tentatively subdivided into four species groups which are believed to include closely related species (Duellman and Fritts, 1972; Duellman et al., 1988; but see Mendelson et al., 2000 for a different view). Since grouping was based on overall similarity, monophyly has not been proven yet. Seventeen out of the currently recognised 49 species are considered members of the *Gastrotheca marsupiata* group which has a predominantly Andean distribution (Frost, 2004). However, this number probably underestimates real diversity, as demonstrated by several new species descriptions (e.g. Duellman and Köhler, 2005). Species distinction is often difficult, if only features of external and internal morphology are used. In contrast, advertisement call features have proved to be a reliable taxonomic tool in anuran systematics (e.g. De la Riva et al., 1995; Sinsch and Schneider, 1996). They may even provide insights in phylogenetic relationships (e.g. Di Tada et al., 2001; Schneider and Sinsch, 1999).

In Hemiphractinae, descriptions of advertisement calls are usually limited to the presentation of an

oscillogram, but quantitative bioacoustic studies considering temperature effects on call structure are scarce. In the *G. marsupiata* group, the vocal repertoire of *G. marsupiata*, *G. griswoldi*, *G. peruana* (Sinsch and Joermann, 1989; Juraske, 1992), and to a minor extent that of *G. piperata* has already been analysed (De la Riva et al., 1995; Duellman and Köhler, 2005). Aims of this comparative study were to identify and quantify taxon-specific bioacoustic features of the advertisement calls of six taxa of the *G. marsupiata* group and to look for bioacoustic support of their specific status.

Materials and methods

The advertisement calls of six Andean *Gastrotheca* taxa were recorded in the field or in captivity: (1) *G. marsupiata*, Huancayo, Dep. Junin, Peru; $n=200$ calls; (2) *G. griswoldi*, Tarma, Dep. Junin, Peru; $n=219$; (3) *G. peruana*, Ondores, Dep. Cerro de Pasco, Peru; $n=31$; (4) *G. spec.* (labelled as *G. peruana* by a commercial supplier), exact locality unknown, Peru; $n=116$; (5) *G. pseustes*, Papallacta, Prov. Napo, Ecuador; $n=245$; (6) *G. piperata*, Sehuencas, Dep. Cochabamba, Bolivia; $n=38$). Calls were analysed using a MEDAV Spectro 2000 signal analysing system. The temporal and spectral structure of advertisement calls was described by seven call variables and corresponding ambient (air) temperature: (1) call duration [ms], (2) intercall interval [ms], (3) call period = call duration + intercall interval [ms], (4) call ratio = call duration / intercall interval, (5) pulses per call [n], (6) pulse rate [Hz], (7) dominant frequency [kHz]. Terminology for call characteristics follows Schneider and Sinsch (1992). Statistical analyses included correlation and regression analysis, factorial analysis (principal component analysis with VARIMAX rotation) and discriminant analysis. Slopes and intercepts were tested for significant differences using ANOVA with the conditional sum of squares.

Results

In each species, advertisement calls recorded were given in series and consisted of a single pulse group

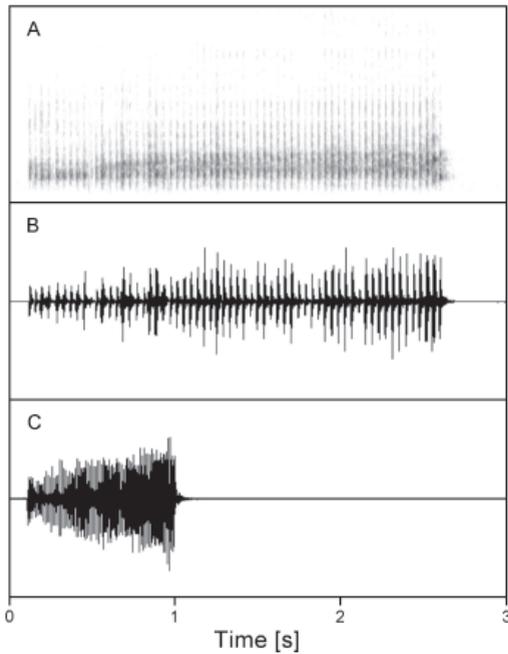


Figure 1. Advertisement calls of a male *Gastrotheca griswoldi*. (A) spectrogram (0 - 10 kHz) of a call recorded at 7.4 °C air temperature; (B) corresponding oscillogram; (C) oscillogram of a call recorded at 26.5 °C air temperature. The calls of the other taxa included in this study were similar in structure and shape.

with numerous, regularly repeated short pulses (Fig. 1). The temperature-adjusted features of the advertisement calls are summarised in Table 1. Factorial analysis showed that the seven measured variables represented merely three independent factors: (1) factor 1 (eigenvalue 3.18, percentage of explained variation: 45.7%) was temperature-

independent and loaded significantly by the variables call duration, call ratio and pulses per call; (2) factor 2 (eigenvalue 2.00, percentage of explained variation: 28.5%) represented the influence of air temperature on call structure and was significantly associated with the variables call duration, intercall interval, call period (linear combination of the first two variables), and pulse rate; (3) factor 3 (eigenvalue 1.13, percentage of explained variation: 16.1%) was also temperature-independent and loaded significantly by the dominant frequency. Thus, call duration had a temperature-dependent and temperature-independent component, as demonstrated by the significant loading of factors 1 and 2. Taxon-specific regression analyses of factor 2 versus corresponding air temperature revealed that the regression lines obtained for *G. marsupiata* and *G. griswoldi* differed significantly with respect to intercepts ($P < 0.0001$), but not to slopes ($P = 0.47$; Fig. 2A). *G. piperata* was excluded from this analysis because calls had been recorded at only one temperature. The regression lines of *G. pseustes* and *G. spec.* were statistically indistinguishable. However, they differed significantly from that of *G. peruana* with respect to intercepts ($P < 0.0001$), but not to slopes ($P = 0.08$; Fig. 2B). The temperature-independent factors 1 and 3 permitted a clear distinction of *G. marsupiata*, *G. griswoldi* and *G. piperata* without *a priori* delimitation of groups (Fig. 3A). Discriminant analysis (*a priori* group delimitation) which was based on the temperature-independent call variables (call ratio, pulses per call, dominant frequency) yielded rates of 97.7% (*G. griswoldi*), 100.0% (*G. marsupiata*), and 100.0% (*G. piperata*) correct classification. In

Call feature	<i>G. marsupiata</i>	<i>G. griswoldi</i>	<i>G. piperata</i>	<i>G. pseustes</i>	<i>G. peruana</i>	<i>G. spec.</i>
Call duration [ms]	450 ± 8	1332 ± 48	1435 ± 63	575 ± 18	628 ± 46	515 ± 9
Intercall interval [ms]	820 ± 41	429 ± 17	1616 ± 99	493 ± 45	368 ± 47	464 ± 41
Call period [ms]	1269 ± 43	1761 ± 49	3063 ± 104	1091 ± 52	1115 ± 103	957 ± 48
Call ratio	0.55 ± 0.02	3.10 ± 0.09	0.93 ± 0.08	1.04 ± 0.03	2.15 ± 0.12	1.04 ± 0.05
Pulses per call [n]	30 ± 0.4	72 ± 1.5	77 ± 3.6	45 ± 0.6	62 ± 1.9	46 ± 0.5
Pulse rate [Hz]	67 ± 0.7	59 ± 0.7	54 ± 0.5	83 ± 0.6	82 ± 1.6	92 ± 0.3
Dominant frequency [kHz]	1.94 ± 0.03	1.98 ± 0.03	2.10 ± 0.03	1.55 ± 0.01	1.87 ± 0.02	1.62 ± 0.01

Table 1. Temperature-adjusted features of the advertisement calls of six taxa pertaining to the *G. marsupiata* group. Means and corresponding 95.0% confidence interval refer to an air temperature of 21 °C.

contrast, the scatter plot of factor 1 scores vs. factor 3 scores did only distinguish *G. peruana* from a homogeneous group formed by *G. pseustes* and *G. spec.* (Fig. 3B). Consequently, the classification success of discriminant functions derived from the temperature-independent variables was low in *G. pseustes* (62.0%) and *G. spec.* (58.6%), but high in *G. peruana* (93.5%).

Discussion

The advertisement calls of all taxa studied had the same general temporal structure, but five taxa were clearly differentiated at species level, as the unique combinations of distinctive call features demonstrate. The partially sympatric Peruvian taxa *G. griswoldi*, *G. marsupiata* and *G. peruana* are easily distinguished by their differences in several call characters, most markedly in pulse rate, a feature known to be used also in other Neotropical anurans for species distinction (Table 1; Sinsch, 1990; Martino and Sinsch, 2002). The largest differences are found between the widely syntopic *G. marsupiata* and *G. griswoldi*, particularly at low temperatures, which is most relevant for species-specific male-female recognition in the natural habitat at high elevations. At sites inhabited by more than one of these species, females can make use of these features at any ambient temperature to identify conspecific males. Bioacoustic distinction allows for reproductive isolation and thus, the species status of these three taxa is supported by their advertisement call features.

G. piperata (Bolivia) was formerly referred to as *G. marsupiata* due to its morphological similarity (De la Riva, 1990; De la Riva et al., 1995, 2000; Duellman and Köhler, 2005). Bioacoustic differentiation of these allopatric taxa is straightforward in most of the variables considered, so that there remains no doubt about their distinctness. Thus, the specific status of *G. piperata* is supported (Duellman and Köhler, 2005). Notably, its advertisement call structure resembles that of the allopatric *G. griswoldi*, but differs from this species by having very long intercall intervals.

The allopatric *G. pseustes* (Ecuador) and *G. peruana* (Peru) are bioacoustically more similar than any of the previously discussed taxa. Still, call ratio and the number of pulses per call allow for a reliable distinction of these two taxa and support their independent specific status. This is definitely not

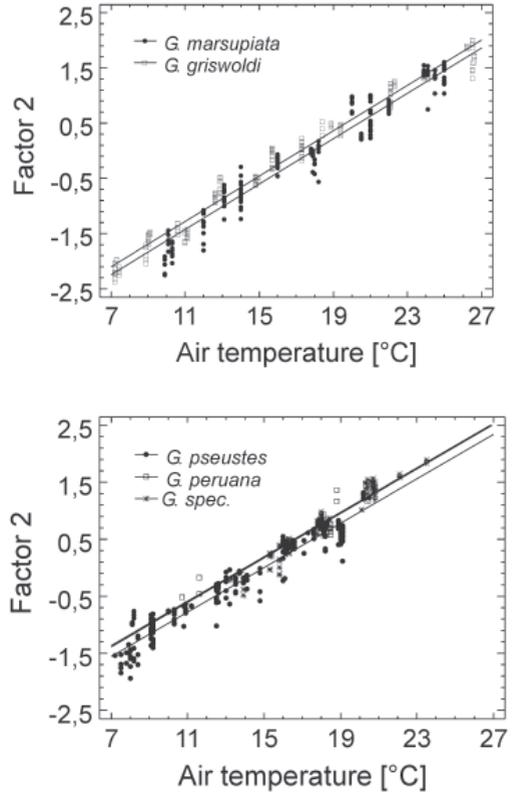


Figure 2. Linear regression of taxon-specific factor 2 scores and corresponding air temperature for *G. marsupiata*/*G. griswoldi* (A) and *G. pseustes*/*G. peruana*/*G. spec.* (B). Statistical details are given in the text. Each symbol represents the factor score of a single call.

the case of the sixth taxon *G. spec.* which had been labelled as *G. peruana* by a commercial supplier and claimed to be of Peruvian origin. The bistriped dorsal colour pattern and size are, in fact, very similar among *G. pseustes*, *G. peruana*, and *G. spec.* so that any taxonomic assignment based on these characters remains doubtful. In contrast, advertisement call features reject the assignment of *G. spec.* to *G. peruana*, and rather suggest conspecificity with *G. pseustes*. Thus, if the geographical origin of these marsupial frogs is given correctly, the geographical range of *G. pseustes* would be extended beyond the Huancabamba depression to Peru. More probable seems a false declaration of origin to avoid the limitations of the strict laws concerning the exportation of protected species in Ecuador.

In conclusion, advertisement call structure supports the specific status of the five named taxa of the

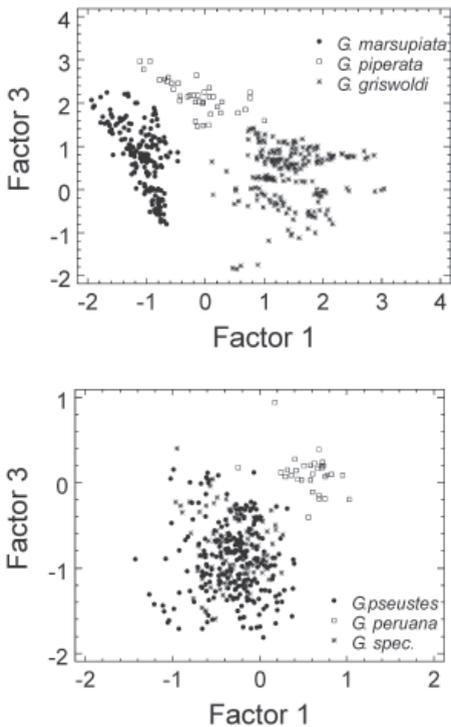


Figure 3. Plot of the temperature-independent factor 1 and 3 scores for *G. marsupiata*/*G. piperata*/*G. griswoldi* (A) and *G. pseustes*/*G. peruana*/*G. spec.* (B). Statistical details are given in the text. Each symbol represents the factor score of a single call.

G. marsupiata group. Sympatric species pairs show stronger variations of the same basic call structure than allopatric ones, but still suggest that these species form a monophyletic group.

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Advertisement calls of hemiphractine marsupial frogs: II. *Gastrotheca plumbea* group

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Abstract. The advertisement calls of five species (*G. espeletia*, *G. monticola*, *G. plumbea*, *G. orophylax*, *G. riobambae*) which pertain to the *G. plumbea* group (Andes of Ecuador) were bioacoustically analysed for taxon-specific differences. Multivariate analyses supported the specific status of all taxa with each taxon showing a unique combination of advertisement call features. The dichotomic phylogenetic arrangement of the *G. plumbea* group in two subclades based on allozymic data is strongly supported by parallel differences in the advertisement call structure.

Introduction

Recent molecular data suggest that Neotropical marsupial frogs (Hemiphractinae) are closer related to the Leptodactylidae than to the Hylidae and even monophyly of these peculiar group is not supported unambiguously (Faivovich et al., 2005). The hemiphractine genus *Gastrotheca* includes currently 49 species which are placed in four groups, the monotypic *G. nicefori*, *G. marsupiata*, *G. plumbea* and *G. ovifera* groups (Frost, 2004). Twelve species form part of the *G. plumbea* group which probably constitutes a monophyletic clade (Duellman, 1974; Duellman and Hillis, 1987; Frost, 2004; but see Mendelson et al., 2000 for a different view). These marsupial frogs inhabit the inter-Andean valleys and Andean slopes of Colombia, Ecuador and northern Peru. Besides the detailed study on the vocalization behaviour of *G. riobambae* (Juraske, 1992), our knowledge on advertisement calls of members of this species group is limited to mostly phonetic transcriptions like “moderately loud ‘bonk-bonk-bonk’ repeated in intervals of 14-20s” in *G. orophylax* (Duellman and Pyles, 1980) and in *G. plumbea* (Auber-Thomay et al., 1990) or “the call consists of a single primary note ‘wraack’ usually followed by two or three secondary notes ‘ack-ack-ack’” in *G. ruizi* (Duellman and Burrowes, 1986). Obviously the taxonomic and phylogenetic potential of bioacoustics has not been adequately explored yet in the *G. plumbea* group. The present comparative study on five species (*G. espeletia*, *G. monticola*, *G. plumbea*, *G. orophylax*, *G. riobambae*) aims to fill this gap and to assess the behavioural significance of the long and short call types.

Materials and methods

The advertisement calls of five *Gastrotheca* species which inhabit the Andes of Ecuador were recorded in the field or in captivity: (1) *G. riobambae*, Chambo/Riobamba, Prov. Chimborazo; $n=193$ calls; (2) *G. espeletia*, San Gabriel, Prov. Carchi; $n=50$; (3) *G. plumbea*, Pilalo, Prov. Cotopaxi; $n=24$; (4) *G. monticola*, purchased from a commercial supplier, exact locality unknown; $n=108$; (5) *G. orophylax*, San Gabriel, Prov. Carchi; $n=42$. Calls were analysed using a MEDAV Spectro 2000 signal analysing system. The temporal and spectral structure of *G. espeletia* and *G. riobambae* advertisement calls was described by seven call variables and corresponding ambient (air) temperature: (1) call duration [ms], (2) intercall interval [ms], (3) call period = call duration + intercall interval [ms], (4) call ratio = call duration / intercall interval, (5) pulses per call [n], (6) pulse rate [Hz], (7) dominant frequency [kHz]. Due to differences in structure the advertisement calls of *G. plumbea*, *G. monticola* and *G. orophylax* were characterized by eight call variables and corresponding ambient (air) temperature: (1) call duration [ms], (2) pulse groups per call [n], (3) pulse group duration [ms], (4) pulse group interval [ms], (5) pulse group period [ms], (6) pulse group ratio = duration / interval, (7) pulses per pulse group [n], (8) dominant frequency [kHz]. Terminology for call characteristics follows Schneider and Sinsch (1992). Statistical analyses included correlation and regression analysis, factorial analysis (principal component analysis with QUARTIMAX rotation) and discriminant analysis. Slopes and intercepts were tested for significant differences using ANOVA with the conditional sum of squares.

Results

Spontaneously given vocalizations of males consisted either of long, pulsed calls (resembling the advertisement calls in the *G. marsupiata* group) and series of short pulse groups (*G. riobambae*, *G. espeletia*) or exclusively of series of short pulse groups (*G. monticola*, *G. plumbea*, *G. orophylax*; Figs. 1, 2). In analogy, we considered the long calls of *G. riobambae* and *G. espeletia* which were produced in series, as the advertisement calls of these species. Some of the calls were irregularly

Call feature	<i>G. riobambae</i>	<i>G. espeletia</i>
Call duration [ms]	814 ± 16	807 ± 66
Intercall interval [ms]	2419 ± 90	2225 ± 528
Call period [ms]	3232 ± 96	3254 ± 550
Call ratio	0.36 ± 0.01	0.39 ± 0.05
Pulses per call [n]	48 ± 1.1	50 ± 2.2
Pulse rate [Hz]	61 ± 0.6	71 ± 1.2
Dominant frequency [kHz]	1.48 ± 0.02	1.26 ± 0.03

Table 1. Temperature-adjusted features of the advertisement calls of *G. riobambae* and *G. espeletia*. Means and corresponding 95.0% confidence interval refer to an air temperature of 21°C.

interrupted by the drop-out of pulses, but still formed part of a single, long pulse group (e.g. Fig. 1B). The temperature-adjusted features of the advertisement calls are summarized in Table 1. Factorial analysis showed that the seven measured variables represented merely three independent factors: (1) factor 1 (eigenvalue 3.67, percentage of explained variation: 52.5%) represented the influence of air temperature on call structure and was significantly associated with the variables call duration, intercall interval, call period (linear combination of the first two variables), pulses per call, and pulse rate; (2) factor 2 (eigenvalue 1.55, percentage of explained variation: 22.1%) was temperature-independent and loaded significantly by the call ratio and partially by the pulses per call; (3) factor 3 (eigenvalue 1.26, percentage of explained variation: 17.9%) was also temperature-independent, but was loaded by the dominant frequency. Regression analysis of factor 1 versus corresponding air temperature revealed that the regression lines obtained for *G. riobambae*

and *G. espeletia* differed significantly with respect to intercepts ($P=0.0015$) and to slopes ($P<<0.0001$; Fig. 3A). In contrast, the scatter plot of factor 1 and 2 scores showed a complete overlap of the taxon-specific scores (Fig. 3B). A significant discriminant function combining call ratio, pulses per call, and dominant frequency distinguished between *G. riobambae* and *G. espeletia* with a rate of correct classification of 70.0% and 86.5%, respectively.

In contrast, long pulsed calls were absent in *G. plumbea*, *G. monticola* and *G. orophylax* which instead produced series of 2-8 short pulse groups (Fig. 1D-F, Table 2). In *G. plumbea* all pulse groups consisted of a single pulse, whereas in the other taxa the number of pulses varied between 1 (98.3% in *G. monticola*; 74.3% in *G. orophylax*) and 5 per pulse group. For factorial analysis we considered calls with a single pulse per pulse group only. As the recording temperature of the *G. plumbea* calls was unknown, temperature dependence could not be analysed. Factorial analysis suggested three independent factors: (1) factor 1 (eigenvalue 2.57, percentage of explained variation: 42.9%) was significantly associated with the variables call duration, pulse group interval, pulse group period, and dominant frequency, (2) factor 2 (eigenvalue 1.65, percentage of explained variation: 27.4%) was loaded by pulse group duration and pulse group ratio; (3) factor 3 (eigenvalue 1.04, percentage of explained variation: 17.3%) was loaded by the number of pulse groups per call. The scatter plot of all factor scores showed a clear resolution of the species from each other (Fig. 4). Two significant discriminant functions combined all call variables and distinguished the three species at rates of 86.5% (*G. plumbea*), 85.1% (*G. monticola*) and 93.5% (*G. orophylax*) of correct assignment.

Call features	<i>G. plumbea</i>	<i>G. monticola</i>	<i>G. orophylax</i>
Air temperature at record	unknown	20.5-21.5°C	11.5-12.8°C
Call duration [ms]	1644 ± 248	1440 ± 118	2465 ± 419
Pulse groups per call [n]	5.6 ± 0.8	3.3 ± 0.2	3.7 ± 0.4
Pulse group duration [ms]	15 ± 0.8	16 ± 0.4	9.2 ± 0.5
Pulse group interval [ms]	337 ± 20	652 ± 20	913 ± 34
Pulse group period [ms]	352 ± 20	668 ± 20	921 ± 234
Pulse group ratio	0.05 ± 0.003	0.03 ± 0.001	0.01 ± 0.0005
Dominant frequency [kHz]	0.97 ± 0.02	0.96 ± 0.02	0.99 ± 0.1

Table 2. Features of the advertisement calls of *G. plumbea*, *G. monticola* and *G. orophylax*. Only calls with a single pulses per pulse group were considered for comparison. Data are presented as means and corresponding 95.0% confidence interval.

Figure 1. Oscillograms of the taxon-specific vocalizations in the *G. plumbea* group: (A) advertisement call of *G. riobambae* followed by short pulse groups (20.0°C); (B) advertisement call of *G. espeletia* with several interruptions (18.9°C); (C) short pulse groups of *G. espeletia* (17.2°C); (D) advertisement call of *G. monticola* (21.5°C); (E) advertisement call of *G. orophylax* (12.5°C); (F) advertisement call of *G. plumbea* (temperature unknown).

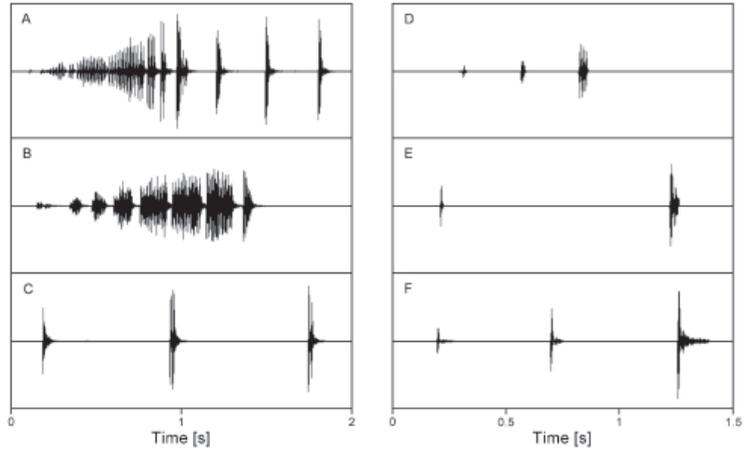
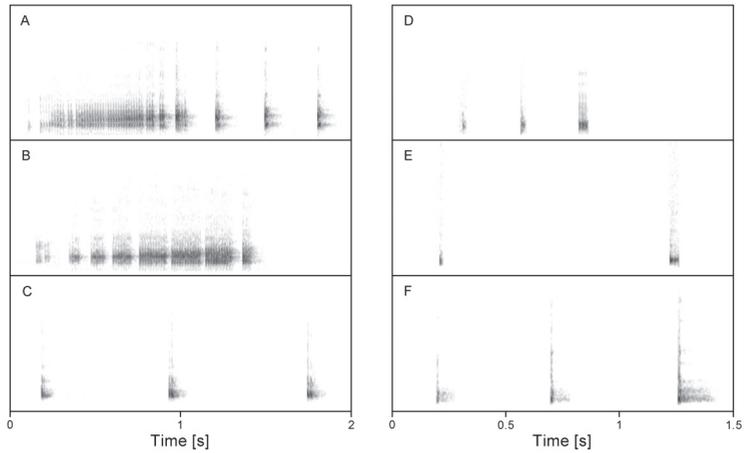


Figure 2. Spectrograms (0 - 10 kHz) of the taxon-specific vocalizations in the *G. plumbea* group: (A) advertisement call of *G. riobambae* followed by short pulse groups (20.0°C); (B) advertisement call of *G. espeletia* with several interruptions (18.9°C); (C) short pulse groups of *G. espeletia* (17.2°C); (D) advertisement call of *G. monticola* (21.5°C); (E) advertisement call of *G. orophylax* (12.5°C); (F) advertisement call of *G. plumbea* (temperature unknown).



Discussion

In contrast to the bioacoustically homogeneous *G. marsupiatia* group with long pulsed advertisement calls, the spontaneously given calls of the five members of the *G. plumbea* group are structurally rather divergent, as already known from the phonetic transcriptions (Duellman and Pyles, 1980; Duellman and Burrowes, 1986; Auber-Thomay et al., 1990). Long pulsed calls similar to those of the *G. marsupiatia* group and additionally series of short pulse groups are present in *G. espeletia*, *G. riobambae* and *G. ruizi* which form a subclade within the *G. plumbea* group (Duellman and Hillis, 1987). Outgroup comparison with *G. marsupiatia* and *G. peruana* (Sinsch and Joermann, 1989; Sinsch, 1990; unpubl. observ.) suggest that the long call represents the advertisement call, whereas the addition of short pulses turns it into an aggressive call. In contrast, long pulsed calls are absent in

G. plumbea, *G. monticola*, and *G. orophylax* which form another subclade within the *G. plumbea* group together with *G. litonedis* and *G. psychrophila* (Duellman and Hillis, 1987). The series of short pulse groups produced by these species provide features for the statistical distinction among species which is partially based on call interval duration. As this variable is known to be influenced by social interactions as well as by motivation of the caller, it remains to be demonstrated that females of these species distinguish conspecific calls from heterospecific ones under natural conditions, as do the statistical functions. Based on this assumption we hypothesize that the short calls represent the advertisement call in this subclade. If this hypothesis is correct and the presence of a long pulsed advertisement call is a plesiomorphic character in *Gastrotheca*, the loss of this call led to a functional change of the calls used before only during

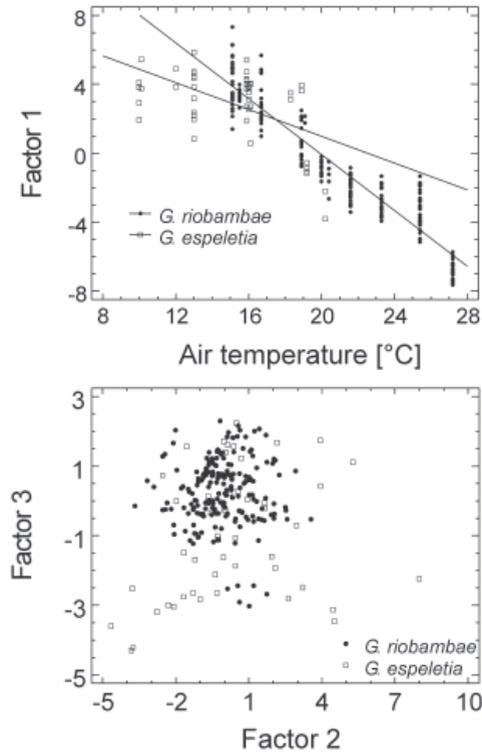


Figure 3. Advertisement call features in *G. riobambae* and *G. espeletia*. (A) Linear regression of factor 1 scores and corresponding air temperature; (B) Scatter plot of the temperature-independent factor 2 and 3 scores. Statistical details are given in the text. Each symbol represents the factor scores of a single call.

aggressive male-male encounters. This hypothesis is supported by the fact that even in *G. riobambae* and *G. espeletia* the more or less frequent drop-out of pulses in the advertisement call may represent the first hint towards a reductive tendency reflecting unknown selective forces in the *G. plumbea* group. In conclusion, the primary dichotomy within this group as proposed by Duellman and Hillis (1987) based on allozymic data is clearly supported by the bioacoustic data presented in this study.

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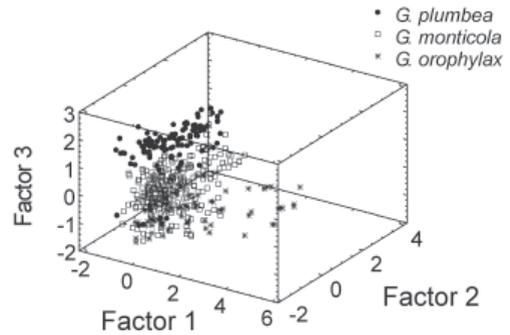


Figure 4. Advertisement call features in *G. plumbea*, *G. monticola* and *G. orophylax*. Scatter plot of the factor 1, 2 and 3 scores. Statistical details are given in the text. Each symbol represents the factor scores of a single call.

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Advertisement calls of hemiphractine marsupial frogs: III. *Flectonotus* spp.

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Abstract. The advertisement calls of *Flectonotus* consisted of pulse groups, one in *F. pygmaeus*, and two or three in *F. fitzgeraldi* and in *F. goeldii*. Despite the striking similarity of call structure in the latter species and their habit to give advertisement calls in series, unique combinations of temporal and spectral call features allow for an unequivocal distinction. Call structure of the Venezuelan *F. fitzgeraldi* suggests a closer relationship to the Brazilian *Flectonotus* species than to the geographically less distant Venezuelan *F. pygmaeus*.

Introduction

The five genera of Neotropical marsupial frogs (Hemiphractinae), *Gastrotheca*, *Flectonotus*, *Hemiphractus*, *Cryptobatrachus* and *Stefania*, share a highly developed parental care for larvae with unique bell-shaped gills, a feature considered as a group synapomorphy (Mendelson et al., 2000; Duellman, 2001). Morphological and molecular evidence suggest that *Gastrotheca* and *Flectonotus* are sister groups (Mendelson et al., 2000; Faivovich et al. 2005). The genus *Flectonotus* currently includes five species which have a disjunct geographical distribution: *F. fitzgeraldi* and *F. pygmaeus* inhabit the Andean slopes of northeastern Colombia, Venezuela, and the adjacent islands of Trinidad and Tobago; *F. goeldii*, *F. ohausi*, and *F. fissilis* are endemics of the mountains and coastal areas of southeastern Brazil (Cochran, 1955; Kenny, 1969; La Marca, 1992; Frost, 2004). Originally, Duellman and Gray (1983) placed the Brazilian species and those of northern South America into distinct genera, *Flectonotus* and *Fritziana*. However, Weygoldt and Carvalho e Silva (1991) demonstrated that available morphological, biochemical and biogeographic evidence did not support the separation in two genera and placed *Fritziana* in the synonymy of *Flectonotus*.

In anurans, advertisement call features have proven valuable tools for the distinction of species (e.g. Martino and Sinsch, 2002) and also for the inference of phylogenetic relationships (e.g. Schneider and Sinsch, 1992; Cocroft and Ryan, 1995). Therefore, we explored the potential of this character complex

in Hemiphractinae. Quantitative bioacoustic studies considering temperature effects on call structure have been published for different *Gastrotheca* species (Sinsch and Joermann, 1989; Sinsch and Juraske, 2006a, 2006b), but for *Flectonotus* few quantitative data have been presented so far (Duellman and Gray, 1983; Weygoldt and Carvalho e Silva, 1991). Here, we attempt to fill this gap with the analysis of the advertisement calls of two Venezuelan and one Brazilian *Flectonotus* species.

Materials and methods

Advertisement calls of three *Flectonotus* species were recorded in the field or in captivity. (1) *F. fitzgeraldi*, Simla Tropical Research Station, Trinidad and Tobago, $n=495$; (2) *F. pygmaeus*, Estación biológica 'Rancho Grande', Estado Aragua, Venezuela, $n=30$; (3) *F. goeldii*, São Conrado, Rio de Janeiro, Brazil, $n=62$. Calls were analysed using a MEDAV Spectro 2000 signal analysing system. Ten temporal and spectral variables of the advertisement calls and the corresponding air temperature were measured: (1) call duration [ms], (2) intercall interval [ms], (3) call period = call duration + intercall interval [ms], (4) call ratio = call duration / intercall interval, (5) Pulse groups per call, (6) pulse group duration [ms], (7) pulse group interval [ms], (8) pulses per pulse group [n], (9) pulse rate [Hz], (10) dominant frequency [kHz]. Terminology for call characteristics follows Schneider and Sinsch (1992). Statistical analyses included comparisons of means (ANOVA), correlation and regression analysis, and factorial analysis (principal component analysis with VARIMAX rotation).

Results

All *Flectonotus* males gave advertisement calls consisting of pulse groups, one in *F. pygmaeus*, and two to three in *F. fitzgeraldi* and in *F. goeldii* (Fig. 1). The latter species frequently grouped the advertisement vocalizations to longer call series with 2-10 single calls in *F. fitzgeraldi* (mean: 4) and with 28-36 calls in *F. goeldii* (mean: 33), whereas *F. pygmaeus* emitted single calls without apparent

grouping. In *F. fitzgeraldi*, call series consisted exclusively of two-pulse group calls and three-pulse group calls were given always singly and separated from the call series. In *F. goeldii*, however, about the first half of a call series consisted of two-pulse group calls and then males shifted to three-pulse group calls, i.e. the longer calls were never given singly. Temporal and spectral features of the calls allowed for an unequivocal distinction among the three taxa, as summarized in Table 1. *F. pygmaeus* differed from the other two taxa by producing a single pulse group with a significantly larger number of pulses and a lower pulse rate (ANOVA, $P < 0.0001$). In contrast, *F. fitzgeraldi* and *F. goeldii* had the same basic call structure, but differed significantly with respect to dominant frequency due to considerable size differences (ANOVA, $P < 0.0001$). Still, a significant increase of dominant frequency from the first to the last pulse group of a call was common to both species (ANOVA, $P < 0.001$).

For the purpose of interspecific comparison, we assume that not only the two-pulse group calls were homologous, but also the three-pulse group calls, though it remains to be proven that the behavioural significance of the isolated calls in *F. fitzgeraldi* is the same as that of the integrated calls in *F. goeldii*. Factorial analyses were run separately on the two call types considered to represent advertisement vocalizations. The data set used for the two-pulse group calls consisted of all ten measured

call variables plus air temperature with 258 observations. Three independent factors accounting for 87.6% of the total variation were identified: (1) factor 1 (eigenvalue 7.4, percentage of explained variation: 56.8%) was temperature-independent and was loaded significantly by call duration, call ratio, and all pulse group 2 variables; (2) factor 2 (eigenvalue 3.2, percentage of explained variation: 24.5%) represented the temperature effect on call structure and was associated with intercall interval, call period and partially with the pulse rate of pulse group 2; (3) factor 3 (eigenvalue 0.8, percentage of explained variation: 6.3%) again was temperature-independent and loaded by all pulse group 1 variables. The factor 2-temperature relationship did not differ between *F. fitzgeraldi* and *F. goeldii*, as the factor 2 scores of the latter species were grouped around the regression line of the first one (SCORE 2 = $-8.15 + 0.37 \cdot \text{Temperature}$, $R^2 = 84.7\%$; Fig. 2A). However, the temperature-independent factors 1 and 3 permitted an unequivocal distinction between the two species without *a priori* delimitation of groups (Fig. 2B).

The data set used for the three-pulse group calls consisted of the variables call duration, duration of each of the three pulse groups and their corresponding number of pulses, pulse rate and dominant frequency plus air temperature with 240 observations. Three independent factors accounting for 82.9% of the total variation were identified: (1)

Call feature	<i>F. pygmaeus</i> n=30	<i>F. fitzgeraldi</i> n=37	<i>F. fitzgeraldi</i> n=38	<i>F. goeldii</i> n=43	<i>F. goeldii</i> n=19
Air temperature	unknown	21.6°C	21.6°C	22.4°C	22.4°C
Number of pulse groups per call	1	2	3	2	3
Call duration [ms]	83 ± 6	68 ± 1	177 ± 5	173 ± 5	161 ± 4
Intercall interval [ms]	-	335 ± 17	-	150 ± 6	176 ± 12
Call period [ms]	-	402 ± 16	-	322 ± 8	338 ± 11
Call ratio	-	0.21 ± 0.01	-	1.16 ± 0.04	0.93 ± 0.07
Pulse group duration [ms], 1 st /2 nd /3 rd pulse group	83 ± 6	19 ± 1 21 ± 1	20 ± 1 25 ± 1 22 ± 1	34 ± 3 43 ± 3	46 ± 3 45 ± 7 42 ± 6
Interval between pulse groups [ms], 1 st /2 nd , 2 nd /3 rd	-	28 ± 1	26 ± 1 85 ± 4	96 ± 7	Not measured
Pulses per pulse group (median, range of 1 st /2 nd /3 rd pulse group)	9, 7-12	3, 2-3 3, 2-4	3, 2-4 4, 3-4 3, 2-4	4, 1-6 5, 4-8	5, 4-7 5, 2-9 5, 2-8
Pulse rate [Hz], 1 st /2 nd /3 rd pulse group	111 ± 2	147 ± 5 145 ± 4	151 ± 3 147 ± 2 145 ± 3	126 ± 5 123 ± 5	115 ± 4 118 ± 2 117 ± 2
Dominant frequency [kHz], 1 st /2 nd /3 rd pulse group	3.06 ± 0.05	3.25 ± 0.05 3.45 ± 0.08	3.22 ± 0.04 3.45 ± 0.08 3.56 ± 0.06	2.14 ± 0.03 2.40 ± 0.04	2.19 ± 0.01 2.22 ± 0.02 2.39 ± 0.05

Table 1. Structural and spectral features of the advertisement calls of three *Flectonotus* species. Data are presented as means and corresponding 95.0% confidence interval. In *F. fitzgeraldi* and in *F. goeldii* the features of the two- and three-pulse group calls are given in consecutive columns.

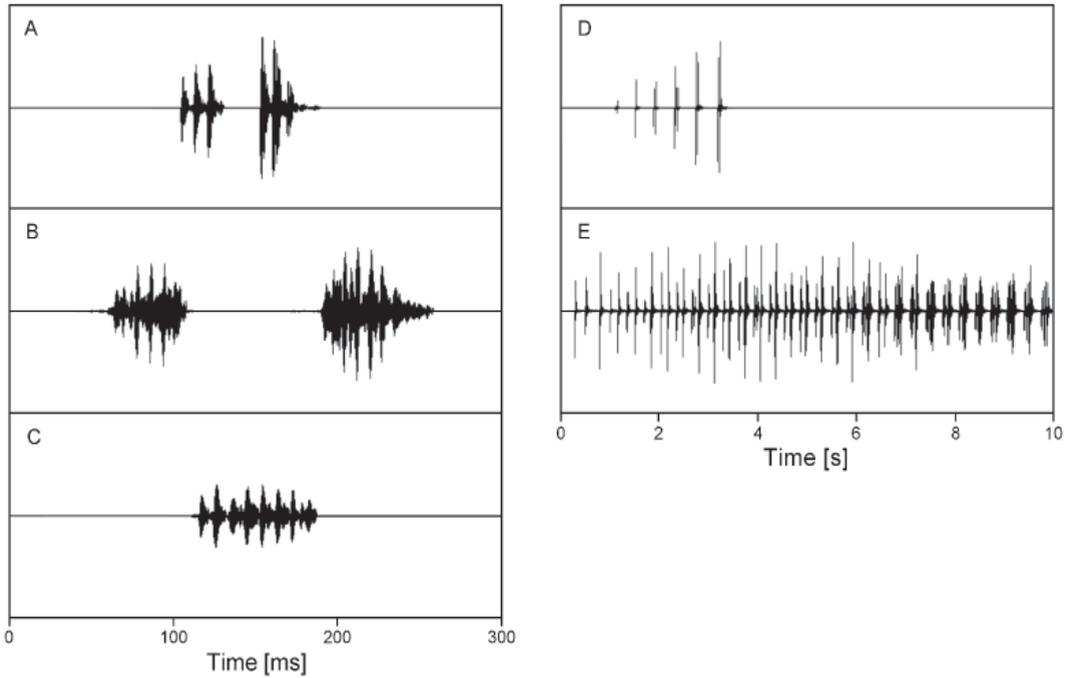


Figure 1. Left: Advertisement calls of *Flectonotus* males, consisting of two pulse groups in *F. fitzgeraldi* (A; air temperature 20.2°C) and *F. goeldii* (B; air temperature 22.4°C) or of one pulse group in *F. pygmaeus* (C; air temperature unknown); Right: Series of advertisement calls of *F. fitzgeraldi* (D; air temperature 20.2°C) and *F. goeldii* (E; air temperature 22.4°C).

factor 1 (eigenvalue 7.8, percentage of explained variation: 55.4%) was temperature-independent and was loaded significantly by the duration and number of pulses of pulse groups 1 and 3 as well as the dominant frequencies of all pulse groups; (2) factor 2 (eigenvalue 2.7, percentage of explained variation: 19.6%) represented the temperature effect on call structure and was associated with call duration and the pulse rates of the three pulse groups; (3) factor 3 (eigenvalue 1.1, percentage of explained variation: 7.8%) again was temperature-independent and loaded by the duration and number of pulses of pulse group 2. The factor 2-temperature relationship did not differ between the two species, as the factor 2 scores of *F. goeldii* were grouped around the regression line of *F. fitzgeraldi* ($SCORE\ 2 = -8.45 + 0.38 * Temperature$, $R^2=91,5\%$; Fig. 3A). Again, the temperature-independent factors 1 and 3 permitted an unequivocal distinction between the two species (Fig. 3B).

Discussion

Despite their disjunct geographical distribution, the advertisement calls of *F. fitzgeraldi* and *F. goeldii*

had the same basic call structure, whereas those of *F. pygmaeus* differed considerably from both. Still, factorial analyses revealed that each of the three taxa emitted advertisement calls with a unique combination of temporal and structural features. Thus, an unequivocal distinction among the species is possible even if only a few calls are available.

A comparison of our results with the brief survey of bioacoustic data of all five *Flectonotus* species published by Duellman and Gray (1983) and that of Weygoldt and Carvalho e Silva (1991) on *F. goeldii* is hampered by the fact that terminology of call description differs among papers. The term “note” as used by these authors is the synonym of “pulse group” used in this paper. We prefer to restrict the use of “note” to the tonal subunits of advertisement calls in *Stefania* to emphasize the fundamental structural difference to the pulsed subunits of *Gastrotheca* and *Flectonotus* calls (Sinsch and Juraske, 2006a, 2006b, 2006c). Moreover, there is disagreement in the delimitation of the advertisement call in *F. goeldii* and *F. ohausi*. According to our behavioural observations the basic units of advertisement vocalizations in *F. goeldii*

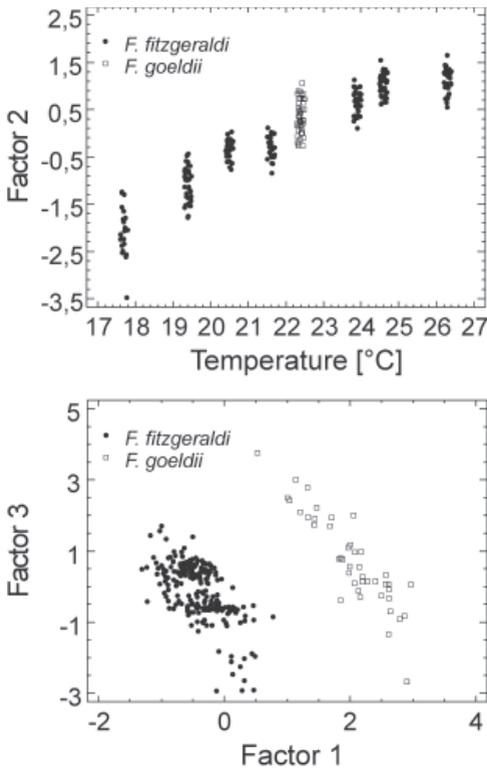


Figure 2. Factorial analysis of advertisement calls consisting of two pulse groups in *F. fitzgeraldi* and in *F. goeldii*. (A) Temperature dependence of factor 2. (B) Scatter plot of the temperature-independent factor 1 and 3 scores. Statistical details are given in the text. Each symbol represents the factor scores of a single call.

are either two or three successive pulse groups exhibiting distinctive features and are repeated in call series. The homology to the two-pulse group call series of *F. fitzgeraldi* is obvious, whereas the assumed homology of the three-pulse group calls requires further evidence. An alternative hypothesis is to assume that the addition of a third pulse group might have caused a functional shift from advertisement vocalization to an encounter call, as observed in the *Gastrotheca marsupiata* group (Sinsch and Joerman, 1989). Therefore, we consider the two- or three-pulse group units of call series as the advertisement call and not the complete series as did Duellman and Gray (1983) and Weygoldt and Carvalho e Silva (1991).

This standardized nomenclature for homologous structures is a prerequisite for any quantitative taxonomic or phylogenetic use of bioacoustic

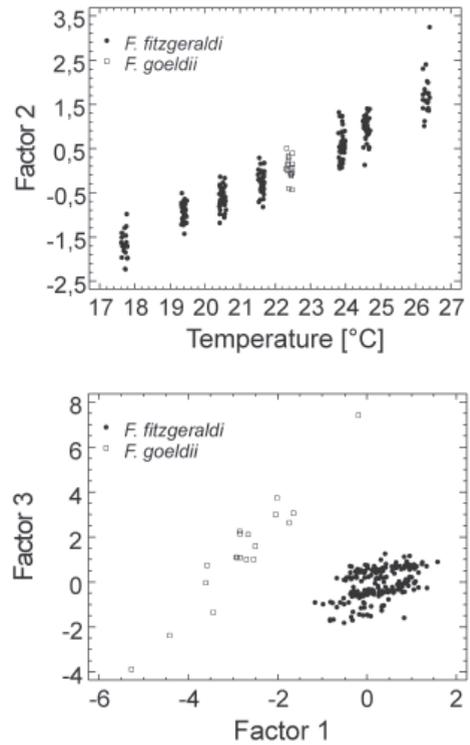


Figure 3. Factorial analysis of advertisement calls consisting of three pulse groups in *F. fitzgeraldi* and in *F. goeldii*. (A) Temperature dependence of factor 2. (B) Scatter plot of the temperature-independent factor 1 and 3 scores. Statistical details are given in the text. Each symbol represents the factor scores of a single call.

data. The taxonomic relevance is obvious, the specific status of the three studied *Flectonotus* which was assigned based on morphological and biochemical evidence (Duellman and Gray, 1983) is corroborated by unique call character combinations. The phylogenetic inference is admittedly more speculative, as evidence independent from bioacoustic data is not yet available. Still, the peculiar structure of *F. pygmaeus* vocalisations may indicate that this species is outgroup to a clade formed by the other four members of *Flectonotus* which share the calls composed of at least two pulse groups. If this phylogenetic inference is correct, i.e. the grouping of the Venezuelan *F. fitzgeraldi* with three Brazilian species, the resurrection of the genus *Fritziana* would not be supported.

Finally, it is worth mentioning that the unique call structure of *F. pygmaeus* within the genus *Flectonotus*

resembles a prototype of the basic advertisement call structure in *Gastrotheca* which consists of a single long pulse group with numerous pulses (e.g. Sinsch and Joermann 1989; Sinsch and Juraske, 2006a, 2006b). This might be a further indication of the sister group relationship between *Gastrotheca* and *Flectonotus* as recently also supported by molecular data (Faivovich et al., 2005).

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Advertisement calls of hemiphractine marsupial frogs: IV. *Stefania* spp.

Ulrich Sinsch, Norbert Juraske

Abstract. *Stefania evansi* and *S. riveroi* produced tonal advertisement calls which consisted usually of a single note, but sometimes of two notes. There were significant differences with respect to temporal and spectral call structure between the two species. *S. riveroi* produced spontaneously also a multi-note call which significantly differed from the two-note advertisement call not only by the note number, but also by further structural features. Its behavioural significance remains unknown. Call features of *Stefania* do not support for a close relationship to *Gastrotheca* and *Flectonotus*.

Introduction

Until recently, Neotropical marsupial frogs (Hemiphractinae) were considered a monophyletic hylid clade which includes five genera *Gastrotheca*, *Flectonotus*, *Hemiphractus*, *Cryptobatrachus* and *Stefania* (Mendelson et al., 2000; Duellman, 2001). Monophyly is supported by two synapomorphies: (1) the presence of bell-shaped gills in larvae; (2) female transport of eggs and larvae in specialised depressions or sacs in the dorsum (e.g. Duellman and Maness, 1980; Gorzula et al., 1983). New molecular data did neither indicate a close relationship to Hylidae nor support unambiguously monophyletic origin of Hemiphractinae (Faivovich et al., 2005). Still, morphological and molecular evidence suggests a sister group relationship between *Stefania* and *Cryptobatrachus* on one side, and between *Gastrotheca* and *Flectonotus* on the other. The latter is also supported by bioacoustic evidence (Sinsch and Juraske, 2006a, 2006b, 2006c), whereas the relationship between both genera groupings remains unresolved.

The genus *Stefania* Rivero, 1968, is restricted to the Guyana Shield of Venezuela and Guyana and several species are endemic to the plateaus of different tepuis. The scientific exploration of this region during the last two decades led to an enormous increase of species richness in this genus: the number rose from seven species (Duellman and Hoogmoed, 1984) to currently 18 species (Señaris et al, 1997; MacCulloch and Lathrop, 2002; Barrio-Amorós and Fuentes, 2003; Frost, 2004). Most studies on *Stefania* focus on morphology, taxonomy, ecology (habitat descriptions) and zoogeography of the genus, whereas reports on the

reproductive behaviour (vocalizations, parental care) are scarce (e.g. Jungfer and Böhme, 1991; Mägdefrau, 1991). Since we had the chance to analyse call records of *S. evansi* and *S. riveroi* we here describe quantitatively the calls of these two species in order to look for taxon-specific bioacoustic features, and to compare them with *Gastrotheca* and *Flectonotus* calls.

Materials and methods

Vocalizations which were produced spontaneously by single males of two *Stefania* species were recorded in captivity: (1) *S. evansi*, La Escalera, Venezuela, $n=81$ (call type 1); (2) *S. riveroi*, Yuruani tepui, Venezuela, $n=78$ (call type 1), $n=20$ (call type 2). Calls were analysed using a MEDAV Spectro 2000 signal analysing system. Descriptions of the temporal and spectral structure of calls were based on five call variables and corresponding ambient (air) temperature: (1) call duration [ms], (2) note duration [ms], (3) internote interval [ms], (4) note ratio (= duration of notes/internote interval), and (5) dominant frequency [kHz]. Terminology for call characteristics follows Schneider and Sinsch (1992). Statistical analyses testing for interspecific differences included ANOVA.

Results

Males of *S. evansi* produced a tonal call which either consisted of a single note ($n=63$; 77.8%) or of two notes ($n=18$, 23.2%; Fig. 1). In the two-notes calls, the second note was considerably louder and significantly longer than the first one (ANOVA, $P<0.0001$; Table 1). With respect to dominant frequency, however, there was no difference between the notes or among single- or two-note calls (ANOVA, $P=0.09$). In *S. riveroi*, the same call type was also present either consisting of a single note ($n=72$; 92.3 %) or of two notes ($n=6$, 7.7 %; Fig. 1) and statistical comparison of these call paralleled the results obtained for *S. evansi* (Table 1). Interspecific call comparison yielded significant differences in the three parameters available for single-note calls (ANOVA, $P<0.0001$). An analogous

comparison of the two-note calls was hampered by the low number analysed, but interspecific differences were significant with respect to the duration of the second note (*S. evansi* < *S. riveroi*, ANOVA, $P < 0.0001$), its dominant frequency (*S. evansi* > *S. riveroi*, ANOVA, $P = 0.0088$), and note ratio (*S. evansi* < *S. riveroi*, ANOVA, $P = 0.0009$). *S. evansi* and *S. riveroi* calls resemble a faint, hoarse dog barking due to their relatively low dominant frequency and the tonal call structure.

In *S. riveroi*, a second call type ($n = 20$) was recorded which consisted of 2-9 tonal notes (average: 5.4 ± 1 ; Fig. 2). Similar to the previously described two-note call, amplitude of notes increased steadily from the beginning to the end. Call features were (average \pm 95% confidence interval; range): call duration 1193 ± 256 ms (386 – 2328), note duration 119 ± 8 ms (63-201), internote interval 121 ± 9 ms (47-218), note ratio 1.15 ± 0.18 (0.39-3.87) and dominant frequency 0.87 ± 0.04 kHz (0.70-1.21). Note duration, internote interval and note ratio differed significantly between this multi-note call and the two-note call of the same species.

Discussion

Duellman and Hoogmoed (1984) inferred from the absence of vocal slits in *Stefania*, that vocalizations

in this genus are probably uncommon or even absent. This prediction failed – *Stefania riveroi* produces two types of tonal calls (e.g. Mägdefrau, 1991; this study). In *S. evansi* and in *S. riveroi*, the most frequently recorded call which is spontaneously given was a single-note call which had species-specific features as well as its rarer two-note variant. Therefore, we propose to consider this call type as the advertisement call. However, these calls do not resemble the advertisement calls of other hemiphractine frogs such as *Gastrotheca* or *Flectonotus* (Sinsch and Joermann, 1989; Sinsch and Juraske, 2006a, 2006b). Without reconfirmation with field observations on reproducing *Stefania* this assignment remains tentative.

S. riveroi also produced a more complex multi-note call which has already been described by Mägdefrau (1991). His description referred to a then unnamed *Stefania* species originating from the Venezuelan Yuruaní tepui. Now, this population was considered to be conspecific with *S. riveroi* (Señaris et al., 1997). We cannot rule out that this composed call, in fact, represents the advertisement call of *S. riveroi*, as implicated by Mägdefrau, but the low frequency with which it is produced does not support this alternative hypothesis. Our study clearly emphasizes that thorough behavioural observations are badly needed to unravel

Call feature	<i>S. evansi</i> single-note call ($n = 63$)	<i>S. evansi</i> two-note call ($n = 18$)	<i>S. riveroi</i> single-note call ($n = 72$)	<i>S. riveroi</i> two-note call ($n = 6$)
Call duration [ms]	111 ± 6 (56 – 168)	1034 ± 143 (474 – 1523)	256 ± 25 (80 – 545)	1086 ± 472 (751 – 1961)
Note 1 duration [ms]	111 ± 6 (56 – 168)	102 ± 18 (59 – 203)	256 ± 25 (80 – 545)	147 ± 16 (80 – 156)
Note 2 duration [ms]	-	128 ± 12 (102 – 172)	-	225 ± 31 (170 – 253)
Internote interval [ms]	-	805 ± 142 (302 – 1327)	-	715 ± 466 (393 – 1589)
Note ratio	-	0.14 ± 0.03 (0.05 – 0.27)	-	0.25 ± 0.09 (0.1 – 0.33)
Dominant frequency of note 1 [kHz]	1.05 ± 0.07 (0.75 – 1.96)	0.98 ± 0.12 (0.75 – 1.86)	0.86 ± 0.04 (0.65 – 1.46)	0.85 ± 0.05 (0.80 – 0.90)
Dominant frequency of note 2 [kHz]	-	1.12 ± 0.12 (0.90 – 1.96)	-	0.78 ± 0.14 (0.70 – 0.95)

Table 1. Quantitative features of the advertisement call of *S. evansi* (air temperature 21.4 – 22.6°C) and of *S. riveroi* (air temperature 17.5°C). Means and corresponding 95.0% confidence interval are presented, ranges are given in parentheses.

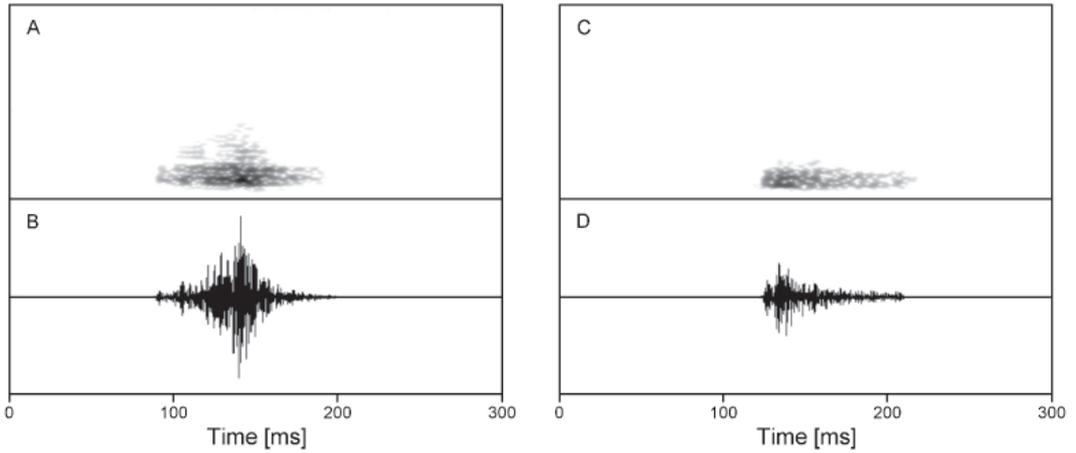


Figure 1. Advertisement calls (= single-note calls) of *Stefania*. *S. evansi* at 22.6°C: (A) spectrogram (0 - 10 kHz) and (B) oscillogram; *S. riveroi* at 17.5°C: (C) spectrogram (0 - 10 kHz) and (D) oscillogram.

the social context of the vocalizations described here. Nevertheless, even the simple tonal structure of the most common calls permits a reliable distinction between *S. evansi* and *S. riveroi* based on bioacoustic features.

Finally, the potential phylogenetic significance of call structure remains to be evaluated. The vocal repertoire (tonal) of *Stefania* deviates so strongly from that of *Gastrotheca* and *Flectonotus* (pulsed) that it appears impossible to homologue call features. Consequently, assuming an independent evolution of vocalizations in *Stefania* and in *Gastrotheca/Flectonotus* seems

more reasonable than a successive modification of the vocalisation of a shared ancestor. While vocalisations of *Cryptobatrachus* and *Hemiphractus* have not yet been analysed, bioacoustics do not contribute to the assessment of phylogenetic relationships between *Stefania* and these genera, but certainly do not suggest a close relationship to *Gastrotheca/Flectonotus*.

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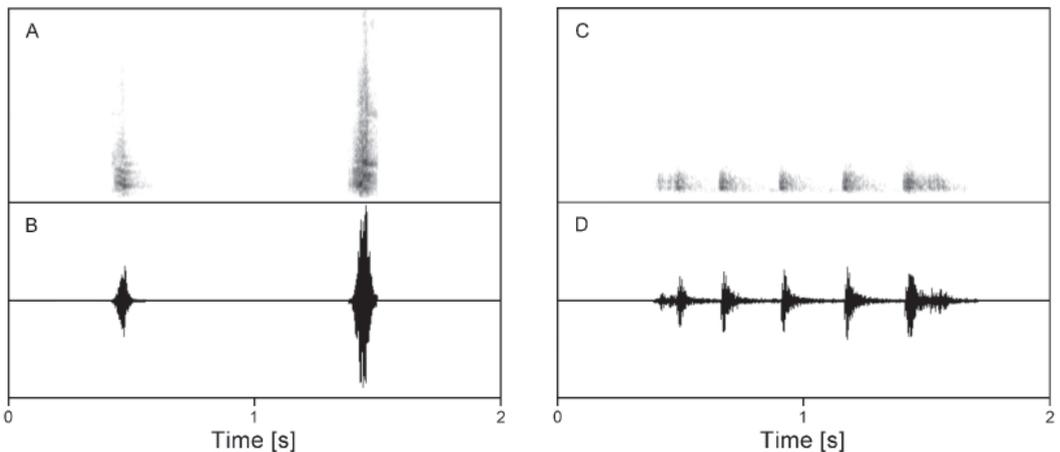


Figure 2. Two-note call of *Stefania evansi* at 22.6°C: (A) spectrogram (0 - 10 kHz) and (B) oscillogram. Multi-note call of *S. riveroi* at 17.5°C: (C) spectrogram (0 - 10 kHz) and (D) oscillogram.

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The homing behaviour of displaced smooth newts *Triturus vulgaris*

Ulrich Sinsch, Rebekka Schäfer, Anja Sinsch

Abstract. Homeward orientation of passively displaced smooth newts *Triturus vulgaris* was tested in four arena experiments at release sites 213m to 3,270m distance from their breeding pond. Newts were unable to assess the home direction from test sites which were located outside the area of familiarity. However, at the shortest displacement distance the mean direction chosen by the newts did not differ from the home direction and the mean vector length was significantly different from zero. It is hypothesised that *T. vulgaris* uses olfactory beaconing to orientate within its area of migratory experience.

Introduction

True navigation is the ability of an organism to orient towards a goal following displacement to an unfamiliar site, without outward journey information and sensory contact with the goal (Able 1980). This ability requires the use of a spatial map and of a compass and has been demonstrated for several vertebrates, including the newt *Notophthalmus viridescens* (Phillips et al. 1995; review: Sinsch 2006). Another four species of newts (*Taricha rivularis*, *T. granulosa*, *Triturus alpestris*, *T. marmoratus*) were shown to actually home following long-distance displacements or to choose the homeward direction in experimental releases (Twitty 1959; Twitty et al. 1964; Diego-Rasilla and Luengo, 2002, 2004; Diego-Rasilla 2003; Diego-Rasilla et al. 2005). Home orientation is hypothesised to rely on a geomagnetic map and celestial and geomagnetic compasses (Fischer et al. 2001; Phillips et al. 2002a, b).

The ability of five newt species pertaining to three genera to navigate home from unfamiliar sites may suggest that this a feature common to all newts. Therefore, we used smooth newts (*Triturus vulgaris*) to test experimentally this hypothesis. Adult smooth newts stay usually within a distance of 20-60m from their breeding pond and only few individuals have been found to move as far as 400m (Baker and Halliday 1999). The maximum dispersal distance of juveniles amounted about 1km (Cabela and Girolla 1994). Early experimental tests suggested that short-distance orientation is based on olfactory cues combined with geotactic behaviour

(Czeloth 1931). In a series of four experiments, we displaced reproductive newts to test sites which were 213-3,270m distant from their breeding pond and monitored their directional choice (= initial orientation) *in situ* in an arena. We expected that at least the less distant test site was within the area of previous migratory experience of most individuals, whereas the most distant site was far outside of the natural migratory range. Aims of this study were to quantify the effect of familiarity on directional choice and to look for indications of a navigational ability.

Material and Methods

Groups of at least 25 adult newts were captured at the breeding pond (Schmittenhöhe, Rhineland-Palatinate, Germany; Sinsch et al. 2003) one day before testing and each group was displaced to a test site with full access to magnetic, olfactory and visual cues during the outward journey. At the test site, they spent the complete night and part the next day within moist plastic boxes and full sensory access to local cues including sky. Boxes (34 x 24 x 10 cm) were shaded during day to prevent overheating. Four test sites were chosen which varied in distance to the breeding pond and were distributed along a south-west axis: (1) 266° (home direction), 213m (distance to home), June 11th, 2005 (date of test); (2) 240°, 496m; (3) 233°, 773m, May 27th, 2005; (4) 239°, 3,270m, May 16th, 2005. After the testing period newts were returned to their breeding pond. All tests were performed during the newts' reproductive period.

Tests consisted in a series of 20 consecutive releases of one individual each in an open unstructured, visually symmetrical plastic arena (80cm diameter, 30cm high) which was placed on flat ground and aligned to the N-S axis. The floor and arena walls were thoroughly wiped with a damp cloth between trials to eliminate directional olfactory cues (Fischer et al. 2001), and then they were wiped dry. All tests were done between 10am and 5pm under a clear day time sky without alteration of the local geomagnetic field. Prior to testing, newts were transferred for at least 10min to a plastic box (34 x 24 x 10 cm) half-filled with water from the newts' breeding pond. Newts were then placed in the arena center under an opaque, cylindrical plastic container (10cm diameter, 15cm high) that served as a release

device. Newts were held in the container for 5min to overcome effects of handling. The release device was then lifted, and the newts were allowed to move freely. Two observers stood motionless at the north and the south pole of the arena and monitored the newt's movements during 5min. An individual trial ended at the first contact of the newt with the arena perimeter. Directional choice (to the nearest 1°) and decision time of 20 newts per test site were recorded.

Directional data were analysed using standard circular statistics (Batschelet 1981). The direction of the mean vector was calculated by vector addition and tested for significance using the Rayleigh test for a non-random distribution. A 95% confidence interval was used to test whether the newts' mean directional choice was significantly different from the homeward direction at the test sites. Sex-specific decision time and median decision time at the four test sites were compared using the Mann-Whitney *U*-test and the Kruskal-Wallis test, respectively. Shapes of distributions were tested for differences by the Kolmogorov-Smirnov test. The level of statistical significance was set at 0.05. All calculations were performed using the program package STATGRAPHICS Centurion.

Results

Independent from the specific test site, orientation behaviour of newts consisted in an initial raising of the head and staying almost motionless in the centre of the arena for 10-40s. Newts gave the impression that they were smelling. Then, newts moved either in rather straight or more often twisted paths to the perimeter of the arena. Males ($n=37$) and females

($n=43$) did neither differ significantly with respect to median decision time (63s, range: 13-153s versus 64s, 7-280s; Mann-Whitney *U*-test, $P=0.59$) nor with respect to the shape of data distribution (Kolmogorov-Smirnov test, $P=0.78$; Fig. 1). Site-specific medians of decision time did not vary either (overall median: 63s; Kruskal-Wallis test, $P=0.44$).

The mean vector length of initial orientation obtained for the three releases at less than 1km distance increased with decreasing displacement distance (Fig. 2): (1) test site: 266°, 213m; mean vector length: 0.388, mean direction: 296.0° ($n=20$, Rayleigh-test, $P=0.043$); (2) test site: 240°, 496m; mean vector length: 0.283, mean direction: 293.7° ($n=20$, Rayleigh-test, $P=0.21$) (3) test site: 233°, 773m; mean vector length: 0.116, mean direction: 351.1° ($n=20$, Rayleigh-test, $P=0.75$). At the site of presumed unfamiliarity, initial directional choice did not differ significantly from random: 239°, 3,270m; mean vector length: 0.359, mean direction: 13.3° ($n=20$, Rayleigh-test, $P=0.073$). Thus, only newts displaced to the close vicinity of the breeding pond were significantly oriented and chose a mean direction, which was not significantly different from the home direction.

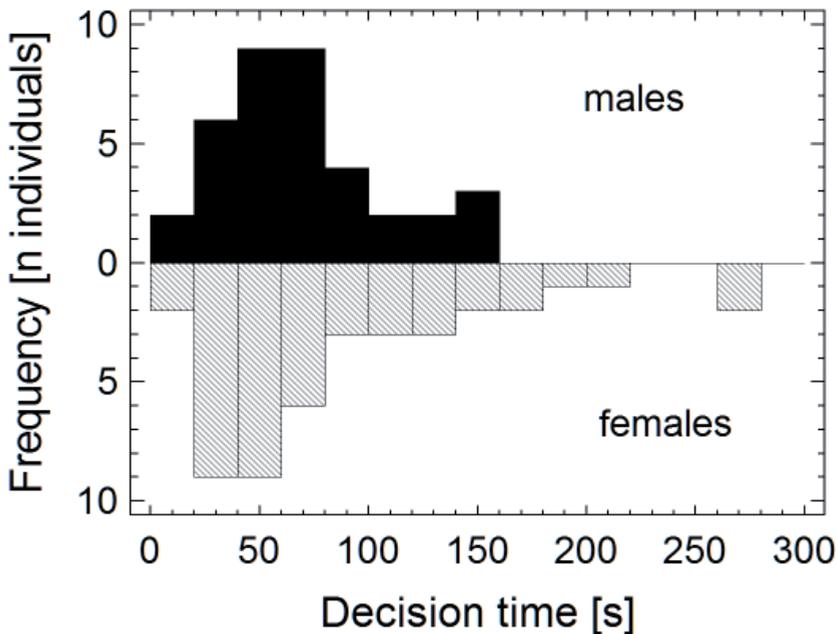
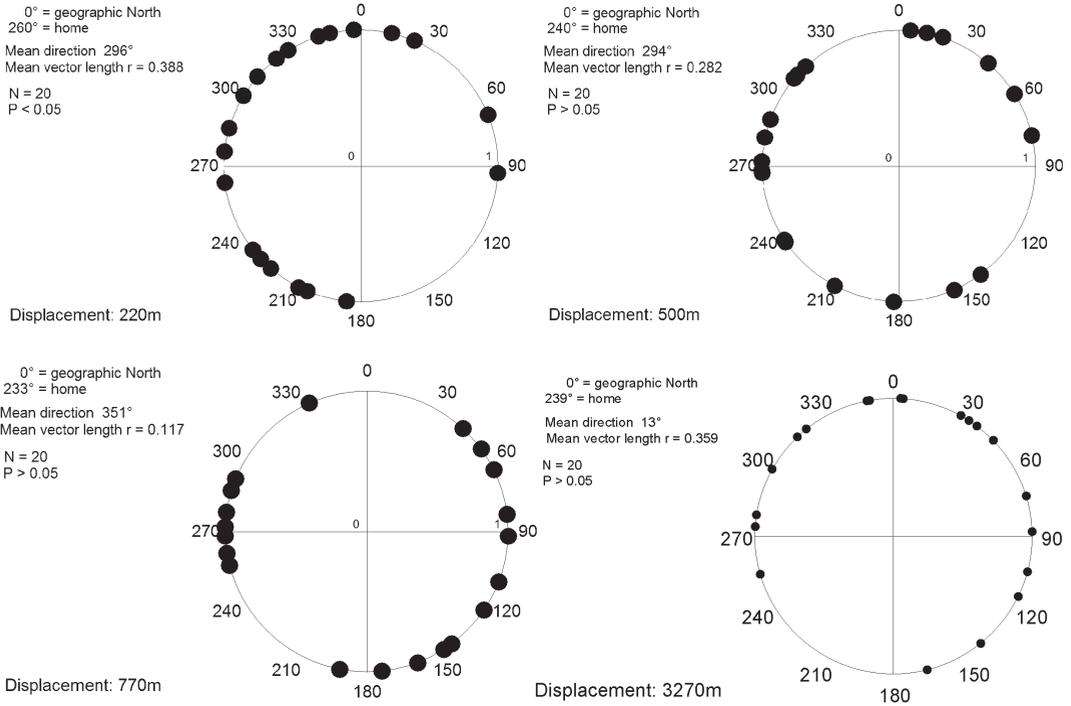


Figure 1. Sex-specific distribution of individual decision time (period between the start of an individual release to the first contact of the individual with the arena wall). Statistical details in text.

Figure 2. Circular diagrams of four tests with increasing distance to home pond. Statistical details are given in the text. Each dot represents the bearing of one individual, the radius of the circle was set to 1.



Discussion

Smooth newts were not able to assess the home direction, if displaced to sites outside their natural migratory range. Not even full access to outward journey information and to local cues at the test sites did improve initial orientation. This result permits to reject the hypothesis that the ability to navigate is feature common to all newts. It is, however, in full agreement with previous studies on the homing ability of displaced *T. vulgaris*. Blab (1986) estimated the maximum displacement distance which allowed successful homing at 550m in males (homing accomplished after 37d) and at 600m in females (after 48d).

Successful homing in smooth newts possibly requires direct sensory contact to the goal, for example by smelling pond odours as suggested by the behaviour of the newts when released in the arena, and by earlier experiments by Czeloth (1931). Close-up releases of alpine newts (*T. alpestris*) also indicated that their homeward orientation over distances of 45-110 meters was based on olfactory cues emanating from the breeding pond (Joly and

Miaud 1993). Thus, olfactory beaconing seems to be a common feature of the orientation system of *T. alpestris* and *T. vulgaris* for short distance homing (Sinsch 2006).

In contrast, the long distance homing ability has only been demonstrated for *T. alpestris* and *T. marmoratus*, but not for *T. vulgaris* (Diego-Rasilla 2003, Diego-Rasilla and Luengo 2002, this study). Pond odours seem to be unsuitable cues for homing from distant unfamiliar sites because the availability of these cues will be affected by wind direction, distance from the pond, and turbulence at ground level (e.g. Sinsch 1987, Baldocchi 1989). Map-based orientation is the most probable mechanism used for homing from unfamiliar sites (e.g. Fischer et al. 2001). The absence of the ability to navigate in *T. vulgaris* may also be related to the fact that the genus *Triturus* is not monophyletic (e.g. Grossenbacher and Thiesmeier 2003; Carranza and Amat 2005). Each of the studied species pertains to another clade which had separated from each other about 14 Myr ago (Carranza and Amat 2005). Therefore, the evolutionary origin of the navigation ability remains

obscure, it may have been secondarily lost in the clade of *T. vulgaris*, or evolved independently in the clades of *T. alpestris* and *T. marmoratus*.

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Urban development and the natterjack toad (*Bufo calamita*) - implementation of the Habitats Directive in The Netherlands

Gerard F.J. Smit

Abstract. *Bufo calamita* is fairly common in The Netherlands and listed on Annex IV of the Habitats Directive. The species and its habitat are strictly protected in The Netherlands. Urban development regularly conflicts with the presence of the species. The implementation of compensation measures in a large-scale project is discussed. For small-scale projects within urban areas the strict interpretation of the Habitats Directive is a bottleneck. This led to the general practice of preventing the species to benefit from temporal situations. A set of practical measures for handling local urban populations is proposed. The set aims to protect the natterjack toad in its most critical stages when construction works are carried out. The potential for a local population sink should be taken into account when deciding to accept or to prevent animals using temporal habitats.

Introduction

The natterjack toad (*Bufo calamita*) is listed at Annex IV of the European Habitats Directive (HD). It is a strictly protected species under the European and national legislation. *Bufo calamita* is rather common in The Netherlands (Bergmans and Zuiderwijk, 1986; Hom et al., 1996). Its natural habitats are found in the coastal dunes, riparian areas and heath land. In The Netherlands it also occurs in polder areas with grassland, arable land or glasshouses and in urban areas. The presence of local populations in urban areas is known, but is mainly documented for Amsterdam (Boomsma and Arntzen, 1985; Melchers and Timmermans, 1991). In The Netherlands urban development areas in general have large surfaces of bare sand as a foundation for constructing buildings. *Bufo calamita*, as a pioneer species, can quickly colonize urban development areas. It benefits from new created ditches and temporal waters that, after rainy periods, remain in local terrain depressions. Urban development projects often conflict with the strict prohibitions of the HD due to the presence of *B. calamita*. The problems can occur at the planning phase when the species is known to be present in the plan area. But also the colonisation of a project area, after the construction work started, has caused procedural problems and project delays. The latter situations raise the question how to deal with protected species in temporal habitats as a result of land development? Taking measures to prevent colonisation is common practice. But preventing colonisation might not be preferable for species that benefit from temporal situations though it offers better procedural prospects

for the project executants. Accepting the colonisation of protected species involves the risk of project delay due to legal procedures, the need for establishing mitigation or compensation plans and the need for field surveys. This results in the dilemma that nature legislation discourages the potential for temporal habitat (Woldendorp and Backes, 2005).

The presence in urban area involves mortality risks of individuals due to local traffic and construction activities. This can cause temporal breeding sites that lack connectivity to suitable terrestrial habitat to act as population sinks. Especially high mortality of juveniles during emigration can be a bottleneck (Rothermel, 2004).

This paper presents the result of a compensation project for *Bufo calamita* and proposes a list of practical measures for urban development areas.

Legislation

Species listed on Annex IV of the HD are strict protected in their natural range. It is prohibited to undertake actions that have a negative effect for their populations. The prohibitions defined in Article 12 of the HD are: all forms of deliberate capture or killing; deliberate disturbance, particularly during the reproduction season, hibernation and migration; deliberate destruction or taking of eggs from the wild; deterioration or destruction of breeding sites or resting places.

For development projects derogation of Article 12 is accepted: (1) provided that there is no satisfactory alternative; (2) not detrimental to the favourable conservation status in their natural range; (3) in the interests of public health and public safety, or for other imperative reasons of overriding public interest, including those of a social or economic nature and beneficial consequences of primary importance for the environment.

The HD has a strict implementation in the Dutch legislation. In addition the principle of 'careful handling' is defined; anyone does, within reasonable limits, what is necessary to prevent damage to protected species. For *Bufo calamita* reproduction and hibernation

sites are protected. In case of damage to reproduction waters, hibernation sites or killing of individual animals the derogation criteria should be applied. There are several examples of large-scale projects where the three criteria are valid. From criteria 2 follows that within those projects possible negative effects are mitigated and compensated. For small-scale projects the third criteria is often not valid and derogation is not accepted.

Development of business area Boekelermeer

Boekelermeer is a polder area in the western part of The Netherlands. The polder is assigned for business development to increase the regional employment. The polder area is 200 hectare and shared by three communities. The city of Alkmaar has the largest interest in the development of the polder. Historical data from the seventies showed that *Bufo calamita* occurred in the polder in low densities (RAVON unpublished data). A field survey in 2001 confirmed that *B. calamita* was still present in the polder. This implied that development of the polder leads to violation of the prohibitions defined in the HD. Because criteria 1 and 3 can be applied a project and compensation plan has been drawn up for the local Municipality (Adrichem, 2005; Van Eekelen and Smit, 2001) to maintain the Favourable Conservation Status of *B. calamita*. This plan includes other (protected) species that are present in the polder as flowers, common amphibian species, small mammals and fish. The Ministry of Agriculture, Nature and Food Quality provided dispensation in 2002 under the condition that all proposed measures in the plan would be put into practice and the results are monitored for a five-years period. The same year the construction work started. The total project period is expected to cover 10 years.

Compensation is done within the plan area and should be realised before the population in the polder is affected. The available surface for nature reduces from 200 hectare of agricultural land to 45 hectare of 'green infrastructure'. Therefore the compensation aims to improve the quality of the remaining habitat.

The project will be developed in phases. The area where most activity of calling males of *B. calamita* were observed, remains undisturbed until the compensation area is realised. This allows for spontaneous

colonisation of the new created area. Colonisation is preferred to translocation of individuals, which is often unsuccessful (Sinsch, 1998).

When planning compensation for Boekelermeer there was little known about the ecology of *B. calamita* in polder areas. The bare land after harvest and fresh cleaned ditches can be understood as equivalents of pioneer habitat. But *B. calamita* also occurs in grassland polders where pioneer stages are lacking. Because of this knowledge gap the habitat of coastal dunes, where the species is abundant, was used as a reference for *B. calamita* habitat rather than the original polder habitat. In general the original habitat can be expected to give the best reference. But because the future business area would alter the landscape completely, the artificial 'dune like' habitat is considered as acceptable. The green infrastructure is designed with a 'dry' *B. calamita* core area situated in the business area and a 'wet' zone optimised for species of grassland. The 'wet' zone is situated along the polder edge where it is in compliance with the adjacent landscape. A pilot of 1,5 ha of dry area is created in 2002. The soil was topped with sand deposits and 3 concrete ponds are created as proposed by Beebee and Denton (2001). Each pond is accompanied by a 20 meter long and 1 meter high wall of bricks covered with sand to provides shelter for vulnerable juveniles within short distance of the breeding site. Though *B. calamita* is expected to be able to colonise the pilot area within a few years (Sinsch, 1998), in October 2003 20 juveniles were collected outside the pilot area and translocated to the shelter walls. Translocation in October was expected to stimulate hibernation in the pilot area. In 2004 no activity of the species was observed in the pilot area. The first calling males were observed in 2005. That year we counted in 1 pond 5 egg strings, the larvae developed successfully (Smit, 2006). In spring 2006, about 300 subadults were counted under stones of the shelter walls. An additional area of 12 ha is created in 2004 and colonised by *B. calamita* in 2005.

Faunatunnels (ACO Pro) with guiding walls to prevent road access are realised where roads dissect the green infrastructure. The separate elements of the green



Figure 1. Artificial pond at Boekelermeer; habitat of *Bufo calamita*.

infrastructure are interconnected and connect with the landscape surrounding Boekelermeer.

Future prospects: Successful reproduction and hibernation indicates that the new created 'core' area is accepted by *B. calamita*. The area is used by a variety of birds, small mammals and plants. The present community is typical for pioneer habitat. The next years will show if the population of *B. calamita* will be sustainable. When construction starts in the old core area, translocation of the remaining local individuals will become inevitable (Marsh and Trenham, 2004). We expect translocation to new shelter sites short before hibernation to be the best approach.

Colonisation in urban area

There are several examples in The Netherlands of *Bufo calamita* colonising urban development area. It recently invaded Maasvlakte, the extensive expansion area of the Port of Rotterdam, from the coastal dunes. It invades sandy project areas of Amsterdam Harbour. At the city of Culemborg, near the river Lek, *B. calamita* is present for several years in the expanding western district. Its natural habitat is the floodplains along the river. *Bufo calamita* did not occur in these project areas before the construction activities started.

The continuing development of Maasvlakte will result in loss of recently colonised reproduction sites which conflicts with the legal prohibitions. A compensation plan has been drawn to facilitate legal procedures. This includes the creation of sustainable reproduction sites (core breeding sites) near terrestrial habitat and a set of guidelines for handling temporal waters and potential shelter sites.

Since the start of developing the western district in Culemborg no conservation measures are undertaken for flora either fauna. In the agricultural land that is used for urbanisation *B. calamita* is not present. It appears in new plots short after the first earthwork has been carried out en new drains are constructed. In the years after finishing construction activities it disappears again. The overall result is a population that shifts with the border of the expanding district. In April 2005, *B. calamita* became active in the river floodplain at 500 meter distance of the western district. In May 15, calling males were observed in a new plot in 1 of 4 ditches that were created in 2004. In May the number grew to a maximum of 25 males and moved to the other ditches and to puddles of rainwater on bare plots. Egg strings were found at two ditches next to bare plots where no construction work was yet carried out. In May and June these ditches contained thousands of larvae. The plots are bare with little debris and do not provide cover for fresh metamorphs. *B. calamita* metamorphs can leave patches used for breeding sites within a few weeks after emergence. Even small toadlets can

migrate distances of several hundred of meters within a short time (Sinsch, 1997; Stevens et al., 2004). The bare soil and lack of cover was expected to facilitate quick movements of toadlets (Stevens et al., 2004). The toadlets were considered to be most vulnerable the first weeks after emergence. When construction work starts in summertime, this could affect the fresh metamorphosed juveniles. But the work was not expected to start before the end of 2005. Therefore, it was recommended to postpone the common practise of mechanical removal of debris from the ditches before the summer holiday, to the end of August when most larvae will be metamorphosed. In August, juveniles were considered to be enough mobile to find suitable land habitat in adjacent areas and were expected to have left the direct vicinity of the water. Thus, no recommendations were given considering metamorphs or juveniles. In August, first juveniles were reported from the gardens of surrounding buildings.

Discussion

Metapopulation theory is an important concept for amphibian conservation. Marsh and Trenham (2000) emphasize the balance between metapopulation considerations and local habitat quality. Habitat connectivity is considered to play a key role in regional viability of amphibian populations. The connectivity is predominantly effected through juvenile dispersal (Cushman, 2006). Breeding sites that lack connectivity to suitable terrestrial habitat may be population sinks due to high mortality of juveniles (Rothermel, 2004). Therefore the design of compensation areas should take into account the quality of breeding sites, the location of these sites close to terrestrial habitat and measures to prevent fragmentation from surrounding populations.

The population size of *B. calamita* in the Boekelermeer was unknown. Estimations in 2001 varied from 100 to 400 individuals. Also knowledge of the effective size of habitat used by the population was lacking. An additional survey carried out in 2003 revealed a core population in the centre of the polder at an area of 6 hectare of a pot flower company. In 2005, a total number of 165 adults were counted during one night in a ditch close to the pot flower company. This was the highest number of individuals observed in the polder. This six hectare area, a glass house for horticulture and a high density of shelter sites, seems to serve as (artificial) habitat for the major part of the polder population. Therefore, the 45 hectare for compensation in Boekelermeer can be considered to be sufficient for a sustaining *B. calamita* population, taking into account that not all the area is optimal habitat and special consideration is given to prevent isolation of the Boekelermeer population.

The compensation area is a popular strolling area for business people in lunchtime. This shows that compensation measures do not necessarily conflict with the public function of green infrastructure in urban areas.

When sink populations can be avoided the common practice of preventing *B. calamita* to benefit from temporal situations in urban development is not desirable and unnecessary. The history of *B. calamita* in the urban development area of Culemborg, shows that it can survive in an area with ongoing construction activities. It appears to benefit from temporal situations, even when no conservation measures are considered. The species seems well adapted to the dynamic situations in urban development areas.

Temporal habitat can support local core populations, but the close distance to natural breeding sites deserves attention. In compliance with the legal principle of 'careful handling' a set of recommendations can be given to protect *B. calamita* in its most critical stages. Natural development of larvae and save migration of juveniles to terrestrial habitat is important to prevent temporal habitat to become population sinks. When natural development and save migration cannot be guaranteed the principle of 'careful handling' requires preventing individuals to access temporal habitat. The following recommendations are proposed: (1) if no work is planned next to water with reproduction of *B. calamita* the water remains undisturbed. Removing of debris is postponed until there are no more larvae or metamorphs present in the water. In general there are no actions necessary when no construction activity is planned between April and August; (2) if work is carried out next to water with egg strings or larvae the water is fenced in, combined with pitfalls for collecting fresh juveniles. The juveniles are translocated to a safer place outside the work area; (3) before construction work starts, debris is manually removed and checked for sheltering animals. Individuals are moved outside the work area; (4) piles of debris or construction material, as potential hibernation sites, are not cleared between October – March. Piles with stock material, which is regular manipulated, are preferably kept on beams. This way they are less suitable as sheltering sites.

It is noted that for translocation of egg strings, larvae, juveniles and adults formal dispensation from the prohibitions of the legislation is required.

The proposed recommendations are intended for handling local urban populations. The strict interpretation of the HD requires that anyone should request for formal dispensation for all actions that violate the prohibitions of the directive. This includes the removal of animals from a construction site or the removal of temporal shelters or breeding sites. The

need for formal dispensation often result in project delays. Woldendorp and Backes (2005) therefore propose a definition of 'temporal nature' and a dispensation or an exempt from the prohibitions for affecting temporal habitat. In case of *B. calamita*, the proposed recommendations combined with an exempt from the prohibitions for 'temporal nature' in development projects serve the conservation goal. If habitat with viable *B. calamita* populations is affected, dispensation will be required and implementing compensation in the new urban landscape is a realistic option. We can allow the natterjack toad to survive in urban areas.

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“Fonoteca Zoológica (www.fonozoo.com)”: the web-based animal sound library of the Museo Nacional de Ciencias Naturales (Madrid), a resource for the study of anuran sounds

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Abstract. We describe the functional structure of the Fonoteca Zoológica (FZ) and introduce its website FonoZoo.com. The animal sounds from FZ are separated into two different collections depending on their origin: the FZ Sound Collection and the Published Sound Collection. The FZ Sound Collection contains recordings obtained by researchers in the Museum and other collaborators, and the Published Sound Collection includes commercially available animal sound guides published from all over the world. Here we present statistical data on the number of species of anurans included in both collections and emphasize the usefulness of the FZ in the study of anurans.

Introduction

Animal vocalizations are useful tools that can be used in ecology, behavioural or taxonomic studies (Baptista, 1993; Tubaro, 1999; Baptista and Martínez, 2002). In addition to basic science, bioacoustics can also have applications for conservation and wildlife management (Tubaro, 1999; Baptiste and Martínez, 2002), and acoustic monitoring programs are becoming increasingly useful (Bridges and Dorcas, 2000; Terry and McGregor, 2002). Animal sound collections are therefore extremely useful to study and conserve biodiversity, and are the basis of significant contributions to the study of animal communication (Kroodsma and Miller, 1996; Baptista and Gaunt, 1997). Animal sound collections also have educational or plainly recreational purposes as they are the basic source of distribution of animal sounds. Anurans are a group of animals that rely heavily on acoustic communication, especially for reproduction, thus collections of animal sounds can be extremely useful to study this particular group.

Fonoteca Zoológica (FZ)

The Fonoteca Zoológica is the library of animal sounds in the National Natural History Museum of Madrid. This collection of sounds has recordings of all groups of animals, from birds to amphibians and mammals and, in a minor degree, insects, fishes

and reptiles; it includes two different databases: the Published Sounds Collection (as of September 2005) includes 655 sound guides, most of them CDs (348) and cassettes (167), and also LP records (137) and CD-Roms (3) too. All of them are being indexed taxonomically, but we have only about 50% currently indexed, adding up a total of 22800 sound samples from 330 volumes.

In the FZ Sounds Collection (as of September 2005) there are 212 cassettes, 127 reels and more than 40 DAT tapes deposited, most of them recorded by the staff of the Museum and other collaborators. The recordings are saved in CD or CD-Rom format after the digitization, usually at a sample rate of 44.1 kHz and a resolution of 16 bits. Almost 100 CDs, with more than 5600 sound samples, have been created from these recordings, having more than 200 cassettes waiting to be indexed.

In the FZ Sound Collection, the dominant group is amphibians, due to the fact that it is the group studied by several researchers at the FZ, all of them interested in bioacoustics. In the Published Sound Collection, the number of bird sound samples is by far the most numerous, in agreement with the fact that most of the published sound guides are dedicated to this group (510 of 655).

Anurans in FonoZoo.com

Anurans are, with birds, the most important group of vertebrates from an acoustic point of view. Traditionally, birds have been more intensively studied than anurans; this, together with the fact that, in total number of species, birds are much more numerous, explains in part why there are many more

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published bird sound guides and many more bird species recorded. But if we compare percentages only, we find that there is also a lower percentage of species of anurans recorded and included in published sound guides: Most orders of birds have at least 75% of the species included in published sound guides, versus only about 20% of all anuran species. In fig. 2 we show the number of species of anurans within each family that are included in published sound guides, a much lower percentage than the number of species of birds per order included in published sound guides (see Solís et al. 2006). This number of species is not

updated with regards of the recordings from the sound guide “The calls of the frogs of Madagascar” (Vences et al. 2006), that includes a total of 238 species from four families (Mantelliidae, Hyperoliidae, Ranidae and Mycrohylidae).

The comparative maps illustrate that there are published bird sound guides from more regions of the world than for anuran sound guides (Figure 3). Finally, for the countries or areas with available sound guides, we graph the percentage of anuran species recorded in each country compared to the total number of species (Figure 4). All the data of the FZ is available

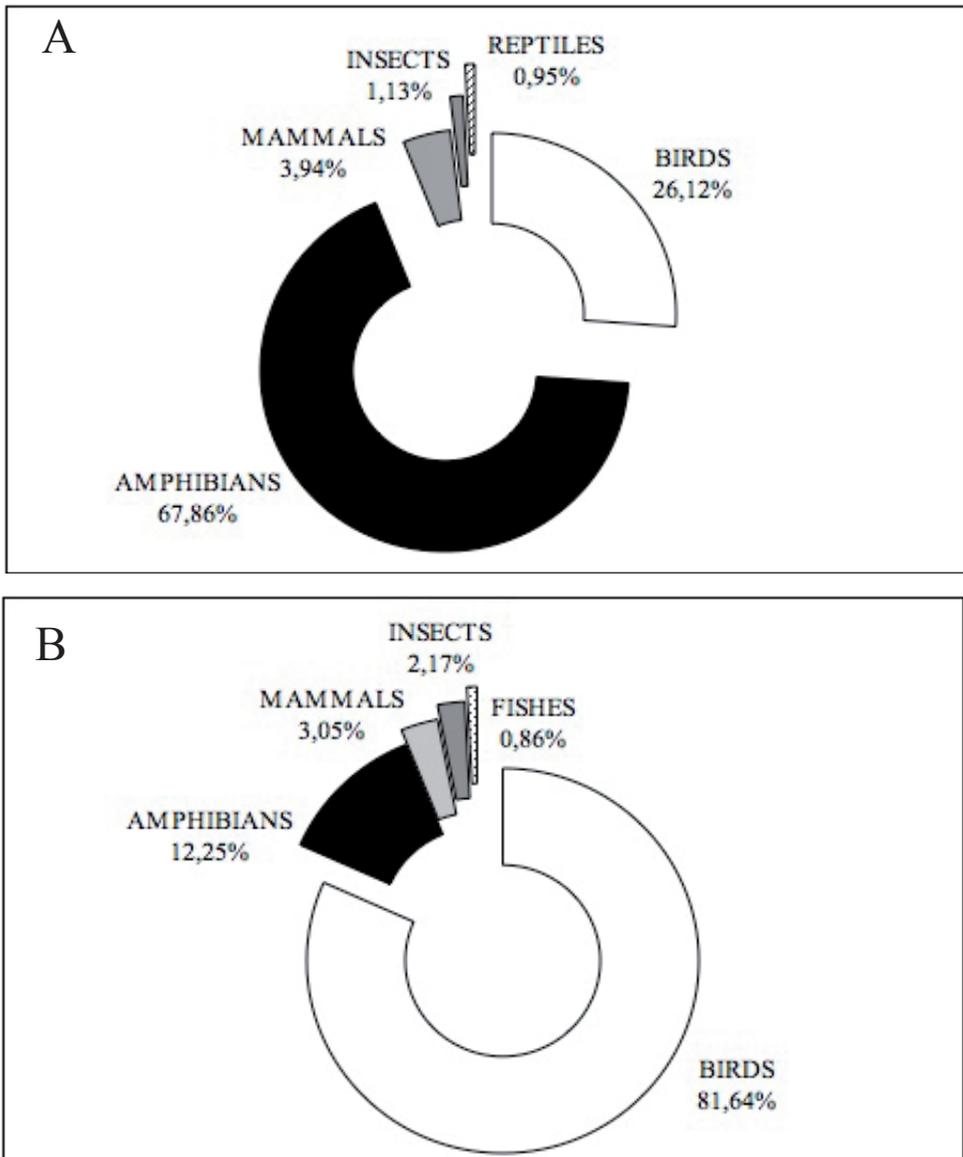
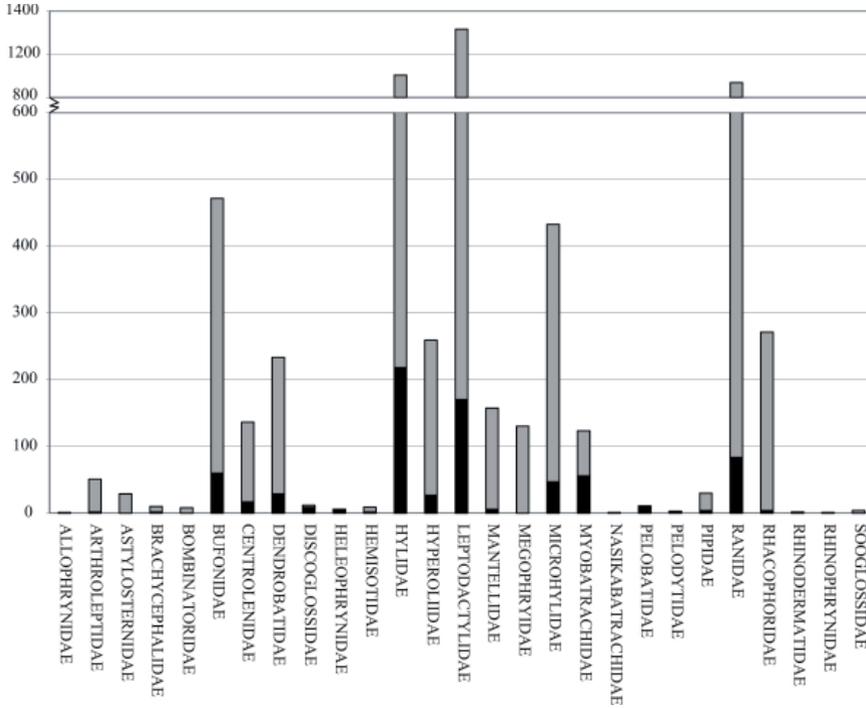


Figure 1. Distribution of recordings included in the sound library. A) FZ Sound Collection. B) Published Sounds

Figure 2. Proportion of anuran species recordings available in published guides. In black, the number of the species recorded, and in grey, the number of species not recorded.



in its user-friendly web page www.FonoZoo.com. It is the first taxonomical search engine for animal sound guides.

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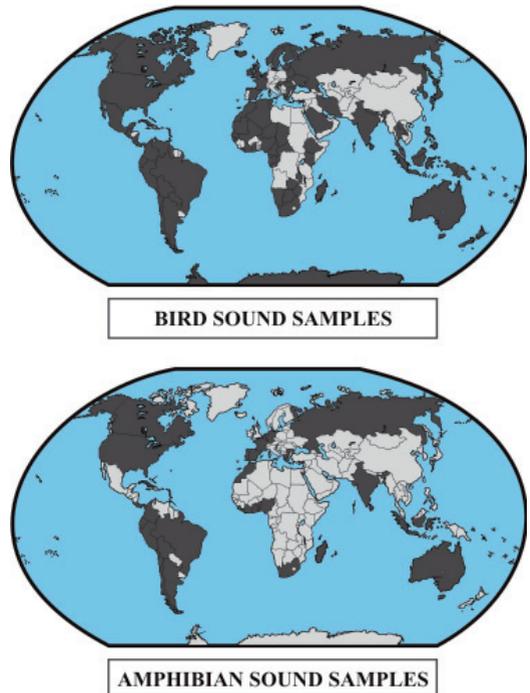


Figure 3. Comparative maps between the countries with published anuran and bird sound guides.

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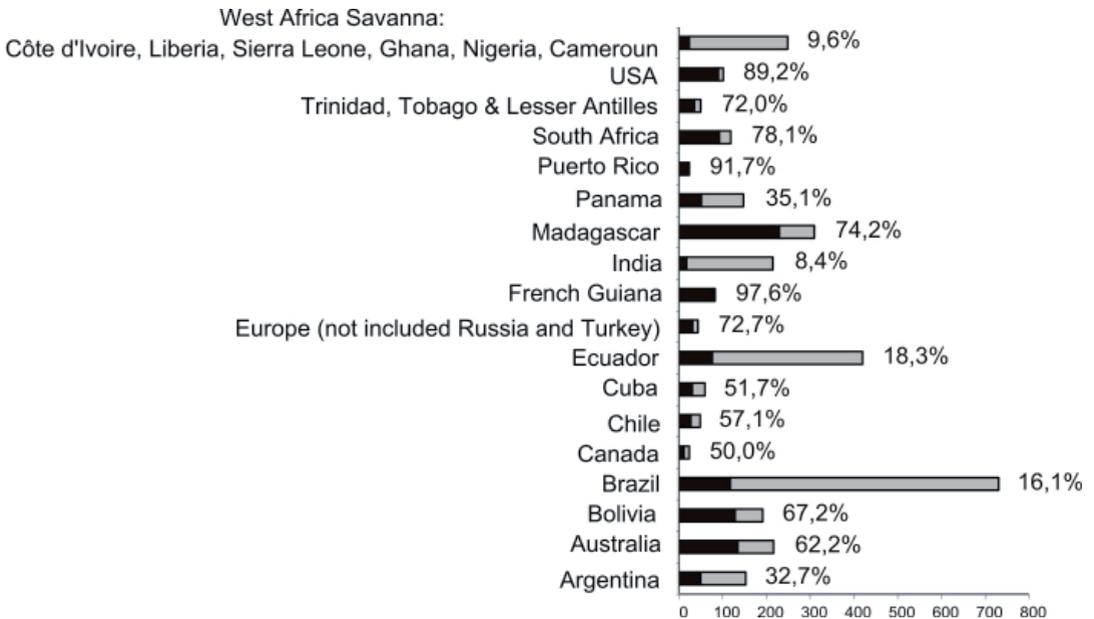


Figure 4. Anuran species recordings available in sound guides per country or region and the total number of species.

Preliminary molecular relationships of Comoran day geckos (*Phelsuma*)

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Abstract. The genus *Phelsuma* contains 42 species of diurnal, mostly green-coloured geckos with a distribution encompassing Madagascar and the Comoro, Mascarene, Seychelles, Aldabra, and Andaman archipelagos in the Indian Ocean. In a first comprehensive assessment of their biogeography we sequenced a fragment of the 16S rRNA gene from representatives of most species. Because the short DNA sequences used were unable to provide an adequate resolution of most nodes, our results remain preliminary, but several biogeographic conclusions are possible: while *Phelsuma* from the Mascarenes and Seychelles, respectively, appear to be monophyletic radiations, the four Comoran taxa were arranged phylogenetically in three separate clades, and appear to have originated from at least two separate colonizations of this volcanic archipelago.

Introduction

Day geckos of the genus *Phelsuma* are mainly confined to Madagascar and surrounding islands of the Comoros, Mascarene and Seychelles archipelagos, and Aldabra (Hallmann et al., 1997). A single species, *P. andamanense*, lives on the Andaman islands close to Myanmar. In contrast to most other geckos, *Phelsuma* species are generally diurnal. Most species are characterized by a bright green colour, although some species, such as *P. breviceps*, *P. masohoala* and *P. modesta*, are greyish. The status of *Rhoptropella ocellata*, a gecko species from the Namaqua Mountains in South Africa that had formerly been assigned to the genus *Phelsuma* is still controversially discussed (e.g., Russel, 1977; Röhl, 1998).

Due to their attractiveness to breeders, species of *Phelsuma* are important in the international reptile trade and are included in the Convention on the International Trade in Endangered Species CITES. Despite this general interest, their intrageneric phylogeny and classification is only insufficiently established. In a recent paper, Austin et al. (2004) provided a molecular analysis of relationships among 19 *Phelsuma* taxa, focusing on the relationships of the taxa from the Mascarene islands (Mauritius and Réunion). Based on sequences from

the mitochondrial 12S rRNA and cytochrome *b* genes and the nuclear *c-mos* gene, they provided evidence that these species and subspecies constitute an endemic radiation on the Mascarenes, and found *Rhoptropella* to be the sister group of the equally diurnal gecko genus *Lygodactylus* rather than *Phelsuma*.

The Comoro islands, located between Madagascar and Africa in the Mozambique channel, are a fully volcanic archipelago that has been colonized by overseas dispersal by a number of amphibian and reptile groups (Arnold, 2000; Vences et al., 2003). In the present paper, we provide new molecular data on *Phelsuma* relationships, based on mitochondrial sequences of the 16S rRNA gene. Our focus was to obtain a broad sampling of species and subspecies, and to analyse the relationships of the species from the Comoro islands in this preliminary screening of *Phelsuma* diversity.

Materials and Methods

Tissue samples (muscle; fresh or preserved in ethanol) were available from 34 *Phelsuma* taxa. We included samples of eight other gecko species for hierarchical outgroup rooting (Figure 1, Table 1). DNA was extracted using the QiAmp tissue extraction kits (Qiagen). We amplified a ca. 560 bp fragment of the mitochondrial 16S rRNA via PCR. The primers and cycling procedures were as follows: 16SA (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16SB (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3'). Initial denaturation step: 90 s at 94°C; 33 cycles: denaturation 45 s at 94°C, primer annealing for 90 s at 55°C, extension for 90 s at 72°C.

PCR products were purified using the Qiaquick purification kit (Qiagen). We sequenced fragments on an ABI 377 automatic sequencer using standard protocols. Sequences were aligned automatically using Clustal X (Thompson et al., 1997). The 16S alignment was slightly refined by eye in the loop regions.

We applied a hierarchical likelihood ratio test for the goodness-of-fit of nested substitution models (for ingroup taxa only) using the program MODELTEST (Posada and Crandall, 1998). Due to the performance of multiple tests, we adjusted the significance level of rejection of the null

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hypothesis via the sequential Bonferroni correction to $\alpha=0.01$ (Rice, 1989). We used the best-fitting model for subsequent phylogenetic analyses.

The data set was subjected to three different methods of phylogenetic reconstruction: (i) neighbor joining (NJ) (Saitou and Nei, 1987) using the specified substitution model; (ii) maximum parsimony with gaps treated as fifth character state; transitions and transversions were given equal weight; heuristic search with the TBR branch swapping algorithm; (iii) maximum likelihood (ML) analysis using the specified substitution model. All analyses were done with PAUP* (Swofford, 2002). Robustness of NJ and MP tree topologies was tested by bootstrap analyses (Felsenstein, 1985), with 2000 replicates each (Hedges, 1992). Due to computational constraints, we used Quartet Puzzling (Strimmer and Von Haeseler, 1996) with 2000 permutations to infer reliability values for ML tree topologies. To gain confidence in Quartet Puzzling, we compared the resulting tree topology and the support values to those derived from 100 ML bootstrap replicates.

We statistically compared the resulting tree topology to alternative topologies (for description see below) applying the non-parametric likelihood ratio test of Shimodaira and Hasegawa (1999) with the following likelihood settings: HKY85 substitution model, ti/tv ratio = 2, empirical base frequencies; one-tailed SH test using 1000 RELL bootstrap replicates) as implemented in PAUP* (Swofford, 2002).

Results and Discussion

The alignment consisted of 580 bp for all taxa, with 311 variable and 228 parsimony informative sites. Empirical base frequencies were $\pi_A=0.3846$, $\pi_C=0.2645$, $\pi_T=0.2247$ and $\pi_G=0.1262$. The strong anti-G bias indicates that no nuclear pseudocopies of the genes have been analysed (Zhang and Hewitt, 1996). Bases were homogeneously distributed among ingroup haplotypes (χ^2 -test: $p=1.00$). The substitution model that fits our alignment best was the Tamura-Nei model with a proportion of invariable sites of $I=0.2635$, a gamma distribution shape parameter $\alpha=0.4926$ and substitution rate $_{[A-G]} = 3.3352$ and $_{[C-T]} = 5.6206$ ($-\ln L_{TN+I+G} = 4690.2271$).

Confirming the results of Austin et al. (2004), our tree (Fig. 1) indicates (1) that the genus *Phelsuma* is a monophyletic group, (2) that the Mascarene *Phelsuma* form a monophyletic radiation, and (3) that *Rhoptropella ocellata* may form a clade with *Lygodactylus* which together is the sister group of *Phelsuma*, although this latter result was neither sufficiently supported in our nor in the previously published analysis. Also the three included Seychellean *Phelsuma* form a monophyletic group in our tree, providing evidence that they constitute an endemic radiation as postulated by Radtkey (1996). As a surprising aspect, *P. andamanense* is positioned sister to *P. breviceps*, a morphologically rather unusual short-snouted, greyish species from Madagascar. This relationship remains to be tested with more extensive

data sets; morphologically, these two taxa bear no obvious similarities.

The phylogeny among Malagasy *Phelsuma* species was virtually unresolved in our data set, as it was in the tree of Austin et al. (2004) who included more nucleotides but less taxa in their analysis. With low support *P. hielscheri* is sister to *P. berghofi*, and *P. abbotti* appears related to *P. madagascariensis* and *P. parkeri*. The latter two species form a highly supported clade and show only one percent sequence divergence. This is among the lowest levels of sequence divergence among all studied *Phelsuma* species.

The four Comoran *Phelsuma* species are scattered among the Malagasy taxa, *P. comorensis* being sister to *P. lineata*, *P. nigristriata* being sister to *P. dubia*, and *P. robertmertensi* and *P. v-nigra* forming a clade with *P. modesta*. The latter two clades are poorly supported (Fig. 1), but several alternative hypotheses of monophyly of Comoran taxa (trees 1-3 in Table 2), proved to be significantly less likely than the best ML tree, providing evidence for non-monophyly of the Comoran taxa. Although relationships of *P. nigristriata* and *P. v-nigra* are not clarified, it seems probable that *P. comorensis* is closely related to the Malagasy *P. lineata*, and therefore colonized the Comoros independently from the other two species. This is further supported by the strong morphological and chromatic similarities between *P. comorensis* and *P. lineata*.

Independent from our study, Rocha et al. (in press) have recently published a phylogeny of Comoroan *Phelsuma* based on sequences the cytochrome b and 12S genes, with a more comprehensive sampling of Comoran populations but including fewer species. They found support for a Comoran clade containing *P. v-nigra*, *P. robertmertensi* and *P. pasteuri*, and, similar to our data, a strongly supported clade containing *P. comorensis* and *P. lineata*.

Close relationships among Comoran and Malagasy taxa are obvious for many organism groups and have recently even been demonstrated for frogs that apparently have been able to colonize Mayotte from Madagascar by overseas rafting (Vences et al., 2003). Our data provide a first indication that, within *Phelsuma*, at least two independent colonization events between Madagascar and the Comoros have occurred, and that the Comoran species of *Phelsuma* - unlike the Seychellean and Mascarene ones - are probably not a monophyletic radiation. More work is necessary to identify, among Malagasy *Phelsuma*, the sister species of each of the Comoran taxa, and to reconstruct the

Figure 1. Maximum likelihood phylogram of *Phelsuma* taxa studied, based on a fragment (580 bp aligned) of the mitochondrial 16S rRNA gene. Numbers at nodes are bootstrap support values in percent, from maximum parsimony, neighbor-joining and maximum likelihood analyses. See Table 1 for localities of origin and codes of samples. Arrows mark the species from the Comoro islands.

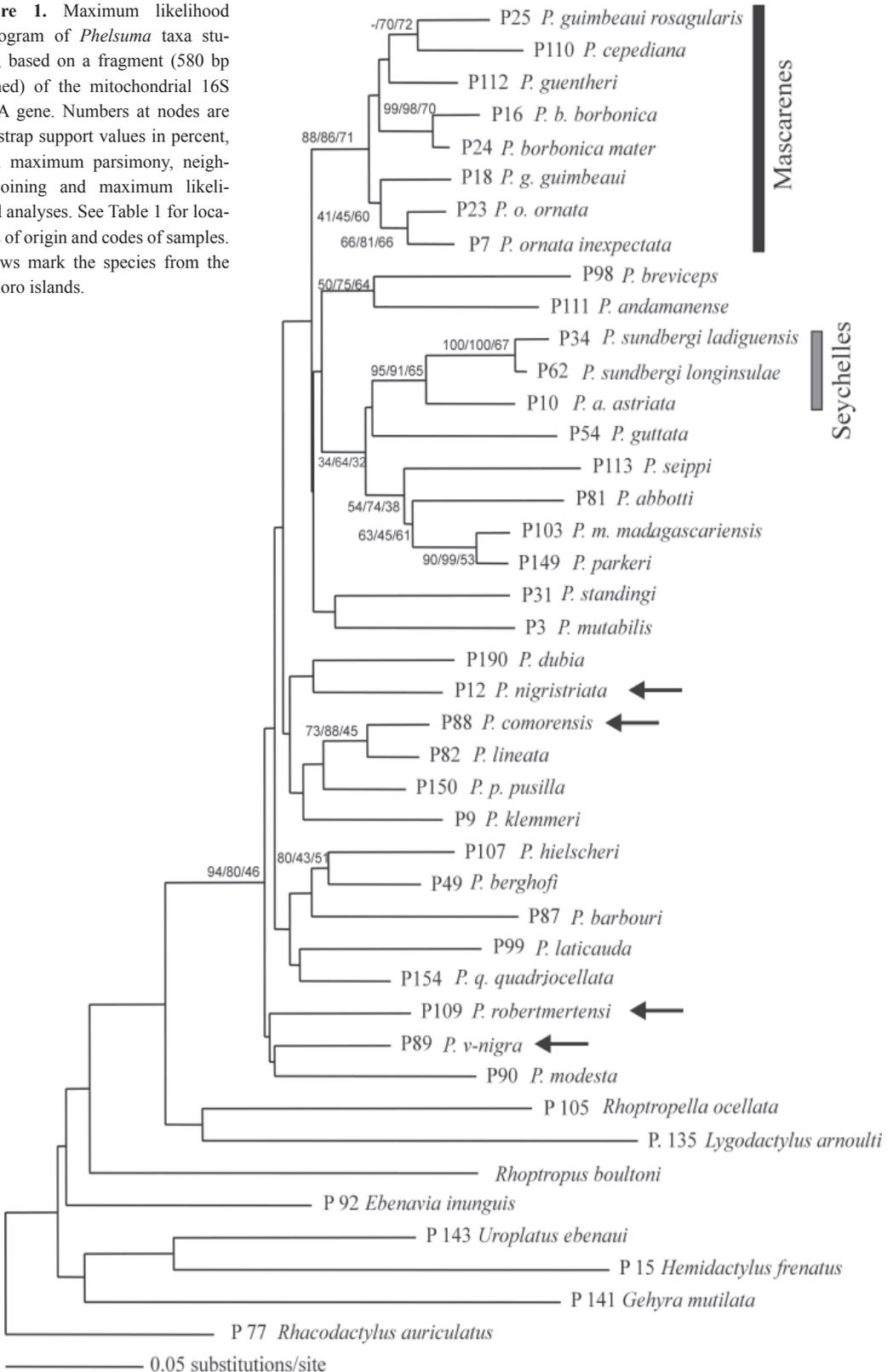


Table 1. Species sequences, voucher specimens (when preserved), localities and Genbank accession numbers for the analysed fragment of the 16S rRNA gene.

Species/Subspecies	Specimen no	Voucher specimen	Locality	Genbank accession
<i>P. astriata astriata</i>	P10	---	---	DQ270582
<i>P. abbotti chekei</i>	P81	---	---	DQ270565
<i>P. andamanense</i>	P111	---	---	DQ270559
<i>P. barbowi</i>	P87	UADBA FGMV 2000.86	Ankaratra, Madagascar	DQ270561
<i>P. borbonica borbonica</i>	P16	---	Le Brule, Reunion	DQ270554
<i>P. borbonica agalegae</i>	P24	---	Basse Vallee, Reunion	DQ270574
<i>P. berghofi</i>	P49	---	Somisiky, Madagascar (23°23'S, 47°32'O)	DQ270556
<i>P. breviceps</i>	P98	UADBA FGMV 2000.564	Arboretum Toliara, Madagascar	DQ270551
<i>P. cepediana</i>	P110	---	Mauritius (highlands)	DQ270563
<i>P. comorensis</i>	P88	ZSM 691/2000	Ivembeni, Grande Comore	DQ270552
<i>P. dubia</i>	P190	---	Comoros	DQ270550
<i>P. guentheri</i>	P112	---	---	DQ270581
<i>P. guimbeaui</i>	P18	---	Grande Case Noyale, Mauritius	DQ270557
<i>P. guimbeaui rosagularis</i>	P25	---	Gorges de la Riviere Noire, Mauritius	DQ270549
<i>P. guttata</i>	P54	---	---	DQ270575
<i>P. hielscheri</i>	P107	---	Morondava, Madagascar	DQ270555
<i>P. klemmeri</i>	P9	---	Type locality, Sambirano, Madagascar	DQ270566
<i>P. laticauda</i>	P99	ZSM 554/2000	Sambava, Madagascar	DQ270578
<i>P. lineata</i>	P82	ZSM 336/2000	Vohidrazana, Madagascar	DQ270570
<i>P. madagascariensis</i>	P103	ZSM 559/2000	Sambava, Madagascar	DQ270569
<i>P. modesta</i>	P90	ZSM 586/2000	Ifaty, Madagascar	DQ270572
<i>P. mutabilis</i>	P3	---	Beroroaha, Makay	DQ270573
<i>P. nigristriata</i>	P12	---	Mayotte	DQ270562
<i>P. parkeri</i>	P149	---	Pemba, Mozambique	DQ270576
<i>P. pusilla pusilla</i>	P150	---	---	DQ270579
<i>P. ornata inexpectata</i>	P7	---	Manapany les Bains/Reunion	DQ270577
<i>P. ornata ornata</i>	P 23	---	Grande Riviere Noire, Mauritius	DQ270567
<i>P. q. quadriocellata</i>	P154	---	Ranomafana, Madagascar	DQ270580
<i>P. robertmertensi</i>	P109	---	Mayotte	DQ270553
<i>P. seippi</i>	P113	---	---	DQ270560
<i>P. standingii</i>	P31	---	South of Isalo, Madagascar	DQ270564
<i>P. sundbergi longinulae</i>	P62	---	Mahé, Seychelles	DQ270568
<i>P. sundbergi ladiguensis</i>	P34	---	La Digue, Seychelles	DQ270558
<i>P. v-nigra</i>	P89	ZSM 714/2000	north of Nioumamilima	DQ270571
<i>Ebenavia inunguis</i>	P92	ZSM 442/2000	Nosy Be, Madagascar	DQ270585
<i>Gehyra mutilata</i>	P141	UADBA FGMV 2000.407	Antananarivo, Madagascar	DQ270588
<i>Hemidactylus frenatus</i>	P15	---	Beroroaha/Makay, Madagascar	DQ270589
<i>Lygodactylus arnouliti</i>	P135	ZSM 394/2000	Ibity, Madagascar	DQ270587
<i>Rhacodactylus auriculatus</i>	P77	---	New Caledonia	DQ270584
<i>Rhoptropella ocellata</i>	P105	---	South Africa	DQ270583
<i>Uroplatus eburni</i>	P143	---	Berara, Madagascar	DQ270586

routes of colonization between Madagascar and the various islands of the Comoro archipelago.

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Table 2. Alternative trees with monophyletic arrangement of the four *Phelsuma* species from the Comoro islands, and the statistical probability with which these were rejected in SH-tests.

	Tree topology (taxon names from Fig. 1 abbreviated)	P in SH-test
Comoroan taxa monophyletic on branch leading to <i>P. nigristriata</i>	((((((((((((P25_P_g_ro,P110_P_cep),(P16_P_b_bo,P24_P_b_ag),(P18_P_gimb,(P23_P_o_or,P7_P_o_ine))))),P112_P_gue),P31_P_stan),(((P34_P_s_la,P62_P_s_lo),P10_P_a_as),(P113_P_sei,(P81_P_abot,(P103_m_ma,P149_P_par))))),P54_P_gutt),P3_P_mutab)),(P98_P_brev,P111_P_and)),(((P190_P_dub,P107_P_hie),P154_P_q_q),(((P88_P_como,P12_P_nigr),P89_P_v_ni),P109_P_rob),P90_P_mode)),(P49_P_berg,P87_P_barb))),(((P82_P_line,P150_P_p_p),P9_P_klemm),P99_P_lati)),(Potu,Lygo)),R_boultoni),Ebenavia),(Uroplatus,Hemidact)),Gehyra),Rhacodact)	P = 0.003
Comoroan taxa monophyletic on branch leading to <i>P. comorensis</i>	((((((((((((P25_P_g_ro,P110_P_cep),(P16_P_b_bo,P24_P_b_ag),(P18_P_gimb,(P23_P_o_or,P7_P_o_ine))))),P112_P_gue),P31_P_stan),(((P34_P_s_la,P62_P_s_lo),P10_P_a_as),(P113_P_sei,(P81_P_abot,(P103_m_ma,P149_P_par))))),P54_P_gutt),P3_P_mutab)),(P98_P_brev,P111_P_and)),(((P190_P_dub,P107_P_hie),P154_P_q_q),P90_P_mode),(P49_P_berg,P87_P_barb))),(((P88_P_como,P12_P_nigr),P89_P_v_ni),P109_P_rob),P82_P_line),P150_P_p_p),P9_P_klemm),P99_P_lati)),(Potu,Lygo)),R_boultoni),Ebenavia),(Uroplatus,Hemidact)),Gehyra),Rhacodact)	P = 0.000
Comoroan taxa monophyletic on branch leading to <i>P. v-nigra</i>	((((((((((((P25_P_g_ro,P110_P_cep),(P16_P_b_bo,P24_P_b_ag),(P18_P_gimb,(P23_P_o_or,P7_P_o_ine))))),P112_P_gue),P31_P_stan),(((P34_P_s_la,P62_P_s_lo),P10_P_a_as),(P113_P_sei,(P81_P_abot,(P103_m_ma,P149_P_par))))),P54_P_gutt),P3_P_mutab)),(P98_P_brev,P111_P_and)),(((P190_P_dub,P107_P_hie),P154_P_q_q),P90_P_mode),(P49_P_berg,P87_P_barb))),(((P88_P_como,P12_P_nigr),P89_P_v_ni),P109_P_rob),P9_P_klemm),P82_P_line),P150_P_p_p),P99_P_lati)),(Potu,Lygo_sp)),R_boultoni),Ebenavia),(Uroplatus,Hemidact)),Gehyra),Rhacodact)	P = 0.002

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A new record of *Vipera ursinii* (Reptilia: Serpentes) from Macedonia

Bogoljub Sterijovski

Abstract. *Vipera ursinii* is recorded from the Bistra Mountains in Macedonia. In addition to the Shar and Korab Mountains, this new record represents the third known locality for the species in the country.

The distribution of *Vipera ursinii* in Macedonia includes western parts of the country, exclusively. The species was first recorded by Doflein (1921) from Kobilica in the Shar Mountains. This site was also mentioned later by Buresch & Zonkow (1932; 1934), Karaman (1938; 1939) and Radovanović (1951). Dimovski (1964) recorded *V. ursinii* at the Korab Mountains (Kobilino). The Shar Mountains in the north-western part of the country range from the Serbian border in the north to the Albanian border in the west. The Korab Mountains spread from the edge of the Shar Mountain to the south, along the Abainan border, with an average altitude of more than 2500 meters a. s. l.

The above mentioned localities were the only records of *V. ursinii* for Macedonia for the last 60 years. During field research in 2003, a new locality was discovered at the Bistra Mountains: Bistra planina, UTM coordinates DM 80-81, National Greed UTM 10 x 10 km Reference) (Figure 1.). This locality represents the southernmost point of distribution of *V. ursinii* in Macedonia.

The Bistra Mountains are part of the Scardo-Pindic group of mountains and extend through the western parts of Macedonia. The highest peak is Medenica with 2163 m a.s.l. The relief is glacial and there are remnants of glaciers and cirques. There are also karstic elements. Vegetation at the mentioned locality constitutes *Calamintho grandiflorae* - *Fagetum* in the lower parts (below 1600 m a.s.l.) and high mountain pastures in the upper parts (above 1600 m a.s.l.). The climate of this region can be considered as continental with cold and wet winters

and warm and dry summers. It is characterized by an average annual temperature of 7 °C (from -2.2 °C in January to 16.3 °C in July), with an annual precipitation of about 1103 mm and 70% relative humidity throughout the year (Lazarevski, 1993).

The habitat of *Vipera ursinii* populations from the Bistra Mountains is situated between 1650-1900 m a.s.l. (approximately). The biotopes are predominantly grassy and represent typical mountain pastures with scattered stone piles and several sites with bushes of *Juniperus communis* and *Vaccinium myrtillus* (Fig. 2.).

Giving the fact that *Vipera ursinii* is included in the CITES list and considered to be endangered in the IUCN Red List, we found that urgent conservation measures are required at national and regional level. But first, more precise data about distribution range, population status and threats in Macedonia are urgently needed.

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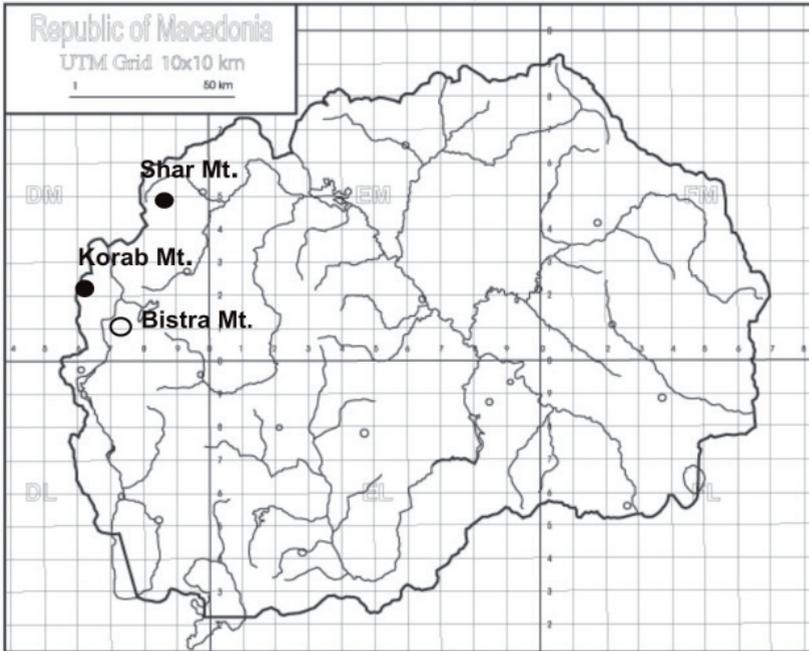


Figure 1. New locality of *Vipera ursinii* in Macedonia (UTM coordinates DM 70-71, National Greed UTM 10 x 10 km Reference) – open circle; previously recorded localities – black circles.



Figure 2. Habitat of *Vipera ursinii* in the Bistra Mountains.

Motor activity of amphibian larvae - from schools to shoals

Galina S. Surova

Abstract. The ability of *Bufo bufo* and *Rana temporaria* tadpoles to form aggregations was studied. In natural ponds, we distinguish different kinds of aggregations and discuss causes of their origin. In the lab, series of tests were carried out to estimate differences in motor activity of single individuals as well as groups of 5 and 30 individuals. In *R. temporaria* tadpoles, the social form of aggregations in nature seems to be connected with thermoregulation with individuals moving independently in a vertical mode. In contrast, *B. bufo* tadpole movements are directed and result in polarized shoals. In laboratory tests, larvae of both species differ by the character of motor activity with respect to the presence of conspecific individuals: tadpoles of *B. bufo* reduce and *R. temporaria* tadpoles increase activity. *B. bufo* tadpoles spend more time close to other conspecific larvae than *R. temporaria* tadpoles and show greater disposition to form aggregations.

Introduction

Larvae of a number of anuran species are known to form different kinds of aggregations. In some cases, these look like an irregular distribution of individuals with permanent rotation (schools), whereas others form larger groups with directed moves and one-way orientated polarized shoals (Beiswenger, 1975; Wassersug et al., 1981). The reasons for these aggregations could be different: external factors, e.g., food, temperature, anti predator behaviour, hydrodynamic features, etc. (Wassersug, 1973; Branch, 1983; Spieler and Linsenmair, 1999) or internal factors, like thermoregulation or preference of familiar individuals (O'Hara and Blaustein, 1981; Surova, 1988; Caldwell, 1989). Wassersug (1973) recognized simple and social aggregations. The first one is based on taxis only (biotic and abiotic) and the reason of its formation is obvious. The second one is based on social mutual attraction. The existence of social aggregations implies an importance of the group as an entire system. Any system implies the existence of some features qualitatively different compared to separately taken elements (Prigogine and Stengers, 2000). Maintenance of integrity in particular biological systems requires rather intensive interactions between its members. Such an approach proposes a new interpretation of the importance of groups in tadpoles. Unfortunately, behavioural analyses of tadpoles are sparse (Lehner, 1996). In this study we characterize the behaviour of tadpoles of two anuran species by

describing their motor activity. The purpose of the study is to examine the alterations in motor activity of *Bufo bufo* and *Rana temporaria* tadpoles in natural ponds and in the lab with and without the presence of conspecifics and/or predators.

Material and methods

The study in natural ponds was conducted at the biological station of Moscow State University in Zvenigorod. The pond with *B. bufo* is located in the forest and had a total surface area of 800 m². The pond with *R. temporaria* is located in flood-land and its size was 1500 m². Abundance was estimated using mesh traps (0.2 m²), fixed with fishing-line to floats. In random samples we recorded the daily motor activity of tadpole aggregations in the ponds and estimated the density of tadpoles in aggregations. Temperature inside and outside aggregations were measured at 2-3 points 10-15 cm apart from each other. Laboratory experiments were done in aquariums (40 x 20 x 30 cm) with 5 cm water level. The bottom was lined with a 36 square grid, each four centimeters wide. After 5 min of adaptation, the squares crossed by tadpoles and the time of stops were registered during a 5 min period. Using these parameters, we recorded the tracks of larvae, the total distance of movement, and the total time spend with movement. We compared motor activity manifested by single tadpoles which were put in aquariums either in groups of 5 or 30 conspecific individuals or alone. Predator experiments were done using large larvae of *Dytiscus marginalis* which were exposed in a small sack to groups of 30 tadpoles. In experiments on vertical activity water level was increased to 20 cm. The observer searched for individuals in a group of 10 and recorded the number, duration and depth of diving incidents. Water temperature in aquariums was 18-20°C. Five replications were carried out for each series of experiment. Significance was estimated by a Mann-Whitney U-Test.

Results

Field study

Aggregations of both species started to form at about 9:00-10:00 h near the first light spot where the water temperature seems to be a little higher compared

Table 1. Motor activity of tadpoles in vertical water column (** = $P < 0.01$).

(mean \pm SE)			
Species	Swimming on the surface (sec)	Duration of diving (sec)	Number of diving
<i>Bufo</i>	166.7 \pm 5.16**	13.0 \pm 227**	5.7 \pm 0.93**
<i>Rana</i>	20.0 \pm 5.91	6.2 \pm 0.30	17.3 \pm 0.26

to other parts of the pond. Later during the day a correlation between tadpole aggregations and sun light was not observed. As the water in the pond was warming up aggregations formed along the shore line and subsequently in the water column. At any time, aggregations included groups from few dozens to several thousand individuals which aggregated around food sources (e.g., water plants, surface of snags where tadpoles eat epibioses, remains of invertebrates, detritus). This kind of aggregation is easy to identify by gnawing moves of tadpoles mouthparts. Aggregations with similar density formed in the warmest parts of the ponds. Here tadpoles did not move at all or they did it slowly in an irregularly shift from the periphery to center. Typical aggregations included those along the shore line before metamorphosis. Tadpoles stayed there the whole day but at night moved to deeper water. The reasons for these three different types of aggregations are almost obvious.

Besides that, both species formed large aggregations in the water column without obvious reasons. These aggregations had an irregular globe shape, sometimes a diffuse shape. They suspended for 10 to 60 min, sometimes longer. Then aggregations completely split or spilled over different parts of the pond. Individuals moved inside aggregations and others out. As a result, we observed that the composition of this aggregation was not constant over time. In *R. temporaria* 30% substitutions happened during 10-20 minutes. The number of aggregations per pond varied from two up to dozens. Sometimes, app. 80% of the population was located in aggregations that occupied about 1.5% of the total pond area, with the density of individuals inside aggregation being several times higher than the average density in the pond (10 and 0.09 individuals/l, respectively). Temperature data show that aggregations may have a pronounced thermoregulatory function (Fig. 1). During cloudy days the temperature inside aggregations is higher compared to ambient ones, but in sunny days vice versa. We suppose that permanent moves of tadpoles up and down create convective streams.

It may optimize the temperature regime and improve aeration. It becomes especially important in ditch-water with high probability of over heating and suffocation. This phenomenon has already been described for fish shoals in ditch-water (Gerasimov, 1983).

Bufo bufo had a constant aggregation structure and tadpoles moved in polarized shoals as a whole group. There were several or just one shoal of toad tadpoles in the pond. Shoals may form globes or spirals to form bands again. Such a band might be up to 12 m long and 0.5 m in diameter. It included practically the whole population of the pond (about 96,000 individuals). Some tadpoles left the shoal and came back but rotation was not active as in thermoregulatory aggregation. Average speed of single tadpoles was 2.4 cm/sec while in shoals it was 3.4 cm/sec ($n = 10$, $P = 0.005$). Shoals obviously increased the capacity of tadpole movements.

Laboratory tests

Figure 2 shows changes in registered parameters of motor activity (distance, time of active moves). Toad tadpoles demonstrate maximum values for single maintenance. After five individuals had been added the activity decreased and after adding 30 animals it sharply goes down. The exposition of a predator did suppress the activity even more and the tadpoles looked congealed. On the contrary, tadpoles of *R. temporaria* were more active in groups of 30 individuals, whereas the activity got lower when only five animals were present in the aquarium and reached its minimum when one tadpole stayed alone. The exposition of the predator suppressed tadpole activity in the group. Thus, both species react completely different on the presence of conspecific neighbours. After each test with the 30 tadpoles groups, we calculated the number of tadpoles located in each grid cell at the bottom and estimated the portion of grouped tadpoles compared to their total number. The portion of crowding tadpoles of *Bufo* was 57%, that of *Rana* was 24% ($P = 0.0007$). One hour subsequent to the tests, we documented the distribution of tadpoles within the aquarium. *Rana* tadpoles were evenly distributed at the bottom of the aquarium while *Bufo* tadpoles were crowded in the corner.

Table 1 shows the results of vertical activity tests. *B. bufo* tadpoles spent more time than *R. temporaria* close to the water surface - they rarely dive but stay longer under water. Visual observations indicate that *Bufo* tadpoles prefer upper levels and swim in groups along the aquarium walls. In contrast, frog tadpoles were mostly ungrouped in all water columns.

Discussion

Our study in natural ponds shows that for *R. temporaria* tadpoles the most effective form of integration is thermoregulatory aggregations (schools) with permanent vertical activity and non-stable composition. In *B. bufo* more organized and long-living units (shoals) with directed movements of the entire group and distinct polarized orientation of individuals prevail. In conclusion, *Bufo* tadpoles obviously interacted. The same shoal type with directed orientation was documented for *Xenopus*, *Scaphiopus*, *Rhinophrynus* and *Phyllomedusa* (Stuart, 1961; Wassersug, 1973; Beiswenger, 1977; Katz et al., 1981; Branch, 1983). In species of *Hyla*, *Rana*, *Pseudacris* and some others very large aggregations (up to several thousand individuals) were reported, but these did not show directed movements (Bragg, 1968; Wassersug, 1973; Caldwell, 1989).

Simple taxis (food, temperature, oxygen) may result in aggregations as a sum of individual responses to an environmental gradient. Movements of animals in such kind of aggregations are not adjusted to each other and do not lead to the appearance of a new function. The types of aggregation in *Rana* and *Bufo* described above differ clearly from this and contain a social component where individuals mutually interact (Wassersug, 1973). As a reason to form non-polarized social aggregations heat accumulation by masses of dark coloured animals and coordination of tail moves during feeding was assumed. This results in directed water flow or stirring silt at the bottom that makes food resources like detritus and plankton available (Beiswenger, 1975). We found another function of non-polarized aggregations, namely active control of the temperature regime. Mutual vertical shifts in aggregations as well as flow of food items require coordination of behaviour and provide an obvious advantage for individuals.

The existence of long-life polarized shoals has to be explained as well. Short distance between animals and a one way direction create very mobile structure, which is able to move quickly. That is why this type of aggregation (especially in *Bufo*) usually is connected with predator escape behaviour (Altig and Christensen, 1981; Spieler and Linsenmair, 1999; Richardson, 2001). Nevertheless, formation of shoals can not impute just utilitarian significance. In our ponds there are not predator fishes and carnivorous insect larvae are rare (we never found them in the center part of the ponds and just occasionally along shore line - note that aggregations mostly are formed at the center). Taking into consideration tadpole and insect distribution, as well

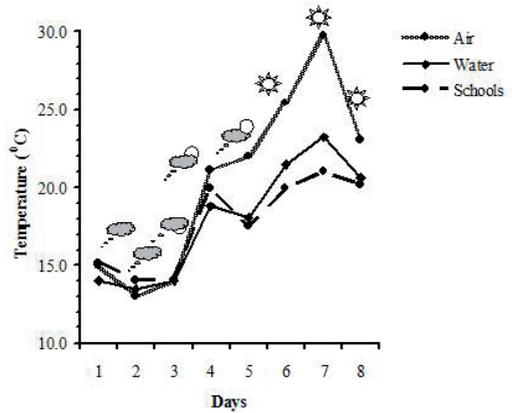
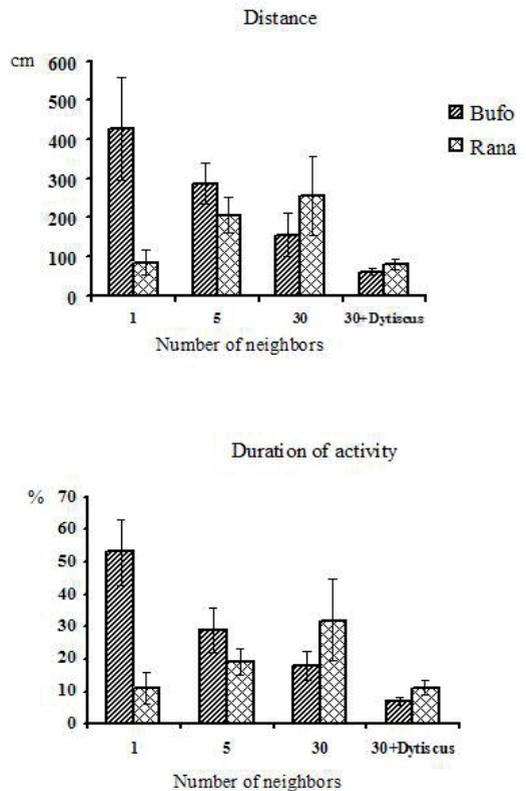


Figure 1. Temperature dynamics in *Rana temporaria* schools and ambient environment.



as total number of tadpoles (at the moment of the study **Figure 2.** Motor activity of tadpoles in differently sized groups and with the presence of predators (larvae of *Dytiscus marginalis*).

the number of tadpoles was estimated to be 100,000), predator pressure can not be a significant factor for distribution and behavioural changes. Our laboratory tests with predators showed decreases of motor activity but did not increase the rate of aggregation formation.

We suppose that shoals of *Bufo* tadpoles do not have significance to supply particular biological functions but carry important information about population condition in the pond. It was demonstrated for fish shoals (Gerasimov, 1983) that living in group greatly decreases oxygen consumption compared to isolated individuals conducted to quite behavior. We noted this phenomenon in our tests with toad tadpoles. We conclude that different reactions to the group size in *Bufo* and *Rana* tadpoles (declining motor activity in groups of *Bufo* and increasing one in *Rana*) reflect different capabilities of studied species to form intra-population aggregations – schools and shoals.

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Dynamics in the sand lizard (*Lacerta agilis*) population at Forteiland, IJmuiden, The Netherlands

Hanneke van Bree, Rosa Plantaz, Annie Zuiderwijk

Abstract. In 2000, an isolated population of sand lizards (*Lacerta agilis*) was discovered on an island in the Netherlands. The population-size was estimated at 57 adult individuals. Subsequently, the area has been improved to make the habitat more suitable for sand lizards. After 5 years of monitoring the population-size was estimated again. There are no indications for major population-size changes, for the population-size is estimated at 59 adult individuals. There is no significant difference in the population structure, although males were more abundant in 2005 than in 2001. Males outnumbered females in both years. The newly made dunes are populated by males, females and sub-adults. Gulls do not seem to conduct a predator stress, as there were no lizards seen there with autotomy. Nevertheless, gulls are believed to negatively influence habitat quality for the lizards. On the short term, cats seem to be the biggest threat to the sand lizards as there are strong indications that they have killed 10 % of the whole population within six weeks.

Introduction

A small island (4 ha) in the mouth of the North Sea Channel inhabits a population of sand lizards that lives isolated from the neighbouring dune-populations since the construction of the North Sea Channel about one hundred years ago.

The population-size was estimated at 57 ± 13 (mean \pm SD) adult individuals in 2001 (Boere et al., 2001). Genetic analysis showed clear indication for isolation and inbreeding (Boere et al., 2002). Habitat alterations in favour of the sand lizard were made as a compensation for economic activities since 2001 (Zuiderwijk, 2002). Possible threats nowadays are: (1) increasing numbers of visitors to the island; (2) loss of habitat for sand lizards; (3) increasing numbers of breeding gulls in the newly created habitats; three wild cats living on the island and preying on lizards.

It was our aim to make a population-size estimation in 2005 and compare these results with former data to see if there has been any change in population size and population structure. Other questions were: (1) Do the lizards use the area that was created especially for them? (2) Are the breeding gulls or the wild cats a direct threat to the lizards?

Population-size estimation

Capture-recapture was based on photos of the unique back pattern of adult lizards. Peterson's method (Begon, 1979) was used: $N = M(n+1) / (r+1)$

N = Estimated total population size

M = Number of already identified animals

n = Number of observed and captured animals in that particular observation day/period

r = Number of recaptures in "n"

Results

The whole island was searched on 13 days during the period 5th of May – 8th of June 2005 (table 1, 2). In 2005, the estimated population size of Sand lizards on the Forteiland was estimated as 59 ± 17 adults (table 2). In 2001, this was 57 ± 13 adults (Boere et al., 2001). The sex-ratio was highly skewed towards males. The ratio male/female 1:1 was rejected using the Binomial test ($p = 0.002$; fig. 2). Also in 2001, relative more males than females were observed, although to a lesser extent (fig. 3).

Fig. 1 shows how the population is distributed over the island. Most lizards were seen on the southern part on southern exposed slopes of the Fortress and Pillboxes. Also the new dunes were occupied, in spite of the presence of a colony of breeding gulls in this area (mainly by herring gulls, *Larus argentatus*).

Discussion

Population structure

157 lizards were observed of which 84 were males, 24 females, 38 sub-adults and 11 adults of whom the gender was unknown. The most outstanding feature (table 1) is the significant high rate of observed males. This ratio can partly be explained by the sampling period. Males are easier to observe at the beginning of the mating season in April and May, while females are easier to observe in June. In that case random sampling of both, males and

Table 1. List of all observations and numbers of different photographed lizards.

Numbers:	Males	Females	Unknown sex	Subadults	Total
Observations	84	24	11	38	157
Photographed individuals	32	11		11	54

Table 1. Estimated population size, Standard deviation (SD) and Relative Standard deviation for each observation day and for two periods (5th of May -23rd of May and 25th of May - 8st of June), following Peterson-Bailey's Method. In bold the two best estimates.

Observation day	M	n	r	N	SD	Rel. SD
5 th of May		2				
10 th of May	2	1	0	4,0	2,0	50,0
12 th of May	3	7	0	24,0	15,9	66,1
13 th of May	9	2	1	13,5	4,5	33,3
18 th of May	10	1	0	20,0	10,0	50,0
19 th of May	11	5	2	22,0	7,8	35,4
23 rd of May	14	2	1	21,0	7,0	33,3
25 th of May	15	8	5	22,5	4,9	21,8
26 th of May	18	3	1	36,0	14,7	40,8
27 th of May	20	3	0	80,0	49,0	61,2
28 th of May	23	10	3	63,3	22,6	35,7
1 st of June	30	9	3	75,0	26,0	34,6
8 st of June	34	6	3	59,5	17,4	29,3
Two periods:						
5 th -23 rd of May		16				
25 th of May-8 st of June	15	30	7	58,1	16,7	28,7

females was not done. However relatively more females than males were recaptured: 18 captures of 11 females versus 45 captures of 32 males. Thus, the recapture rate was higher in females(1.6) than in males(1.4). This supports that there are indeed significantly more males than females.

From a DNA-analysis it became clear that the population on the Forteiland is inbred (Boere et al., 2002). In sand lizards females are the heterogametic sex

(ZW), not males (ZZ). In that situation the production of daughters is associated with an increased risk of offspring inviability due to the expression of paternal, detrimental recessives on the Z chromosome. Inbreeding (and outbreeding) can cause offspring sex ratios biased towards sons (Olsson et al., 2005; Berglind, 2005). It is possible that this phenomenon is happening on the Forteiland.

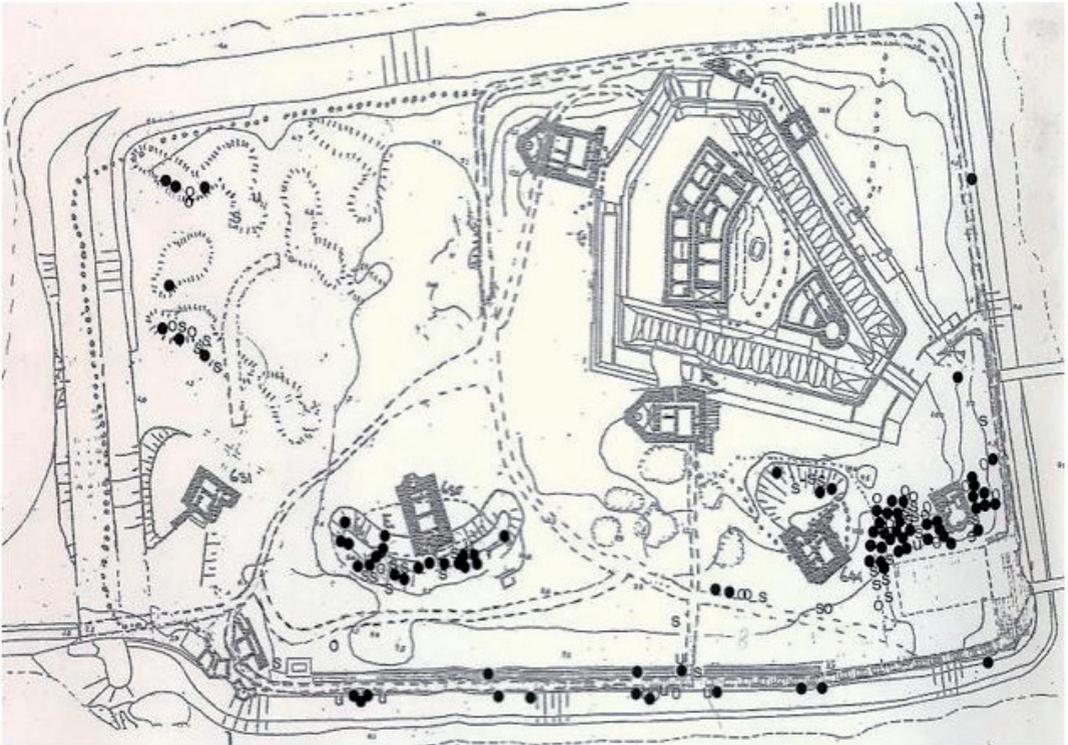


Figure 1. Forteiland IJmuiden. Spots are indicated where lizards were observed in 2005. Black dots are males, open circles females and subadults were given an “s”. The big building (NE) is the fortress. Other smaller buildings are pillboxes. The northwestern quarter of the island has the newly built dunes.

Population size and predators

The population, in between 44 and 76 adult sand lizards (13 per ha), has been stable since 2001. Positive and negative alterations occurred since then. Negative is the increased pressure of predators and human activities. Positive is the laying-out of 1 ha of dunes. Sand lizards occupied them, however more animals did so as well. 140 pairs of gulls were breeding there in 2005. Gulls are said to be predators of lizards but this is highly rarely

observed by bird-catchers (Zotaar, 2005, pers. com.) nor was it observed by us. The percentage of autotomy we found in the new dunes was much lower than the 33% that we found in general on the island. We presume that the gulls have another negative effect, namely in reducing the suitable habitat for the lizards. The faeces of the gulls enriches the soil and makes it less suitable for European beach grass (*Ammophila arenaria*). Open

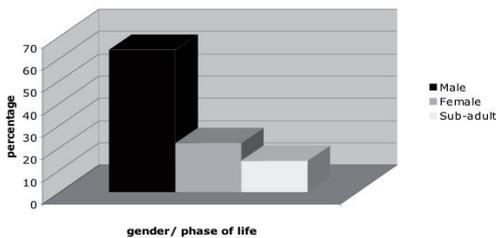


Figure 2. Relative occurrence of marked males, females and sub-adults on the Forteiland in 2005.

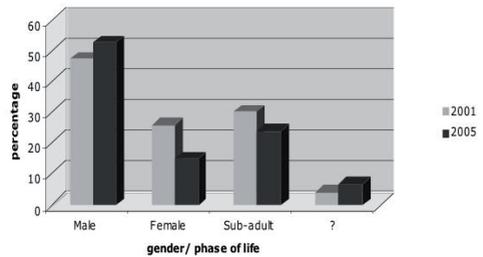


Figure 3. Comparison of the relative number of males, females, sub-adults and unknown gender for the years 2001 and 2005

spots get overgrown faster and nesting sites disappear quicker.

Cats were recognised as the most important predators. Four cats occur on the small island. They are fed by people but three of them hunt in the wild mostly. Six dead lizards were found during the six weeks of research, which is 10% of the whole estimated population. Cats were most probably the cause of death, because two times a cat was seen with a lizard in its mouth and the other cadavers were found on typical cat spots and recognised as cat prey.

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Capture and recapture of Grass snakes near Amsterdam

Jierry van Roon, Iris Dicke, Rosalie Brinks, Annie Zuiderwijk, Ingo Janssen

Abstract. Within the Grass snake (*Natrix natrix*) metapopulation near Amsterdam several sub-populations were investigated using mark-recapture methods in order to examine the effects of conservation measures and habitat destruction/degradation. Of the three sub-populations included in this study, two have increased in numbers and one showed a decline. This study shows how Grass snakes can profit from conservation measures and are affected by habitat-degradation within a short timespan.

Introduction

Along the shores of lake IJmeer, the south-western corner of Lake IJsselmeer, the Grass snake, *Natrix natrix*, borders Amsterdam. Snakes live here in several locations connected by dikes and the lake itself, resulting in a network of patches, i.e. in a metapopulation structure.

Living in the vicinity of Amsterdam, snakes have to cope with a highly dynamic, ever changing landscape. Typical Dutch landscape characteristics as dikes, ditches and allotments play a vital role in sustaining the grass snake populations in this (sub)urban area providing the snakes with hibernacula, foraging sites and egg deposit sites: the three main habitat requirements of the Grass snake (Madsen, 1984; Völkl, 1991).

In the late 90's the city of Amsterdam began constructing new artificial islands in Lake IJmeer to solve the housing-shortage, thereby disconnecting the northern- and southern branches of the metapopulation from each other (fig.1).

During the spring and summer of 2005 capture-recapture studies were carried out in several patches, as was done in previous years. Results show how the Grass snake reacts to conservation measures and habitat destruction/degradation.

Methods

During favourable weather conditions Grass snakes were caught from the end of March until June along linear structures (dikes, ditches), the best places for catching Grass snakes near Amsterdam as previous studies have shown (Zuiderwijk et al., 1999). As Grass snakes are individually distinct by their belly pattern (Zuiderwijk

and Wolterman, 1995), individuals were marked by photographing or drawing the pattern of the first twenty ventralia.

Permits to catch and handle captured Grass snakes, listed as *Vulnerable* in the Dutch Red List (Hom et al., 1996), were provided by RAVON (Reptile, Amphibian and Fish Research Netherlands). (Sub)population size estimates were calculated using Bailey's modification of the Lincoln-Petersen method (Begon, 1979).

Results

In total 41 individual Grass snakes from the subpopulations A, B and G were caught and thus marked during surveys. Recaptures of adult snakes were scarce. Snakes with a snout-vent-length (SVL) < 40 cm were regarded as sub adults and excluded from the Lincoln-Peterson estimates (Table 1.).

Due to the small sample size and the limited number of recaptures Standard Errors are large and give no additional information: estimates should be regarded as approximations rather than calculated population sizes.

Discussion

Before construction of the new islands in Lake IJmeer in 1998, the southern branch of the metapopulation consisted of several larger populations (E, F and G) whereas the populations of the northern branch (A and B) were regarded as small (Zuiderwijk et al., 1999). Besides the new islands themselves there were other infrastructural interventions in Grass snake habitats. The islands need a new approach to the motorway, sport accommodations, a park and other requirements new residential areas need nowadays. A gunpowder factory, situated in an old forested marsh just behind the dike of population G, an important foraging site for snakes, was closed in 2004 due to the political consequences of the Enschede Fireworks disaster of 2000. Dismantling of this important part of the habitat of population

Table 1. Lincoln-Petersen estimates of the examined subpopulations.

Subpopulation	1998	2005
	Zuiderwijk <i>et al.</i> , 1999 Lincoln-Petersen estimate	<i>this study</i> Lincoln-Petersen estimate
A (northern branch)	12	80
B (northern branch)	8	18
G (southern branch)	54	15

G almost immediately led to fewer sightings and smaller population size estimates.

The small populations of the northern branch were of special concern after the splitting of the metapopulation, as small populations can not survive on the long term (Soulé, 1980). Conservation measures such as the construction of breeding heaps, creation of dams leading to shallow water and a change in mowing regime carried out by several organisations in the last 4 years start to pay of as shown by the estimates of 2005.

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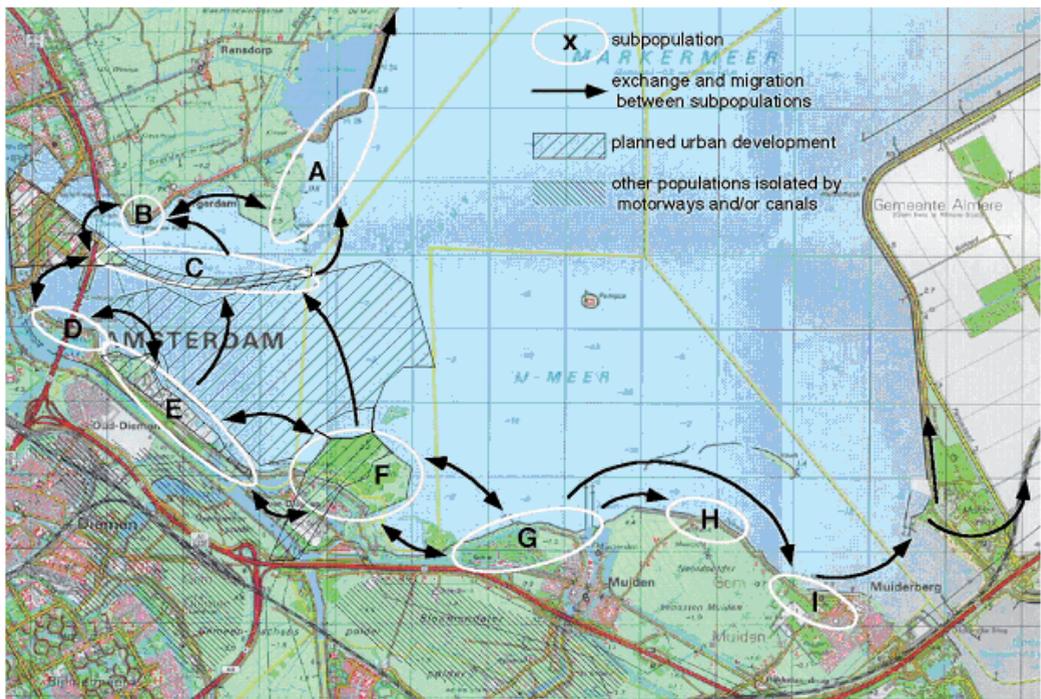


Figure 1. The structure of the metapopulation with subpopulations A-I and the city-development plans.

The current status of genetic exploration in amphibians: taxonomic and geographical disparities

Miguel Vences¹, Jörn Köhler²

Abstract. We evaluated the current amount of available gene sequences in Amphibia based on public online resources and analysing the number of sequences at the level of species and genera, and according to their distribution at continental level. There are significant disparities considering the level of genetic exploration in different taxonomic groups of Amphibia as well as in different geographical units. Total number of sequences per species as well as taxonomic species coverage was highest in salamanders and lowest in caecilians, and was highest in taxa from Europe, North America and Madagascar, and lowest in Latin America.

Introduction

Today, living amphibians comprise more than 6000 known species. In the last 11 years (1992-2003) this number has increased by 26.3 % and many undiscovered species still remain in species-rich tropical countries (Köhler et al., 2005). At the same time, an evaluation according to IUCN red list criteria revealed that amphibians are suffering severe declines with about 32.5 % of the known species being globally threatened (Stuart et al., 2004).

The establishment of molecular genetics as a tool in systematics and the reconstruction of phylogenetic relationships has led to accumulation of many gene sequences of amphibians and the compilation of a complete phylogenetic tree of the Amphibia has been proposed by initiatives such as AmphibiaTree (<http://www.amphibiatree.org>). The need for a better understanding of the systematics of this highly threatened vertebrate class and the proposed goal of completion of its phylogenetic tree propelled us to draw up an interim balance of available gene sequences to detect deficits, progresses and future needs at the current stage.

Materials and methods

The number of DNA sequences of amphibians available from public resources (<http://www.ncbi.nlm.nih.gov/Genbank>) was analysed as of June 2005 at the levels of species (5758 species considered), genera, families and orders, without considering genome projects currently

underway. We compiled a database with amphibian genera currently recognised as valid, including their allocation to order and family, as well as their geographic distribution at continental level by using AmphibiaWeb (2005) and Frost (2004). We used a scheme slightly modified from geographical definitions of continents and set the geographic categories as follows: sub-Saharan Africa, Latin America (including the Caribbean), North America, Australia/New Guinea, Asia, Madagascar and Europe (including northern Africa) (Fig. 1). Genera with intercontinental distribution were split among continents according to respective species distributions. The analysis was facilitated by the fact that most amphibian genera and almost all species have distribution areas restricted to a single continent. The number of species sequenced as well as the total number of nucleotide sequences available per genus was evaluated using the GenBank taxonomy browser (June 2005). Sequences from genera not considered valid taxa anymore were transferred to the respective valid genus.

A recently published major contribution on amphibian systematics (Frost et al., 2006) provided additional gene sequences of about 500 amphibian species, many of them not sequenced before. These sequences were not included in our analysis and therefore may in part contribute to a picture different from the results presented herein. However, we argue that the identified differences among taxonomic groups and geographical regions constitute a pattern not significantly altered by the sequences accumulated by Frost et al. (2006).

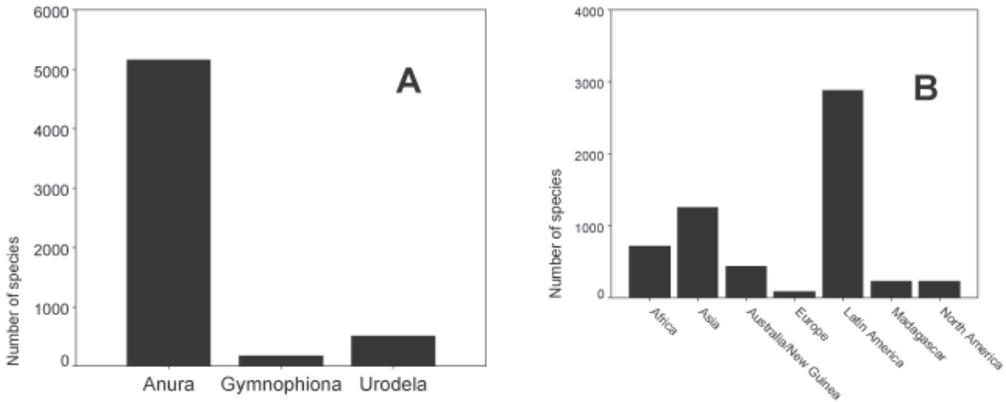
Results

Our analysis revealed that among amphibians, salamanders are best studied with respect to the number of available nucleotide sequences. At average, more than nine sequences are available per species within the Urodela. This is clearly contrasted by the respective mean value for Anura with approximately two available sequences per species and Gymnophiona with approximately one available sequence per species at average (Fig. 2). Generally, European amphibians are best studied genetically, with an average number of available sequences per species of approximately 22. With regard to this character, Europe is followed by North America (approximately 17 available sequences

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Figure 1. (A) Numbers of species recognised by order; (B) Numbers of species recognised per continent.



per species at average), and Madagascar (approximately 7 Sequences per species). Africa, Asia, Australia/New Guinea and Latin America display considerably lower mean values ranging from one to three available sequences per known species (Fig. 3). However, the Australian situation is likely to change soon due to the intensive, yet unpublished, ongoing sequencing projects by several working groups (S. Keogh, personal communication).

Referring to the number of species for which gene sequences are available, about 86 % of the known

species of Gymnophiona lack any sequence data as do approximately 76 % of the recognised anuran species, but only 29 % of species of Urodela (Fig. 4). When considering the number of species studied genetically at continental level, Latin American amphibians are the least explored ones with about 83 % of the known species lacking sequence data. This value is also comparably high in Australia/New Guinea (81 %), Africa (76 %) and to some lesser extent in Asia (68 %). In contrast, only 12 % of European species are without any sequence data (Fig 5).

Conclusions

In summary, there are obvious disparities in the degree of genetic exploration between certain taxonomic groups and when comparing different geographical regions. Generally, species-rich tropical regions are ‘under-represented’, as is the diverse order of Anura when compared to the less species-rich and comparably well-studied order Urodela. Among the amphibians without available sequence data are many which are indispensable for the understanding of amphibian systematics and evolution. In view of the goal to compile a complete phylogenetic tree of Amphibia and the exposed threat to this vertebrate group, the following actions are needed: (1) global coordination of sampling activities to avoid major overlap of efforts, (2) intensified field work in remote tropical regions, (3) specific search for key taxa and highly endangered species.

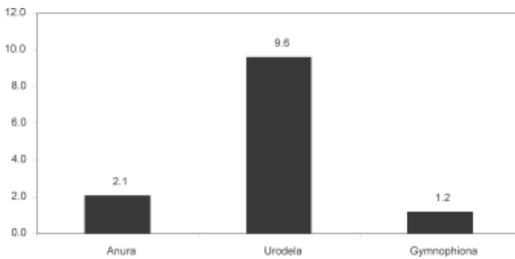


Figure 2. Average number of available sequences per species by amphibian order.

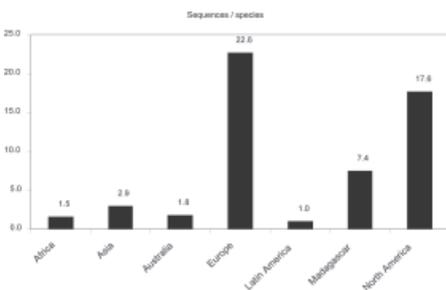


Figure 3. Average number of sequences available per species by continent.

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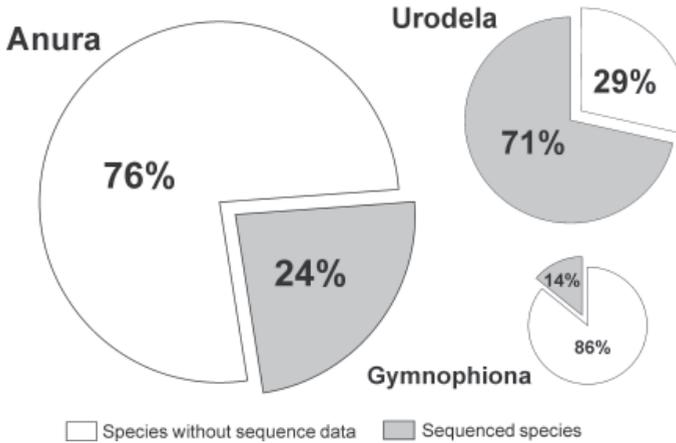


Figure 4. Portions of sequenced amphibian species by order (by June 2005).

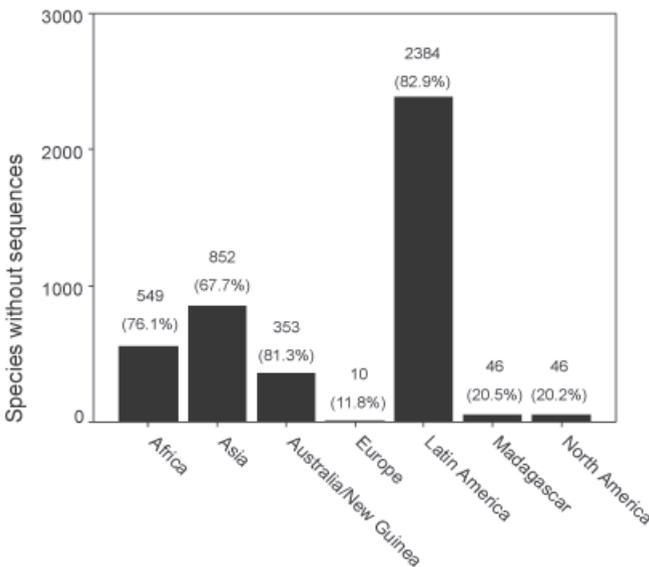


Figure 5. Number and percentage of species without sequences in Genbank by continental distribution.

Significance of recessive and dominant mutations in adaptive processes of the genus *Rana* in the modern biosphere

Vladimir. L. Vershinin

Abstract. The frequency of striata morph individuals in *R. arvalis* (44.9%) and *R. ridibunda* (92%) populations in Yekaterinburg increases along with the degree of urbanization. The reason of this process lies in the physiological specificity of the genotype. According to our data, the sodium permeability of the skin of the striata morph in *R. arvalis* is more than three times lower than in others. The frequency of iris depigmentation in the city populations of moor frogs was higher than in the forest population – 1.62% and 0.39% respectively. The probably underlying genetic mechanisms and the adaptive significances of these characters is discussed.

Introduction

From the orthodox point of view on processes of microevolution, it is supposed that during the process of mutating, recessive alleles usually accumulate in populations in heterozygotic state and later, when some environmental changes occur, can be used in adaptational processes as a hidden resource of internal variability (Chetverikov, 1926). Vice versa – dominant mutations usually are eradicated by natural selection because of their immediate appearance in phenotype and their harmful influence on survivalship of the specimens.

During a long study (1976-2005) of city and forest populations of three anuran amphibian species we found some facts that show a different destiny of some dominant and recessive mutations and their different role in the adaptation process under the effect of urbanization.

Materials and Methods

The work was done on the territory of city agglomeration (Yekaterinburg) that is situated in Middle Ural - right on the border of Europe and Asia. Control sites were located 23 km from the city.

I studied populations of three species of anuran amphibians from the genus *Rana*: *R. ridibunda* Pall., *R. arvalis* Nilss., and *R. temporaria* L. that lived in an urban agglomeration. Frequencies of two characters were investigated – striata morph and iris depigmentation. The first so-called striata morph, which is phenotypically expressed as presence of a

light dorsomedian stripe, has been found in several species of the genus *Rana*. It is determined by the dominant allele of the diallelic autosomal gene “striata” with complete dominance (Schupak, 1977; Berger and Smielowski, 1982).

The second feature, absence of normal iris pigmentation in one or both eyes, is widespread in many amphibian species, both anuran (Cain and Utesch, 1976) and urodelean (Dubois et al., 1973; Engelmann and Obst, 1976). Hybridization experiments with *Rana esculenta* (Rostand and Darre, 1970) have shown that this anomaly is determined by a recessive mutation (Rostand, 1953). It has also been described in the brown frogs *R. temporaria* (Rostand, 1953) and *R. arvalis* (Vershinin, 2004a). In fact, it may be regarded as partial albinism.

Results and Discussion

It has repeatedly been noted that the frequency of striata morph individuals in *R. arvalis* (44.9%) and *R. ridibunda* (92%) populations in Yekaterinburg increased ($p < 0.01$ by chi-square) along with the degree of urbanization (Fig. 1).

The reason of this process lies in the physiological specificity of the genotype. According to our data, the sodium permeability of the skin of the striata morph in *R. arvalis* is more than three times lower than in others ($F = 5.39$, $p < 0.0023$). The decrease in the skin permeability for many substances (including oxygen) favors an increase in the importance of lung respiration, which leads to an increase in the blood oxygen capacity due to an increased amount of hemoglobin. The high hemoglobin concentration accounts for the high amount of iron in the body of striata morph frogs. The increased lung function intensity resulted in an increased metabolic rate and, hence, decreased

life span of striata morphs (2.8 ± 0.16 years ($n=71$) vs. 3.3 ± 0.19 years ($n=42$) in other frogs; $F=4.1$, $p < 0.045$). The shortened life cycle of striata frogs promotes an increase in the rate of evolution. Frogs with this dominant mutation are preadapted to natural and artificial geochemical environmental anomalies (Vershinin, 2004b). The second feature, recessive mutation, is the evidence for inbreeding depression in urban populations and high mutagenesis in the urban environment (Vershinin, 2004a).

The frequency of iris depigmentation in the city populations of moor frogs was higher (chi-square=28.9; $p < 0.001$) than in the forest population – 1.62% and 0.39% respectively. We found that among the probable factors responsible for fluctuations in the frequency of this anomaly, attention should be focused

on ambient temperature in the period of spawning and embryonic development: in the years characterized by the absence of frogs with iris depigmentation no frosts occurred in this period. Apparently, cold spells in spring promote the phenotypic manifestation of this anomaly (Vershinin, 2004a). In the city and in the forest populations of moor frogs, the frequencies of this deviation decreased in adults compared with young of the year animals (Fig. 3).

The total frequency of the feature in all juveniles is 4.9 times higher than in all adults (chi-square=12.39; $p < 0.001$). We suppose that homozygosity of these specimens can negatively influence survivalship. Apparently, there is a complex of recessive traits that, being in the homozygous state, may be responsible for a low survival due to inbreeding depression (Simberloff,

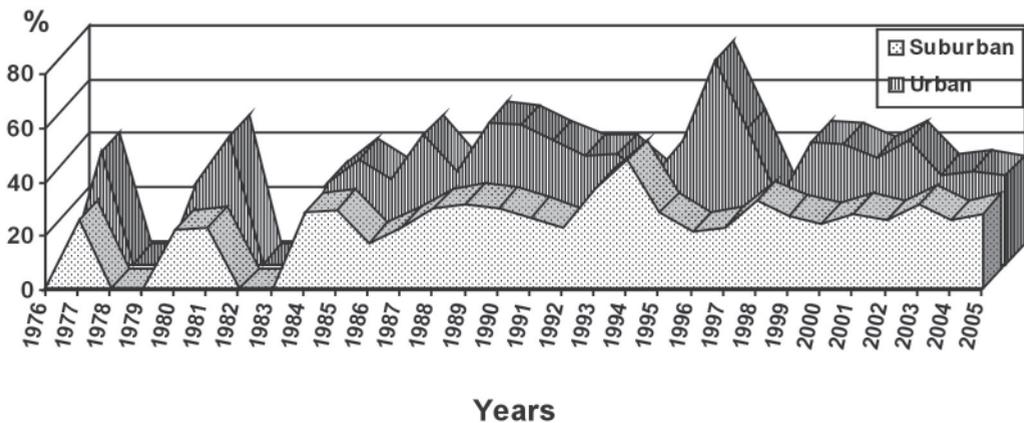


Figure 1. Frequency of striata morph in urban and suburban populations in juvenile *R. arvalis*

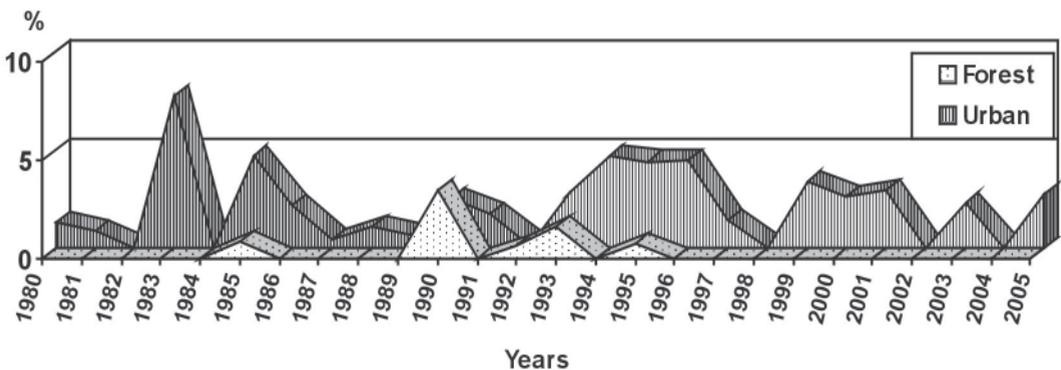


Figure 2. Frequency of iris depigmentation in urban and forest populations in juvenile *R. arvalis*

1983). It appears that striata morph frequency in the city populations slightly increases in mature animals (Fig. 4).

The relatively lower capacity for bioaccumulation determined the increased frequency of striata frogs in areas of natural and artificial geochemical anomalies. The striata morph is absent in *R. temporaria*, which is sympatric to *R. arvalis*. Apparently, the ancestors of *R. temporaria* lost this morph; this was related to the fact that wintering *R. temporaria* tolerate hypoxia by using skin respiration.

High resistance to anthropogenic transformation of environment is characteristic for *R. arvalis* and *R. ridibunda* - the species in which the striata morph

exists as a genetic variant. Phenotypic manifestation of dominant - striata differs from recessive iris depigmentation (Fig.1, 2) by more stability and its penetrance is independent from seasonal factors. Thus, dominant mutations can immediately increase adaptive success of their carrier. On the contrary - recessive mutations reduce chances of individual to survive.

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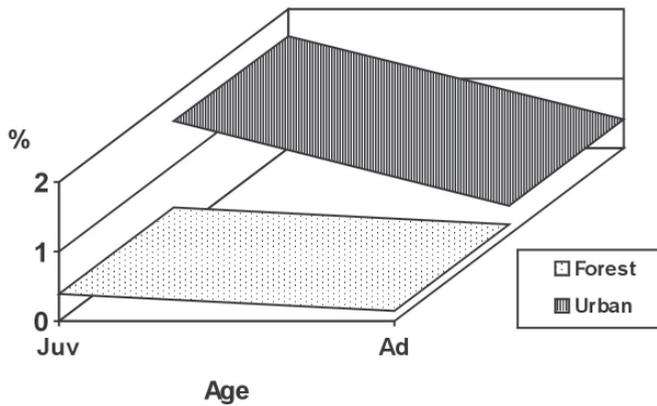


Figure 3. Age changes in iris depigmentation frequency in city and forest populations *R. arvalis*

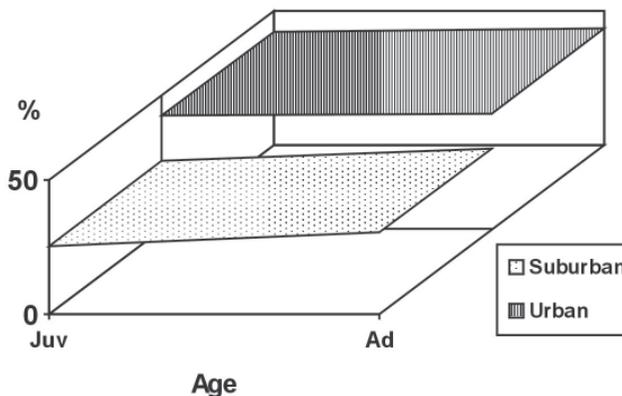


Figure 4. Age changes in frequency of the striata morph in urban and suburban populations *R. arvalis*

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Seasonal changes in the diet of *Rana ridibunda* Pallas, 1771 (Anura: Ranidae) from the Gorele River, Giresun, Turkey

Zeliha Colak Yilmaz¹, Bilal Kutrup²

Abstract. We analyzed the stomach contents of 252 specimens of the marsh frog, *Rana ridibunda*, in the Gorele River habitat of Giresun, Turkey. We found that this species consumed mostly arthropod prey, as well as some prey items belonging to Mollusca, Nematoda and Annelida. Based on prey number, frequency and volume, the most important prey category were insects with beetles, flies and ants predominating. Not only invertebrates but also some vertebrates were found in the stomachs. The highest prey diversity was found in July (62 prey categories) and the lowest in August (38). The highest average prey number was observed in July. Frogs were found to consume large prey items more in June than in the other months. Diet compositions of recaptured individuals varied slightly during the study but confirmed the highest prey number in July. The results of all analyses suggest that the diet of this species varied monthly and correlated with the prey availability.

Introduction

Most anuran species are known to be general predators consuming mainly invertebrates, with some vertebrates being ingested by large frogs (Pough et al., 2001). Since anurans are poikilotherm animals, their activity is related to temperature. In addition, they breed in specific months; therefore, repeating behavioural and ecological observations at different times is important to avoid bias due to daily temperature changes (Houston, 1973). There is also a relationship between the abundance of prey in the environment and in the diet of anurans (Turner, 1959; Houston, 1973).

When studying on the diet composition of any anuran species, the monthly diet variation and the correlation of food composition to prey availability in large sample sizes are important factors. Although there are many records about feeding habits of anurans (e.g., Werner et al., 1995; Ugurtas et al., 2004), only few included these patterns. For example, to detect diet composition of *Rana ridibunda*, Atatur et al. (1993) used only 19 adult specimens; Ruchin and Ryzhov (2002), in a study on this same species, ignored effects of season and prey abundance.

R. ridibunda is distributed in Northern Africa, Middle

and Southern Europe and Western Asia (Ozeti and Yilmaz, 1994) and its reproductive season is during the first days of May and the first half of June (Maxwell, 1962). Here we present results of a study on effects of season and prey availability on the diet of *Rana ridibunda* in Turkey.

Materials and Methods

We selected a population of *R. ridibunda* inhabiting slow flowing water near Giresun, Turkey. The study site (41°02'N, 39°01'E) was near hazelnut and alder trees, and there were no other amphibian species present.

To detect seasonal diet variations, we collected 252 marsh frogs in May ($n=40$), June (53), July (59), August (52) and September (48) in water or near the water between 09.00 and 16.00 in daylight. After capturing we anesthetized the frogs with diethyl ether as soon as possible and removed stomach contents by flushing method at least three times for each specimen to remove all stomach contents (Legler and Sullivan, 1979). We preserved all contents in 10% buffered formalin for further analysis and measured frogs SVL. After marking frogs individually by toe-clipping, we waited until the anesthetised frogs seemed fine and then released them to their original habitats. On each collecting day, we made sweeps in, on and near the water (up to 0.5 m above) to determine the prey abundance.

We counted all prey items flushed out and identified them to the most practical taxonomic level according to keys by Chu (1949) and Chinery (1993). Larvae and adults of holometabolous insects were regarded separately, and empty stomachs were excluded from further analyses. We measured maximum width and length of each item with a calliper (to the nearest 0.1 mm) to estimate their volumes using the formula for an ellipsoid: $\text{Volume} = 4/3 (\text{Length}/2) (\text{Width}/2)^2$ (Dunham, 1983). For partially digested preys, we used the predetermined length-width regression formulas (Hirai and Matsui, 2001). We calculated the percentages of number, volume and frequency of each prey category. Besides, we recorded the following variables per stomach content: total, average, smallest and largest prey volumes, which were compared using Kruskal-Wallis test (χ^2) and Mann-Whitney U tests.

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Table 1. Frog SVL, prey numbers and volumes from the stomach contents of 206 marsh frogs in the population studied.

	SVL (mm)	Prey Number	Total Volume (mm ³)	Minimum Volume (mm ³)	Maximum Volume (mm ³)	Mean Prey Volume (mm ³)
Mean	66.5	9.1	528.7	9.0	258.4	74.1
<i>n</i>	206	206	206	158*	158*	158*
Minimum	19.9	1	0.2	<0.1	0.4	0.3
Maximum	97.6	121	4101	123.2	2330	506.9
SE	1.3	1.1	50.3	1.3	29.4	7.3

* only stomachs with at least three prey items considered

Kendall's rank correlation coefficient (τ) was calculated to detect the relationship between numerical percentage of preys in the environment and percentage of frequency of the same preys in the stomachs. We also used Ivlev's electivity index (Ivlev, 1961) to find out the prey taxa preferred or not preferred by this frog: $E_i = (n_i - r_i) / (n_i + r_i)$ (n_i : relative abundance of prey taxa i ; r_i : abundance in the environment; Cogalniceanu et al., 2000). Statistical analyses were conducted with SPSS 10.0 version.

Results

From 206 stomach contents of 252 frogs (46 were empty), 1864 prey items representing 46 prey categories were identified. Mean prey number and volume were, respectively, 9.1 and 528.7 mm³ per stomachs (table 1). Prey items were belonged into four invertebrate phyla, Arthropoda (having the largest proportions in the diet composition; N%=91.0, F%=98.0 and V%=70.6), Mollusca, Nematoda and Annelida), and one vertebrate taxon (Anura). Insects were the dominant prey (fig.

1). Larval and adult Coleoptera made up the largest fractions in number followed by Diptera, Formicidae and Araneae. In addition to invertebrates, 27 marsh frogs (21 tadpoles and 6 post metamorphic juveniles) were also encountered.

Kruskall-Wallis test showed that prey number, as well as minimum and mean prey volume, changed significantly among months (table 2). For example; we found that more prey items were consumed in July than in the other months, with the highest difference between July and August (*U*-test, $z=3.149, P<0.01$). The frogs were found to ingest largest prey in June, followed by September, May, August and July. The highest prey diversity was detected in July (62 different taxa identified) and the lowest in August (38).

The most common prey taxa were adult and larval Coleoptera, adult Diptera, Formicidae and Araneae in all months, but in varying proportions. In frequency, number

Months		Prey Number	Total Volume (mm ³)	Minimum Volume (mm ³)	Maximum Volume (mm ³)	Mean Prey Volume (mm ³)
May	Mean	8.3	555.6	3.4	346.4	82.8
	<i>n</i>	32	32	25*	25*	25*
	Minimum	1	0.2	<0.1	8.9	6.4
	Maximum	37	2460.3	21.9	2330.0	437.8
	SE	1.5	121.4	1.1	115.8	22.6
June	Mean	5.7	686.0	13.4	338.5	95.3
	<i>n</i>	44	44	36*	36*	36*
	Minimum	1	0.7	<0.1	11.2	13.1
	Maximum	17	3800.0	62.8	2260.8	407.4
	SE	0.6	131.2	2.6	74.9	14.2
July	Mean	19.1	593.7	5.8	180.8	46.5
	<i>n</i>	49	49	41*	41*	41*
	Minimum	1	0.2	<0.1	0.4	0.3
	Maximum	121	4101.0	91.7	1364.0	506.9
	SE	4.3	123.9	2.3	38.3	12.8
August	Mean	4.7	346.3	7.8	196.3	66.8
	<i>n</i>	43	43	29*	29*	29*
	Minimum	1	3.2	<0.1	1.4	1.1
	Maximum	27	1339.8	36.8	732.4	191.5
	SE	0.7	53.5	1.5	36.2	11.0
September	Mean	5.6	446.5	14.4	254.5	87.8
	<i>n</i>	38	38	27*	27*	27*
	Minimum	1	3.4	0.1	10.1	3.9
	Maximum	18	2959.1	123.2	1099.2	421.9
	SE	0.6	105.2	5.2	54.4	21.4
Kruskall-Wallis test	χ^2	12.546	4.245	20.411	4.601	14.205
	<i>P</i>	<0.01	0.374	<0.001	0.331	<0.01

* only stomachs with at least three prey items considered

Table 2. Prey numbers and volumes of marsh frogs in the population studied, separately per month.

and volume, Ephemeroptera, Isopoda, Carabidae, and Adult Diptera had important proportions in May and June whereas they showed a striking decrease in the other months (fig. 2). Orthoptera and Gastropoda were dominating both in frequency and number in August. In July and September, respectively, Scarabaeidae and Oligochaeta were heavily consumed (fig. 3). In insects, larval specimens were seen especially in July. Frogs were consumed in May, June and July (fig. 2) and the volumetric proportion of this prey type was highest in June. According to diet composition of 20 individuals captured more than once, we did not detect any important differences among the five months, but their mean prey number was also highest in July.

The prey types collected in the environment and found in the diet composition were similar but their proportions differed (fig. 4). Although the correlation between the prey availability in the environment and the stomach contents of abundant prey taxa (including 11 categories) was significantly positive ($r=0.514$, $P<0.05$), when larval Lepidoptera and Odonata were excluded from the analysis because of their small fractions in numeric proportions in the environment, the general value of correlation was low and not significant ($r=0.310$, $P=0.249$). The electivity index calculated suggested that this frog had preferences on some prey types. For example, Heteroptera ($E_i=-0.72$), Ephemeroptera (-0.91) and Formicidae (-0.56) were apparently avoided by the marsh frogs studied, whereas larval insects (0.52) were preferred to adult insects (-0.23).

Discussion

The results of stomach content analyses of 252 specimens of *R. ridibunda* showed that its diet consisted mainly of Arthropoda followed by some other invertebrate taxa, with an expressed cannibalistic tendency to consume immature individuals of their own species. We found that this species was mostly insectivorous and its foods changed from month to month, weakly correlated with the prey availability except for some prey taxa being not preferred by this frog.

The food types of marsh frogs we detected were similar with those found in ranid frogs in general (Simic et al., 1992; 1995; Cogalniceanu et al., 2000; Ruchin and Ryzhov, 2002), although Atatur et al. (1993) recorded a numeric proportion of insects (97.8%) which was relatively higher than we found (76.1%). The same is true for some other studies (e.g., Ruchin and Ryzhov, 2002). This difference may result from the small sample size ($n=19$) used by Atatur et al. (1993).

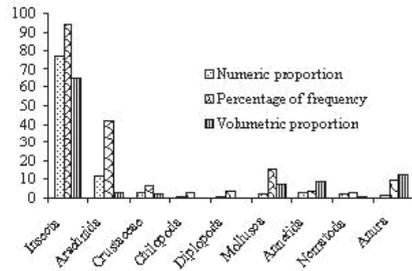


Figure 1. The proportions of the major prey taxa in the diet composition of the marsh frog.

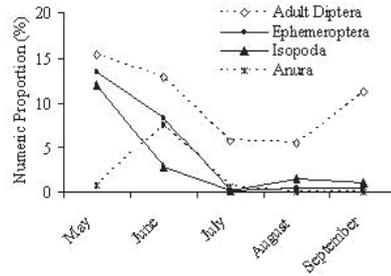


Figure 2. The numeric proportions of some prey taxa especially dominating in May and June.

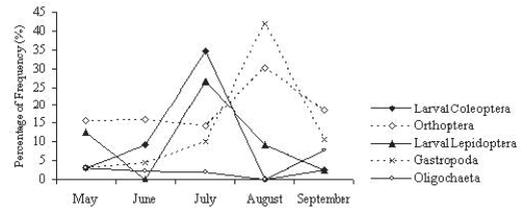


Figure 3. The percentages of frequency of some prey taxa (larval Coleoptera and larval Lepidoptera were dominating in July; Orthoptera and Gastropoda in August; Oligochaeta in September).

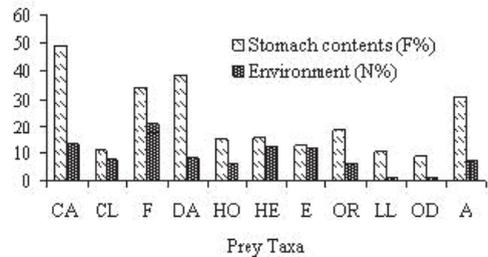


Figure 4. The proportions of some prey taxa in the stomach contents and in the environment (CA, adult Coleoptera; CL, larval Coleoptera; F, Formicidae; DA, adult Diptera; HO, Homoptera; HE, Heteroptera; E, Ephemeroptera; OR, Orthoptera; LL, larval Lepidoptera; OD, Odonata; A, Araneae).

As for example in *Rana catesbeiana* (Smith, 1977), it is known that large frogs occasionally eat vertebrates (Pough et al., 2001). We confirm cannibalism in *R. ridibunda* as reported previously (e.g., Ruchin and Ryzhov, 2002). Rastyatin (1974) and Ruchin and Ryzhov (2002) also found fish and even some mammals such as rats in the stomachs of this species, not detected by us.

In spite of the fact that anuran diets vary monthly (Houston, 1973), there is only one record (Simic et al., 1992) comparing seasonal variations in the diet of marsh frog. There were similarities as well as differences between their results and ours. Similar to our results, prey diversity was highest in July. However, in Simic et al. (1992), Coleoptera and Hymenoptera were dominating in spring and autumn whereas in the population studied here, these prey taxa were dominated in all months with small variations. Crustaceae were found mainly in summer by Simic et al. (1992), whereas we observed this prey mainly in May and June.

Several studies have characterized ranid frogs as general predators, their diets being correlated to prey availability (Turner, 1959; Houston, 1973; Hirai and Matsui, 1999). In contrast, the correlation of these two variables observed in this study was rather weak and several prey taxa were found to be positively or negatively selected by the frogs. For example; the electivity index showed that marsh frog had positive selectivity on larval insects such as larval leaf beetles (Chrysomelidae) whose numeric proportion, in July (N=42.1%), was considerably higher than the results reported so far (Simic et al., 1992; Ruchin and Ryzhov, 2002), indicating a possibly higher prey electivity in this generalist species in particular populations or seasons.

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Habitats of *Vipera berus nikolskii* in Ukraine

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Abstract. In one of the best-known parts of its distribution range (eastern and central Ukraine), *Vipera berus nikolskii* inhabits ecotones of broad-leaved forests connected with river banks. Sometimes, normally close to such forests and particularly towards the southern border of its range, *V. b. nikolskii* occurs in flood-plain deciduous forests or bogs inside pine forests on sandy, alluvial river terraces. Exceptionally, *V. b. nikolskii* can also exist on steppe slopes with bushes and sparse trees. Common syntopic species recorded were *Anguis fragilis*, *Lacerta agilis*, *Natrix natrix* and *Coronella austriaca*; more rarely encountered were *Zootoca vivipara* and *L. viridis*, and extremely rare was *Viper renardi*. The structural components of the *V. b. nikolskii* habitats had much in common with those of *V. b. berus*, however, there is great overlap between the preferred habitats of these two subspecies. Differences in habitat selection may reflect different habitat availability within their ranges, but despite their general habitat overlap they remain ecologically separated in the contact zones. This could be interpreted as competitive exclusion with a subsequent shift of their ecological optima. Alternatively, *V. b. nikolskii* could have had a temporal advantage in persisting in broad-leaved forests and better abilities to colonize new territories as compared with *V. b. berus* which on contrast might have arrived later on from more northern and western Pleistocene refugia.

Introduction

Nikolsky's viper was described as a full species – *Vipera nikolskii* Vedmederja, Grubant et Rudaeva, 1986 – but is now considered as a subspecies of the Common Adder, *V. berus nikolskii*, distributed in the forest-steppe zone of Eastern Europe from Moldova to Tatarstan (Milto & Zinenko 2005). Differences between the habitats of *Vipera berus berus* Linnaeus, 1758 and *V. b. nikolskii* were mentioned already in the first paper on the taxonomic status of *V. b. nikolskii* (Grubant et al. 1973), but until now only very sparse data have been published. They concern the connection with broad-leaved forests of the right banks of rivers in Eastern and Central Ukraine (Grubant et al. 1973; Vedmederja et al. 1986; Strelkov 1997) and other parts of its range: Moldova (Tsurkanu 2006), southwestern Ukraine (Tabachishin & Shljakhtin 2003), various regions in Russia (Bozhansky 2001; Tabachishin et al. 2003; Sokolov 2005) and in the whole range (Milto & Zinenko 2005); connection to different biotopes and landscapes between *V. b. berus* and *V. b. nikolskii* in contact zones (Loparev & Sytnik 2003; Sytnik & Loparev 2003; Loparev & Sytnik 2004; Zinenko 2004).

The aim of this paper is to give more detailed description of *V. b. nikolskii* typical habitats in Central and Eastern Ukraine, which are the best known parts of *V. b. nikolskii* range. Thus, the description will make the comparison of habitat preferences of *V. b. berus* and *V. b. nikolskii* possible.

Materials and methods

Data on habitat structure and biotopes were collected during 1997-2004 in Central and Eastern Ukraine and the adjacent Belgorod region of Russia in a forest-steppe zone. Descriptions of the habitats in Lubny vicinities in the Poltava region were given by a personal report of O. M. Rudyk, in Slavjanogorsk vicinities in Donetsk region – by a personal report of T. I. Kotenko and literature, vicinities of Staritsa village in Volchansk district in Kharkiv region and Kochetok vicinities in Chuguev district in Kharkiv region – by personal reports of V. M. Pashkov and D. G. Strelkov. Specimens from all these localities are stored in the Museum of Nature at V. N. Karazin's Kharkiv National University (MNKNU, Kharkiv) and the Zoological Museum of National Museum of the Natural History (ZNMNH, Kyjiv) of Ukraine National Academy of Sciences. Data on the habitats of 30 populations of *V. b. nikolskii* were investigated, 23 are to the east of the Dnipro in Ukraine, 5 – to the west, 2 – in the Belgorod region of Russia. Data on the distribution and structure of forest were obtained from vegetation and forests maps of Ukraine and literature (Andrienko 1977; Marynych et al. 1982; Isachenko & Shljapnikov 1989).

The conclusions concerning systematic position of the majority of the studied populations were based on the analysis of morphological variability in the redescription of *V. b. nikolskii* (Milto & Zinenko 2005) but intermediate hybrid populations were considered as belonging to one of the subspecies with closer affinity to these populations: populations in Kaniv vicinities on the right-bank of the Dnipro in Cherkasy region, in Novaja Sloboda vicinities Putivl' district, Sumy region and from vicinities of Sumy were considered as *V. b. nikolskii*; populations from the left bank of the Dnipro in Kaniv vicinities, left bank of Dnipro in Kyjiv vicinities and vicinities of Spadshchina in Putivl' district in Sumy region were considered as *V. b. berus* populations.

Results

V. b. nikolskii inhabits ecotones of open and closed biotopes with bushes (86.7%), pronounced slopes (80.0%), eroded areas (ravins, gullies – 53.3%), plenty

of dry vegetation (branches, grass – 63.3%) and fallen trees (53.3%), stubs (33.3%), rodents holes (20.0%) and southern and south-eastern or western expositions. Bare rocks are absent in Ukraine forest-steppe east from the Dnipro. Dry climatic conditions prevent the development of thick layer of moss, which is also common in habitats of *V. b. berus* in Central Europe (Völkl & Thiesmeier 2002). Instead, thick layers of dead leaves provide good thermoinsulation and are a suitable substrate for spring basking.

The range of *V. b. nikolskii* almost completely coincides with the forest-steppe and broadleaved deciduous forest zones in eastern and central Ukraine (the second one is very narrow and fragmented in eastern Europe). Localities of *V. b. nikolskii* (86.7% of the populations) are strictly connected with large areas of prevailing type of natural forests in the forest-steppe zone: broadleaved deciduous oak forests on the water sheds and upwarded right banks of the rivers Ingulets', Dnipro, Udaj, Khorol, Sejm, Psel, Vorskla, Siversky Donets' with tributaries (Fig. 1). For such forests the dominance of *Quercus robur*, *Tilia cordata*, *Acer platanoides* with presence of some other tree species (*Fraxinus excelsior*, *Ulmus* sp., *Malus sylvestris*, *Pyrus communis*, etc.) is typical. West of the Dnipro in Ukraine, *Carpinus betulus* is another main tree species in such forests, but east of the Dnipro it occurs only sporadically. As a rule, the understory is represented by *Corylus avellana*, *Swida sanguinea*, *Acer tataricus*, *Euonymus verrucosa*, including grasses and herbaceous plants like *Carex* sp., *Asarum europaeum*, *Aegopodium podagraria*, *Galium* sp., *Astragalus glycyphyllos*, *Stellaria* sp., *Poa nemoralis* etc. The forest edges are usually dominated by small trees (*Betula pendula*, *Malus sylvestris*) and bushes (*Crataegus* sp., *Euonymus verrucosa*, *Ulmus suberosa*, *Prunus spinosa*). In some localities of its central range (Krasnokuts'k district of Kharkiv region, Akhtyrka and Trostjanets' districts in Sumy region) and at the southern border of distribution (in Kharkiv, Donets'k and Lugans'k regions: Kotenko 1983; own data) *V. b. nikolskii* can also live in flood-plains, in ecotones of deciduous forests or in the ecotones between wet floodplain biotopes (wet meadows or *Alnus glutinosa* forest) and a sandy terrace. In addition *V. b. nikolskii* also inhabits small bogs with *Sphagnum* hummocks and *Betula pendula* within small depressions on sandy terraces inside dry pine or mixed forests. As a rule, such populations are situated near to large typical habitats. In the vicinity of the large forests on the right bank of the Siversky Donets' river in Donets'k region

Nikolsky's viper inhabits also the slopes with chalky bare rocks, steppe and dry meadow vegetation altered with bushes and forest with oaks and endemic *Pinus cretaea* (Kotenko 1983; Zinenko & Kotenko 2005; T. I. Kotenko, personal report). However, such habitats should be considered as secondary.

Open biotopes in habitats of *V. b. nikolskii* can be more diverse than forest biotopes. Before the human transformation they consisted of dry and wet grasslands. Within cultural landscapes adders still can be abundant in ecotones of previously described forests with fields, gardens, rural building areas and pastures. Inside forests the open sites are represented by the banks of forest lakes and ponds, upper swamps, glades, clearings, and road margins.

Syntopic reptile species usually are the sand lizard (*Lacerta agilis exigua* Eichwald, 1831 or *Lacerta agilis chersonensis* Andrzejowski, 1832 – in 76,7% of all cases) and the grass snake (*Natrix natrix* L., 1758 - 60.0% of all cases). The Slow-worm, *Anguis fragilis colchicus* Linnaeus, 1758 and Smooth snake, *Coronella austriaca* Laurenti, 1768 are common (73.3% and 26.6% respectively) in the typical habitats of *V. b. nikolskii*. These four species form a specific assemblage for such biotopes. The Common Lizard, *Zootoca vivipara* (Jacquin, 1787) is only exceptionally syntopic with Nikolsky's viper (13.3%), due to its absence in deciduous water-shed forests within the investigated region. On the slopes of the right bank of the Dnipro, in the ecotones of oak-hornbeam forest with bushes, the Green lizard, *Lacerta viridis*, Laurenti, 1768 is another species syntopic with Nikolsky's viper (6.7% of all cases, but present in two of tree investigated populations of *V. b. nikolskii* on the right bank of the Dnipro). Among amphibians, usually Crested newts, *Triturus cristatus* (Laurenti, 1786), Common toads, *Bufo bufo* (Linnaeus, 1758), Tree-frogs, *Hyla arborea* (Linnaeus, 1758) and Moor frogs, *Rana arvalis* Nilsson, 1842 inhabit the same forest tracts as *V. b. nikolskii*.

Discussion

The habitats of Nikolsky's viper are characterized by the presence of ecotones, a mosaic structure, well warmed substrate with good thermo-insulating properties, open sunny places, and also eroded slopes. The connection with broad-leaved forests on watersheds and the right banks of the rivers in East-European upwarded forest-steppe and broad-leaved forest landscapes appears also very clear. Habitats of Nikolsky's viper in floodplain forests, in bogs in small depressions on alluvial sandy

Figure 1. Localities of *V. b. berus* and *V. b. nikolskii* in the central and eastern Ukraine.



terraces of the rivers or in comparatively dry slopes with bushes and sparse trees are rare and probably colonized from adjacent typical habitats. The type of the open biotope in *V. b. nikolskii* habitat is more variable than forest type and apparently is less important.

Our data have revealed significant differences in frequency of the occupied biotopes between *V. b. berus* and *V. b. nikolskii*. Thus, the typical plain habitats of *V. b. berus* in Central and Western Europe are edges of raised bogs and fens, heaths, sparse wet coniferous forests and wet mixed forests (Benson 1999; Völkl & Thiesmeier 2002). Almost the same set of biotopes exists in habitats of *V. b. berus* in European Russia and Byelorussia (Belova 1976; Pikulik et al. 1988; Bozhansky & Orlova 2001), Northern Ukraine (Grubant et al. 1973; own data). These habitat differences are present in the contact zone of *berus* and *nikolskii* and lead to a partial spatial separation (Loparev & Sytnik 2003, 2004; Sytnik & Loparev 2003; Zinenko 2004). Subspecies also differ in the preferred hibernation places: winter shelters in *V. b. nikolskii* were observed in dense oak-maple forest on eroded slopes with fallen trees and stubs (Gaidary vill., Zmijiv dist., Kharkiv reg.) and in old glades with *Corylus avellana* bushes inside dense oak-forest (Kharkiv vicinities, Strelkov D. G.,

pers. comm.). Typical winter shelters of *V. b. berus* (see Bozhansky & Orlova 2001; Völkl & Thiesmeier 2002) are not used by *V. b. nikolskii* according to our observations.

Does *V. b. nikolskii* prefer other biotopes or are these differences caused by a different set of available biotopes in the region?

Habitats overlap in the two *V. berus* subspecies. *V. b. nikolskii* can live in very humid habitats characteristic for *V. b. berus*, like edges of raised bogs and wet meadows or on the hummocks in shallow water in relict bogs of sandy alluvial terraces depressions (North-West of Kharkiv region – V. I. Vedmederja, personal report; own observations), though in our opinion such habitats are secondary. Vice versa, *V. b. berus* can, however, at least elsewhere inhabit similar habitats as *V. b. nikolskii*: e.g. ecotones of broad-leaved forest and bare rocks and heath (Southern Sweden: Andren & Nilson 1981). Thus, the observed habitat differences could be caused by other sets of available biotopes in the subspecies areas: heaths are completely absent, bogs, natural coniferous and mixed forests are extremely rare and marshes have a small area and are not numerous in forest-steppe zone of central and eastern Ukraine.

The range of *V. b. nikolskii* has different climatic characteristics when compared with that of *V. b. berus*: less annual precipitation (430-550 mm), a larger temperature range (average temperature of January: 7-8°C, average temperature of July 19-21°C) and primary equal balance between precipitation and evaporation (Marynych et al. 1985). However, a shift to more humid habitats in the region, known in the southern part of the range in *Zootoca vivipara* (Korshunov & Zinenko 2003) or *Lacerta agilis* (Sand Lizard, Jablökow 1976) does not occur. On the contrary, the rareness of syntopy with *Zootoca vivipara* and *Lacerta viridis* to the west of Dnipro reflects the preference of more warm and dry biotopes by *V. b. nikolskii*. On the contrary, only in 3 of 14 populations of *Z. vivipara* in the Kharkiv region *V. b. nikolskii* was recorded (Zinenko, unpubl.).

The separation of the subspecies in different biotopes and landscapes in the contact zone of *V. b. berus* and *V. b. nikolskii* (Loparev & Sytnik 2003, 2004; Sytnik & Loparev 2003; Zinenko 2004) could be interpreted as competitive exclusion, due to a shift of ecological optima and possible active preferences of different habitats (ecological displacement). But the hybridization in a contact zone (Zinenko & Ruzhilenko 2003; Zinenko 2004), with a wide mutual intergradation of morphological characters (Milto & Zinenko 2005), as well as some indications of gene flow at least from *V. b. berus* to *V. b. nikolskii* (Kalyabina-Hauf et al. 2004) show that these differences in ecology and habitat preferences do not lead to the isolation of taxa. Moreover, wide introgression could also reduce ecological isolation of subspecies because of emerging of hybrids with intermediate morphological but also ecological characters.

Another explanation of subspecies habitat separation in a contact zone could be the history of colonization. Originally connected with refugia of broad-leaved forests in upwarded landscapes, *V. b. nikolskii* thus had advantages in colonization time. The Common Adder has dispersed from refugia in Central Europe (Carlsson 2003, Kalyabina-Hauf 2004) across newly formed and homogenous biotopes and has reached a forest-steppe zone when it was already inhabited by *V. b. nikolskii*, despite the comparatively higher dispersal capacities of *V. berus berus* (cf. *V. b. bosniensis* and *V. b. berus* in northern Italy: Kalyabina-Hauf et al. 2004; Ursenbacher et al. 2006).

Thus, *V. b. berus* and *V. b. nikolskii* have overlapping habitats. Together with the lack of reproductive isolation mechanisms it makes introgression possible.

Separation between different biotopes and landscapes in a contact zone most likely is caused by postglacial colonization history, but by different habitat selection and competitive exclusion due to ecological differences of subspecies can be considered as secondary factors.

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Chemical marking behaviour in the psammophiine snakes *Malpolon monspessulanus* and *Psammophis phillipsi*

Cornelius C. de Haan¹, Alexandre Cluchier²

Abstract. In colubrid snakes of the subfamily Psammophiinae a “self-rubbing” behaviour is known that serves as a preliminary to chemical marking of conspecifics, nest and hunting routes, and most notably, marking of a genuine territory. We here review this behaviour and the organoid structures probably related to it.

Psammophines, a colubrid subfamily of mostly African snakes, consisting of 8 genera with about 44 species, are characterized by a tiny, quasi filiform and 3-4 Sc short hemipenis (Bogert 1940), and in both sexes, by a valvular nostril enabling “self-rubbing” (De Haan 2003a). Moreover, a lack of significant sexual dimorphism in tail length seems to be generalized in this clade. However, some species show tails slightly longer in females. Impressed by the research and tempting hypothesis of Dunson et al. (1978), several colleagues reported that in psammophines “self-rubbing” or “self-polishing” or “grooming” serves to limit evaporative water loss through the skin.

In fact, this behaviour serves as a preliminary to chemical marking of conspecifics, nest and hunting routes, and most notably, marking of a genuine territory (for certain Montpellier snake males in may-june, see Darevsky 1956, De Haan 1982-2003a). So, the Montpellier snake seems to be the only snake being territorial and additionally the most social one, though seemingly only during its 4-6 weeks lasting mating period. At other times (and always being an opportunistic predator), it also proved to be rather cannibalistic, much more than apparently less social but equally opportunistic *Psammophis phillipsi*.

Two “self-rub” systems are to be distinguished (De Haan 1982): M (like *Malpolon*, terrestrial) and P (like *Psammophis*, terrestrial + arboreal). The P-system permits a snake to perform self-rubbing not only on firm ground but also when balancing on branches. In both systems a watery, colourless secretion, containing

proteins and fatty acids comes from a special nasal gland. It is leaving from an outlet situated on the external side of the so-called *narial valve* having closed the nostril. The secretion is applied ‘gracefully’ to the belly and tail, where it quickly dries to form a transparent film, ready for being scraped off and left behind as slightly sticky particles.

A male territory, chemically marked upon substrate between visual landmarks and optically surveyed by the marking individual, can easily be entered by females. An entering male, however, is quickly bitten away or tired out in a 1-3 hour lasting ritual combat. The territorial male mostly wins and during the rest of the spring a loser rarely dares another intrusion as soon as reminded of his defeat by linguovomerally retasting secretion particles left by the landlord. Certain losers, however, may be gathered in. They serve as “vassals” guarding the landlady when the lord is marking around and are ready to replace him every way if he should perish.

The ‘territorial female’ Montpellier snake, protected by a male which not only successfully fought with other males, but also hunts *with* her or *for* her and does not take food for himself, uses her perfumed cloacal scent gland secretion to rub with her hind-part, tail held upwards, one of the flanks of ‘her’ male as soon as a conspecific outsider female gets nearby or touches him. Such a rubbing equals a direct chemical marking of a conspecific and results in a peaceful, immediate retirement of the newcomer female (De Haan 1999, 2003a).

Understanding these three ways of rubbing helped to discover (so far only in psammophines), periodically present, extrabuccal, infralabial secretion outlets (ILOs) in snakes. No study has yet analysed the ILO-secretion or revealed the type and location of its source, but behavioural observations on *Psammophis phillipsi* showed that it is used with a fourth way of rubbing, viz. head moving with the ILOs pressed onto a conspecific

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individual, most visibly done by a female to a male. Obviously, it substitutes for functions of both the special nasal and the cloacal scent glands, when the related rubbing behaviours, especially the cloacal scent rubbing with the snake's hind-part, are inhibited by lack of firm substrate. This is usually the case in central Africa between twigs and stems some meters above ground, where *P. phillipsi* frequently moves or rests in balance. ILOs were found in psammophines which, even if desert dwelling, may move easily upon stems and twigs, viz. in representatives of the P-system self-rubbing genera *Dromophis* and *Psammophis*, as well as - surprisingly - in the Malagasy *Mimophis*, the latter still being one of the six M-system (terrestrial) self-rubbing genera (De Haan 2003 a and b). The following synopsis is proposed as an aide-mémoire on the organoid structures found within the psammophine snake group, and being subject here and in the following article in this volume:

1. On the head of the snake

PP Parietal Pit(s): sporadically present sense-organ-like pit(s) [see following article]

NVO Narial Valve Outlet: 'self-rub' secretion outlet upon closed nostril

ILO Infralabial Outlet(s): secretion outlet(s) periodically present/absent in 4th or 5th infralabial shield

2. On the hind part of the snake

CSO Cloacal scent Outlet: two secretion outlets next to cloaca

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3 types of secretion outlets serve **chemical marking**, of which

2 are permanent (NVO and CSO),

1 is periodical (ILO).

4 patterns of rubbing behaviour, of which

2 (self-rub) patterns are preliminary (via NVO) to chemical marking,

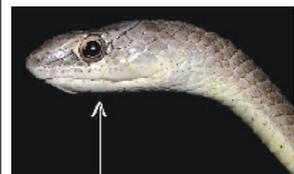
2 other patterns serve direct chemical marking (via CSO, resp.ILO).

(For further details see De Haan 2003-a)

Concerning the PPs (Parietal Pits):

their function is unknown, and moreover, they are 'only' *sporadically present* (see DeHaan 2003-b).

NVO PP(s)



ILO(s)

~ ~ ▶ CSO

Sense-organ-like parietal pits, sporadically occurring, found in Psammophiinae (Serpentes, Colubridae)

Cornelius C. de Haan

Abstract. Minute, sense-organ-like parietal pits are reported for several psammophine snakes of Bogert's (1940) African colubrid group XVI. Such pits do not occur not in all individuals, and unrelated to sex. If present, one to four pits are detectable per head, which most often are symmetrically disposed. Further research is required to clarify the function and why these pits are only sporadically occurring.

Minute, sense-organ-like parietal pits (PPs) appear to occur in several psammophine snakes which belong to the Bogert (1940) African colubrid group XVI. The pits have so far been found in *Dromophis lineatus* (Lined Olympic snake), *Malpolon monspessulanus* (Montpellier snake), several *Psammophis* species and *Rhamphiophis rubropunctatus*. However, they do not occur in all individuals, which is unrelated to sex. Their sense-organ-like internal structure has only been documented by microscopic photography of its "print" which is detectable in shed skins (De Haan, 2003-b). If present, PPs are in numbers of one to four per head; most frequently two, one in each parietal shield. They are most often symmetrically disposed, as shown in Fig.1(right), in a *Malpolon monspessulanus*. In this species, distributed in mediterranean type climate in N-Africa, SW-Asia and S-Europe, the frequency of PP-occurrence found in 100 randomly chosen, mostly wild individuals, and examined through their shed skins, was 9%. In 100 mostly congenial, captive *Psammophis phillipsi* individuals it was 60%. In *Psammophis schokari*, no PPs were found in the only ones examined so far: 21 rather congenial, captive individuals.

The reason why the PPs are sporadically occurring is fully obscure. Their equally enigmatic function does not seem to be related to any 'rubbing' behaviour typical for psammophines (cf. previous note), nor to the classically reported snake upper-head 'pits' (as in *Dasypeltis* and *Mehehya* sp.), which for the rest are not genuine pits (cf. De Haan, 2003-b).

Noticing PPs in dark pigment-spotted parietal shields as in Fig.1(left) is not evident. In Fig.1, the typical

female did not present any 'hidden' pit in the parietal shields, while the typically unspotted male upper-head showed presence of PPs in an evident way. (In *M. monspessulanus*, pronounced sexual dimorphism both in pigmentation and growth has been pointed out by De Haan, 1993-1999).

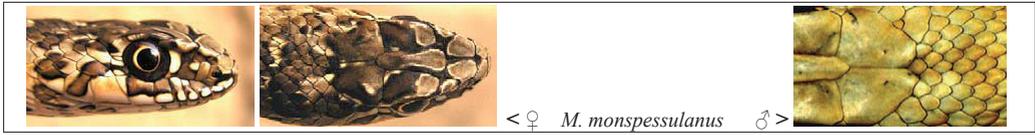
PP orifices vary in shape per individual snake, but do not change otherwise, except in growing with the overall head growth: their usual \emptyset is about 0.1 mm in small (30cm sv+t L) to 0.6 mm in big (>150cm sv+t L) individuals. Pits with a \emptyset twice as large as usual or even shaped as rather long slits exist, but seem to be rare. However, Chirio and Ineich (1991) presented a drawing of the holotype of the psammophine species *Rhamphiophis maradiensis* in which the parietal shields show symmetrically, which I presume to be two common PPs ('pits') and two uncommon ones ('slits').

If pits or slits in parietal shields are not symmetrically disposed, there is some more chance that they are not the PPs at issue, but banally due to injuries. Identifying their quality is relatively easy in live psammophines, that are kept in captivity for at least one skin shedding period in order to obtain and examine microscopically their subsequent slough.

Otherwise, preserved shed skins found in the field, e.g. 6 to 60 at the same time and place mid-summer, from about ten days young *M. monspessulanus* (6 to 12 indiv. per lay), may also be of use to PP-discoveries (Details in De Haan, 2003-b).

So far, low-budget histological examinations of some PP presenting psammophine heads, did not provide any unriddling result. Looking for and reporting on presence of parietal pits (PPs *sensu* De Haan, 2003-b) in psammophine individuals or any other snake, encountered in the field or preserved in a museum collection, as well as investigation of the PP-structure by sensorial physiologists, would be extremely useful.

Figure 1. *Malpolon monspessulanus*. Head of a female (left) and the parietal region of a male with PPs (right).



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Addendum

Even though we have not seen any thorough histological research on both the periodical ILOs and the sporadic PPs, it seems to me that an ILO (infralabial outlet) is induced when a *Psammophis* bites itself with one or two mid-maxillary teeth while keeping its mouth closed. The secretion that comes out extrabuccally may be 'regular' saliva, perhaps mixed with a product from Duvernoy's or another special gland. Based on continuing behavioural observations, the supposed function of the ILO secretion is more and more plausible, viz. marking of conspecific individuals, balancing in tree or bush, by rendering them vomerolingually identifiable. In contrast, the enigmas

around the PPs (parietal pits) still remain, except perhaps the one which is their sporadic occurrence.

Recently, findings possibly dealing with ILOs and PPs (*sensu* De Haan 2003) came to my attention, in particular those described and pictured by Miralles & Ieich (2006)*). The authors state that they have discovered structures in the Asiatic non-psammophine colubrid *Atretium schistosum* which are similar in shape and occurrence to the ILOs and the PPs described for Psammophiinae by De Haan (2003a+b). Indeed, two out of 12 in alcohol preserved *A. schistosum* show parietal pits (PPs), which from the outside look like those described by De Haan (2003b). Since naturally shed parietal epiderm or histological insight were not available, nothing is said about the *Atretium* PPs' internal aspect. In the meantime I (unpubl.) have found sporadically occurring PPs in two out of 9 individuals of another Asiatic non-psammophine colubrid, the *Orthriophis taeniurus* (formerly *Elaphe taeniura*). Thanks to shed skins presenting "prints" of their internal sides, the PPs show microscopic traits of an organoid structure. This internal structure requires further investigation, but so far does not seem similar to that of the psammophine PPs. Externally, however, the *Orthriophis* PPs are similar to common parietal pits and less common slits in both psammophines and *Atretium*.

On the other hand, concerning the "gular pits" of *Atretium*, I must stress that these are quite wrongly placed in the same paper by Miralles & Ieich (2006). Indeed, as the authors report gular pits equivalent to the "exclusive" psammophine "gular pits", found in 10 out of the same 12 *Atretium schistosum*, they confusingly call these pits also "small protuberances" and picture them as 1-3 minute circles on almost each scale of the snake's gular region. So, the *Atretium* gular pits may be novel pits or classic protuberances, but are in no way similar to the psammophine infralabial outlets (ILOs), not even when pseudonymized as "psammophine gular pits".

Apart from unfortunate quoting De Haan's (2003a+b) papers and indicating possible novelties, the *Atretium* article offers a plausible atavism hypothesis to explain the sporadic occurrence of the PPs actually known, and those expected to be found in more snakes, especially from basal lineages.

*) Miralles, A., Ieich, I. (2006): Presence of gular and parietal pits in *Atretium schistosum* (Serpentes, Colubridae), a singular trait not exclusive to psammophine snakes. C. R. Biologies 329 (2006): 180-184.

Surface structure of the infrared sensitive pits of the boa *Corallus hortulanus*

Jill Ebert, Anke Schmitz, Guido Westhoff

Abstract. Snakes of the subfamilies Boinae, Pythoninae and Crotalinae developed an infrared (IR) sense. The IR sensitive receptors (terminal nerve masses: TNMs) of most of these snakes lie in labial (boas and pythons) or loreal (pit vipers) pit organs. The surface structure of the epithelium in the IR receptor organs reveals a characteristic array of microscopic pores, which are associated with the IR sensitive TNMs. So far, only a few studies have been conducted to investigate these microscopic pores and none has been carried out for boa species with labial pits. In this ongoing investigation, the surface structure of one pit of the boa *Corallus hortulanus* was exemplarily examined. Scanning electron microscopy revealed the presence of two different types of microscopic pores in the pit fundus. This is the first record of microscopic pores in a boa with pits. Furthermore, one pore type has not been described yet.

Introduction

Infrared (IR) sensitive snakes occur in the family Boidae (Boinae and Pythoninae) as well as in the family Viperidae (Crotalinae). Snakes of all three subfamilies developed their facial infrared sensitive organs independently. Their IR receptors are terminal nerve masses (TNMs), which are connected via nerve bundles and the trigeminal nerve to the central nervous system. Crotalinae possess two loreal pits (e.g. *Crotalus atrox*), in which the IR sensitive receptors lie in a thin membrane suspended between an inner and outer chamber. In contrast to the Crotalinae, the boas and pythons possess their receptors in specialized labial scales, which differ in number, size and shape from species to species. The IR sensitive receptors lie either in labial pits (e.g. *Python reticulatus*) (Gopalakrishnakone 1984) or in the epidermis of the labial scales without specialized structures (e.g. *Boa constrictor*) (von Düring 1974). The surface structure of the epithelium in the IR receptor organs of Crotalinae, Boinae and Pythoninae reveals a characteristic array of pores, described as microscopic pores (Amemiya et al. 1995). The pores were only found in association with the IR sensitive TNMs. According to the family and snake species, differences in dimension and distribution of the pores exist. Amemiya et al. (1995) hypothesized the function of the microscopic pores to reflect visible light in order to enhance the resolution of the IR radiation. However, experimental evidence is lacking. Several morphological investigations on the IR sensitive organs of crotaline species and pythons have been carried

out, whereas little is known about boas. Among boas, nothing is known about species that possess labial pits. The Amazon Tree Boa *Corallus hortulanus* appears to be a suitable experimental animal since it possesses large IR sensitive pits. As this snake hunts at dawn and night (Bartlett 2003) a good resolution of the IR detection would be of great advantage. According to the existing hypothesis, specialized surface structures in the pits, e.g. microscopic pores, are to be expected.

Material and methods

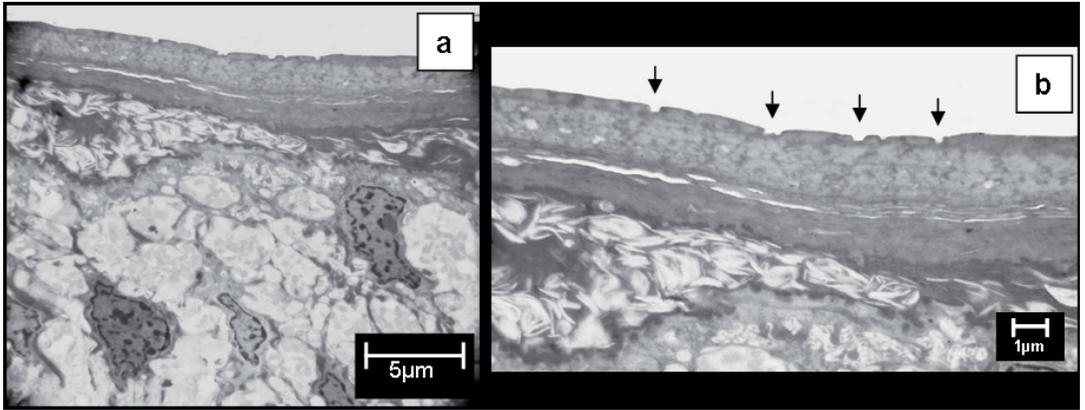
The labial scales of an anaesthetized and then dispatched juvenile *C. hortulanus* were dissected. The scales were dehydrated with ethanol and treated with hexamethyldisilazane (HMDS) for scanning electron microscopy (SEM). The HMDS treatment avoided shrinking or distortion of the tissue and thus well preserved the surface details. The labial scales were mounted on stainless steel stubs and sputter-coated with a gold layer (thickness: 30 nm) before the material was investigated with the SEM (Leo 440i, Leica, Nensheim).

For the transmission electron microscopy (TEM) examination, the dissected upper labial scales were kept in fixative (2.5% glutaraldehyde in 0.1M phosphate buffer) overnight. The material was washed with phosphate and cacodylate buffer, immersed in 2% osmium tetroxide for one hour, dehydrated in ethanol and embedded in Epon. Semi-thin sections (0.5 µm) stained with toluidine blue were used for light microscopy and for selecting characteristic areas for TEM. The ultra-thin sections for the TEM were stained with uranyl acetate and lead citrate and examined with a Zeiss 109 microscope.

Results

The 6th upper labial scale was exemplarily examined with light and transmission electron microscopy. In the pit of this scale TNMs were found (Fig. 1a). These TNMs were densely packed with mitochondria (light grey matter) and several bundles of TNMs were interspaced with epithelial cells (dark grey matter). In addition, indentations (microscopic pores) were discovered in

Figure 1. a: Electronmicrograph of the epidermal region of the 6th upper labial scale. b: Higher magnification of the surface of the Stratum corneum. Arrows indicate indentations.



the stratum corneum of the epidermis (Fig. 1b). The pores exhibit a diameter of less than 0.5 µm. In order to gain more information on the distribution and ultra structure of the pores, SEM studies were exemplarily undertaken with the 9th and 10th upper labial scales. The pit is situated between the scales and is one of the most pronounced pits of the caudal labial scales. The pit is covered with two different types of pores: i) pores with a diameter of about 0.3 - 0.5 µm and ii) small pores with a diameter of less than 0.2 µm (Fig. 2). The smaller pores are irregularly distributed over the pit fundus (Fig. 2b

and c). The larger pores can only be found caudally in the pit of the 10th scale and are evenly distributed (Fig. 2d).

Discussion

The investigations of the labial pits of *C. hortulanus* revealed TNMs in the pits. Furthermore, this study was the first to demonstrate that the pits are covered with different types of microscopic pores. However, the exact TNM area size and congruence with the labial pits was only demonstrated for one upper labial scale so far, and

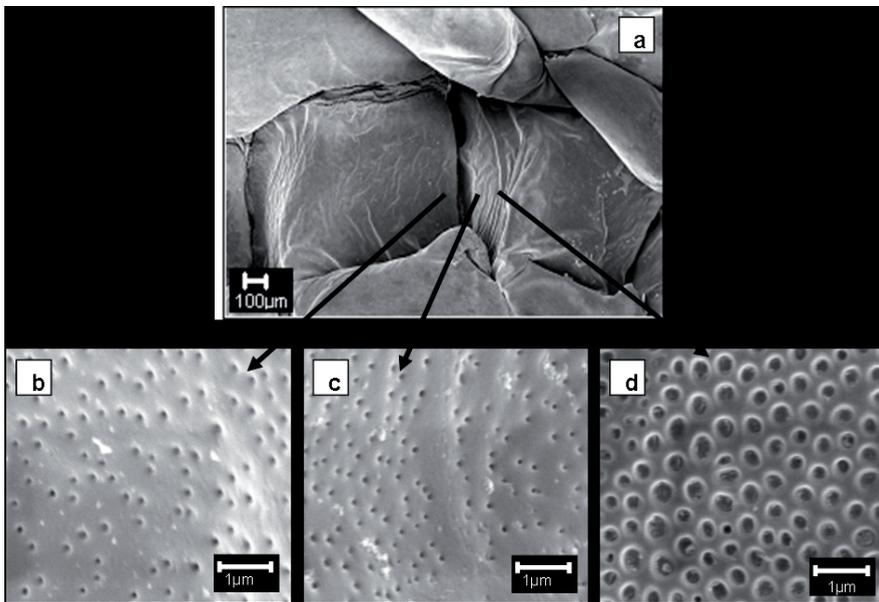


Figure 2. a: SEM picture of the labial pit between the 9th & 10th scale. The lower part leads towards the mouth, the upper part to the eye. b-d: Higher magnifications of the scale surface. Note the two different types of pores in the pit and their different distribution.

has to be thoroughly investigated for all labial scales in a forthcoming study. This information is necessary for an exact description of the IR sensitive areas and will serve as the base for the estimation of the sensitivity and resolution of the IR sense in this species.

The results of the SEM investigations revealed indentations in the Stratum corneum with diameters of less than 0.5 μm . These structures are similar to the microscopic pores of the crotaline and boid species examined so far (Amemiya et al. 1995). Amemiya and co-authors revealed only one pore type with a diameter of about 0.1 μm to 0.25 μm which was found in the pit fundus of the boid snakes *Python regius* and *P. molurus*, as well as on the membrane of the crotaline snake *Agkistrodon blomhoffi*. In these species the TNMs lie only in regions where pores are present. *Boa constrictor*, however, possesses larger pores (0.3 – 0.5 μm) which are not confined to the IR sensitive areas of the labial scales (Amemiya et al. 1995). The larger pore type in the pits of *C. hortulanus* looks similar in size, shape and distribution to the pores found in *B. constrictor*. However, *C. hortulanus*' pit fundus is also covered by much smaller pores (about 0.2 μm in diameter), which have not been described so far.

The present study shows that boas with labial pits possess specialised surface structures (two different types of pores) in the IR sensitive regions. Therefore, these findings support the hypothesis that microscopic pores possibly enhance IR vision in snakes. Efficient

absorption of IR radiation will be different between the loreal pits of crotaline snakes, the labial pits of boids and the IR sensitive scales of pitless boids. The form of the labial pits combined with the distribution of the pores and the TNMs probably allows and enhances a directional perception of the IR radiation. Further investigations of the differences and similarities of microscopic pores in different snake taxa will increase our knowledge on IR detection in snakes.

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Modelling imaging performance of snake infrared sense

Andreas B. Sichert, Paul Friedel, J. Leo van Hemmen

Abstract. Several snake species use infrared-sensitive pit organs to localise prey. These sensory organs enable the snake to successfully strike prey items even in total darkness or following the disruption of other sensory systems. The pit organ has traditionally been thought to function as a pinhole camera. The need, however, to gather a reasonable amount of thermal energy per time unit (second) necessitates the “pinhole” of the pit organ to be very large, thus greatly reducing its optical performance. Although the image that is formed on the pit membrane has a very low quality, the information that is needed to reconstruct the original temperature distribution in space is still available. In this paper, we present an explicit mathematical model that allows the original heat distribution to be reconstructed from the low-quality image on the membrane.

Introduction

Pit vipers and boids possess infrared (IR) sensitive organs that play an important role in their sensory ecology. Newman and Hartline (Newman and Hartline 1982) suggested that rattlesnakes can strike a heat target with a precision of 5°. Furthermore, the infrared-sensitive pit organs of rattlesnakes project into the *Tectum opticum* forming a spatiotopic map (Newman and Hartline 1981). These findings suggest a considerable spatial resolution of the neuronal image that is formed by the IR organs.

Several snake species have a very high sensitivity to IR radiation detecting temperature differences down to 0.003°C (Bullock and Diecke 1956; for further references see Molenaar 1992). In contrast to the retina, the IR sensing mechanism seems not to be based on a photochemical reaction. Rather, the temperature of the membrane causes the nerve cells to respond (de Cock Buning et al. 1981). In addition, structural specialisation of the periphery containing the receptor cells enhances the sensitivity. Following (Molenaar 1992) we may distinguish three different types of infrared sensitivity in snakes:

1. “*Boa* type” organs: IR-sensitive cells are found on the supra- and infralabial scales, and no specialisation of the scale surface is present;
2. “*Python* type” organs: the sensitive cells are again found supra- and infralabial within grooves formed by the scales;
3. “*Crotalus* type” organs: the heat-sensitive cells lie within a thin (15 µm) membrane that is suspended in the pit organ, lowering the detection threshold; see figure 1.

Only one pit organ is present at each side of the head, whereas in the first two types, multiple IR-sensitive sites may be present at both sides of the head.

The *Python* and *Crotalus* type of sensors are traditionally thought to function as pinhole cameras: an incoming light ray passes through the opening (aperture) of the organ and hits the light-sensitive membrane. The superposition of all incoming light rays then forms an image of the original heat source on the membrane. If the aperture of the organ is very small, the image of a light ray coming from one point is approximately a point. That is, if the opening is small, one point on the membrane corresponds to exactly one point in space outside. If on the other hand the aperture of the organ is large, the image of a point source of heat is disc-shaped rather than point-like. Since, however, the size of the disc-shaped image may be determined by the detectors on the membrane, it is still possible to tell from which direction the radiation comes, ensuring directional sensitivity of the system; cf. figure 1.

The above approach breaks down if the organ is stimulated with real input. Unlike in the laboratory case, point sources of heat are hardly found in nature. Not only do prey animals subtend a significant angle within the view field of the organ, but distraction and noise may be present as well. Several sources of heat then project onto the membrane at the same time and the resulting picture will not at all be disc-shaped. The question we wish to answer in this paper is how the snake may be able to extract information on the location of the prey from the blurred image that is formed on the pit-membrane.

Optical treatment of the problem

To understand the nature of the problem we are dealing with, it is necessary to take a closer look at the input

signals of the IR organs. Any source of heat, such as a prey animal, emits IR radiation. The radiation intensity is distributed over a wide range of wavelengths, the exact profile depending on the surface temperature of the source. Warm objects emit more energy than cool objects and the emitted radiation intensity is concentrated around smaller wavelengths. The radiation emitted by an animal with a surface temperature of 30°C can be described accurately by Planck's law of radiation. We then find that the radiation intensity is maximal for a wavelength of 9.5 μm , which lies

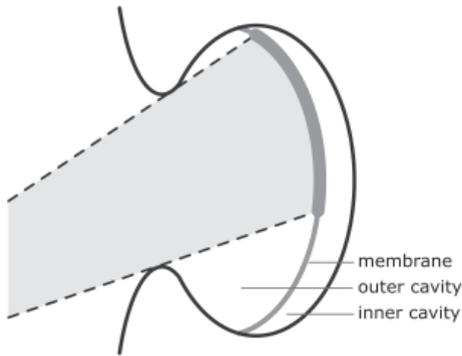


Figure 1. The snake infrared-sensitive pit organ consists of a cavity where a thin membrane containing heat-sensitive nerve receptors is suspended. Because the membrane is suspended, heat reaching the membrane cannot diffuse into adjacent tissue and the membrane is more effectively heated, enhancing the heat sensitivity. An incoming light ray will illuminate a disc shaped region on the membrane. The opening of the organ and its depth measure about 1 mm.

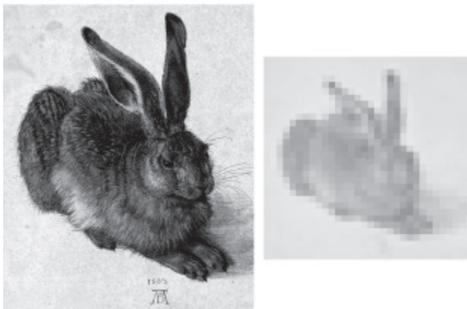


Figure 2. This engraving by the German artist Albrecht Dürer from 1502 (left) has been used as a test for the reconstruction model. The image was converted into an artificial heat distribution, shown on the right. The heat distribution is not biologically realistic. It only serves to demonstrate the capabilities of the proposed model.

exactly within the atmospheric transmission window for far-IR radiation (8-12 μm). Since a typical IR organ measures about 1 mm, the characteristic wavelength of the incoming radiation is much smaller than the organ size and therefore, we may neglect bending and refraction effects as the radiation enters the organ (see e.g. Tipler and Mosca 2004); all light rays entering the organ follow straight lines. This may seem trivial but the condition that the organ size is large compared to the radiation wavelength is not automatically fulfilled for small systems detecting IR radiation and hence should be checked.

As mentioned above, the image on the membrane resulting from the total heat distribution in space will be some complicated shape that consists of the sum, i.e., the superposition of the contributions of all heat sources. This point is made clear in figure 3. We took the famous engraving by Albrecht Dürer depicting a hare and converted this into a “heat intensity profile” (see figure 2). We then calculated the resulting heat distribution on the membrane (see equation 4). In panel A of figure 3, we see what happens for a very small aperture. Since every point in space corresponds to effectively one point (pixel) on the membrane, the heat distribution on the membrane is just a scaled upside-down version of the heat distribution in space. The case is rather different if the aperture of the organ is large. Then, the image on the pit-membrane does not at all look like the original heat distribution (panel B of figure 3).

The obvious question therefore is: Why does the pit organ have such a large aperture? The answer to this question is simple. If the aperture was very small, the amount of energy per unit time (second) reaching the membrane would also be small. This means that the snake would have to wait a long time before enough energy entered the organ to allow for a reliable neuronal response. Even worse, as neuronal integration times are finite, the heat object would hardly ever become observable. So there is a trade-off here. Either the snake ensures that the organ gets strong enough input (large aperture), or the snake forms a sharp image on the membrane (small aperture). Since prey animals are generally moving, it is not an option to wait for enough heat energy to enter the organ. Without the ability of real-time imaging the IR organ would be of little use for the snake.

In the next section, we will argue that it is possible to *reconstruct* the original heat distribution using the blurred image on the membrane. Hence the snake can afford a large aperture organ without losing the important information contained within the signal.

The reconstruction model

We start by presenting the necessary mathematics to gain a minimal understanding of the model. An extensive and mathematically rigorous version of the model has been published elsewhere (Sichert et al. 2006).

Before we can reconstruct the heat distribution in space, we must know the heat image on the pit-membrane. First, we consider the membrane image that is formed when a single point j in real space with intensity \mathbf{O}_j is considered. For the heat intensity at one membrane point i we have

$$(1) \quad \mathbf{I}_i = \mathbf{T}_{ij} \mathbf{O}_j$$

That is, the intensity \mathbf{I}_i at the membrane point i is given by the intensity in space \mathbf{O}_j multiplied by a *transfer function* \mathbf{T}_{ij} . To find the total heat image on the membrane, we must sum the contributions from all points in space,

$$(2) \quad \mathbf{I}_i = \sum_j \mathbf{T}_{ij} \mathbf{O}_j$$

The transfer function describes how effectively heat from a point j can reach the membrane point i . If the point j in space is not visible from the membrane point i (the pathway may be blocked by the organ edge), then there is no heat transfer possible and \mathbf{T}_{ij} vanishes. If the point j is visible, the law of conservation of energy determines the transfer function. The heat reaches the membrane scales with the inverse square distance to the heat source \mathbf{r}_{ij} (since the radiation energy spreads out over a spherical surface area), and with the cosine of the angle ϕ_{ij} . This takes into account that the effective area of the membrane that is “seen” by the incoming radiation is dependent on the incidence angle. We have thus

$$(3) \quad \mathbf{T}_{ij} = \begin{cases} \frac{\cos \phi_{ij}}{|\mathbf{r}_{ij}|^2} & \text{if } j \text{ is visible from } i \\ 0 & \text{otherwise} \end{cases}$$

To include omnipresent noise, we add a stochastic term to the membrane intensity as given by (2) and obtain

$$(4) \quad \mathbf{I}_i = \sum_j \mathbf{T}_{ij} \mathbf{O}_j + \chi_i$$

The variable χ_i has a Gaussian distribution with zero mean and standard deviation σ_{χ_i} . The membrane intensity in (4) is just what the IR sensors on the membrane measure. The next step is trying to reconstruct the *original* spatial heat distribution from the image on the membrane. That is, we try to find a reconstruction, which can be formed from the measure membrane intensity,

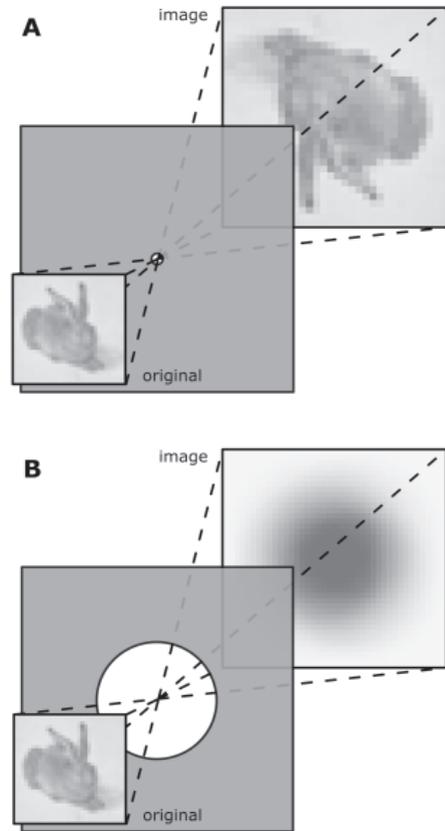


Figure 3. Schematic display of the imaging process. The image chosen here is not biologically realistic. The image only serves to demonstrate the capabilities of our model.

A. If the aperture of the organ is very small, each point in space corresponds to exactly one point on the membrane. Apart from rotation and scaling, the membrane image directly represents the spatial heat distribution.

B. If the aperture is large (comparable in size to the organ depth), the image that is formed on the membrane is blurred. Because light coming from different directions may hit the same point on the membrane, the information content of the spatial heat distribution is smeared out over the membrane.

$$(5) \quad \hat{\mathbf{O}}_j = \sum_i \mathbf{R}_{ji} \mathbf{I}_i$$

The matrix \mathbf{R}_{ij} must now be determined. However, the mapping that was defined in equation (4) has no inverse since some of the information that was present in the original heat distribution is lost and, in addition, equation (4) is of a stochastic nature. The best we can do is to find optimal values for the components of \mathbf{R}_{ij} . We define the error of our estimate (5) as follows,

$$(6) \quad E = \left\langle \sum_i (\hat{O}_i - O_i)^2 \right\rangle$$

To calculate (6), we only have to compute the statistical properties of the input \mathbf{O}_i . The value of \mathbf{O}_i itself need not be known. In our model, we assume that the original heat distribution is totally uncorrelated: knowing the value of \mathbf{O} at any point does not say anything about its value at other points. In reality, the spatial heat distribution will tend to vary smoothly, meaning that neighbouring points in space tend to have heat intensities that are closely alike. We assume, however, that the snake does not “know” this and therefore expects an *a priori* uncorrelated distribution. After reconstruction, the spatial heat distribution may well turn out to be highly structured, as indeed it will in reality often be. In fact, assuming uncorrelated input makes reconstructing the signal *more difficult* and we are dealing with a “worst case scenario”.

We may now calculate the components of the matrix \mathbf{R}_{ij}

to minimise the error (6). Having found the right matrix, we can calculate the “best estimate” of the spatial heat distribution, given the measured heat distribution on the membrane.

Results and discussion

The results of one sample calculation are displayed in figure 4. For the original heat distribution, we used the Dürer picture as in figure 2. The resulting membrane image is shown in figure 4 on the left. The image is severely blurred because the information present in the original heat distribution is distributed over the entire membrane surface. It seems unrealistic that a spatiotopic map could be built using this measured membrane input. On the other hand, after application of equation (5), the image quality is enhanced spectacularly, as shown on the right in figure 4. The original image is clearly recognizable, even in the low resolution that was used in the calculations.

We hope that at this point we made clear that, although the imaging capabilities of the pit-organ are poor, it is still possible to reconstruct the spatial heat distribution. The model we used here is of a mathematical nature. The assumptions that went into the calculations are a “worst case scenario”. For instance, we assumed that the input to the pit organ is totally uncorrelated, meaning that the snake has no idea what heat distribution to expect. In reality, important information about the environment is always available. For example, typical temperature and size of a prey animal may be encoded in the neuronal

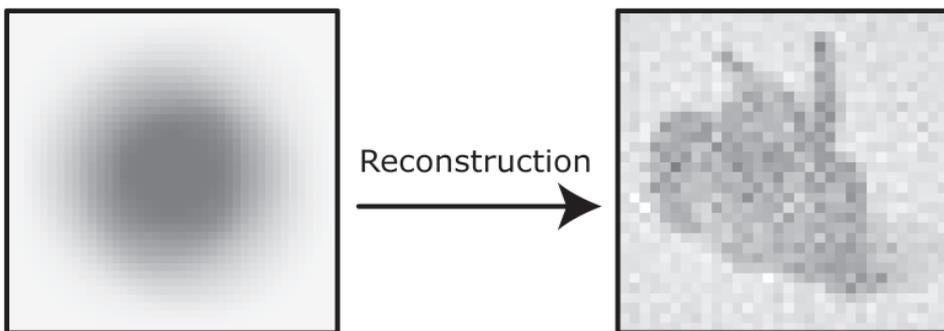


Figure 4. The membrane image on the left still contains enough information to reconstruct the original heat distribution (compare figure 2). The quality of the reconstruction is quite amazing. For computational reasons, the heat distribution and the membrane have been taken as squares, but the model works equally well if applied to a realistic membrane shape. Computational parameters are: organ aperture: 0.8 mm; depth of membrane: 0.86 mm; size of membrane: 1.2 mm x 1.2 mm; number of sensitive cells on the membrane: 41 x 41; spatial heat distribution resolution: 32 x 32 points.

processing structure. If the snake “knows” what kind of images to expect, the reconstruction process can be enhanced considerably.

A neuronal implementation of the model is fairly straightforward. In neuronal terms, the matrix \mathbf{R}_{ij} would correspond to a network of connections between the membrane IR detectors and tectal neurons building the map. The strength of the individual connections would just be the value of the corresponding entry in \mathbf{R}_{ij} . Optimisation of the coefficients in the matrix could be obtained by comparing the information from the IR system with the visual map in the *Tectum opticum*, thus learning the right neuronal connection strengths (see for instance Franosch et al. 2005).

The obvious next step would be to formulate the model in biological terms, using realistic, spiking neurons, realistic input and physical properties of the pit organ. Adding these components to the model would presumably not change the main conclusion of this paper: the information needed for reconstruction is still present in the membrane image.

At present it is not known how accurate the snake IR system really is. It has been shown that the IR system suffices for localising prey without using other sensory input. Furthermore, a tectal map is formed that lies on top of the visual map (Newman and Hartline 1981). These findings suggest that considerable accuracy is indeed possible using the IR sense. We have shown that such accuracy can be obtained using the (low-quality!) imaging hardware that snakes possess, with some basic neuronal processing.

The experiments described in (Newman and Hartline 1982) are highly simplistic. A soldering iron was used as a heat source in experiments measuring the strike accuracy. Being point-like and having a temperature that does not correspond to biological reality, a

soldering iron can hardly be considered as an accurate prey animal model. These experiments therefore cannot be considered as conclusive. Many questions about the IR system still require a truly quantitative answer: What is the striking accuracy if snakes must rely on the IR system *only*? What is the detection range of the IR sense? What is the influence of background noise and distraction? Nevertheless we hope that this paper made a contribution to the interesting debate as to how snake infrared localisation works.

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Infrared detection in the rattlesnake *Crotalus atrox* – from behavioural studies to midbrain recordings

Guido Westhoff, Marco Morsch, Jill Ebert

Abstract. We investigated the infrared detection range of *Crotalus atrox* for a mouse like stimulus for the first time in a behavioural approach. The results propose a detection range of merely 1 m which greatly exceeds all detection distances previously reported for Crotalinae. In addition we report from physiological experiments based on multi unit recordings and recordings of evoked potentials within the midbrain of rattlesnakes while applying infrared stimuli. The two different ways of measuring response profiles revealed evidence for an enhancing mechanism in the midbrain Tectum opticum. This enhancing mechanism is proposed as a possible reason for differences reported in detection ranges for mouse-like infrared stimuli.

Introduction

Pitvipers (Crotalinae) like rattlesnakes have a name giving pit organ between their nostril and eye on each side of the face (Bullock, 1957; Lynn, 1931; Noble and Schmidt, 1937). These organs enable the snakes to detect minute thermal differences within their environment as they are highly sensitive to infrared (IR) radiation (Bullock and Diecke, 1956; de Cock Buning, 1983a, b; Molenaar, 1992). In physiological experiments different detection thresholds have been determined in crotaline snakes (Bullock and Diecke, 1956; de Cock Buning, 1983b). Based on these values the calculated detection range for crotaline snakes for a mouse-bourne stimulus varies from less than 5 cm (Jones et al., 2001) to 66 cm (de Cock Buning, 1983b). Thus, the actual detection distance remains more or less unclear. In an attempt to fill this gap we investigated the IR detection threshold and sensitivity of the rattlesnake *Crotalus atrox* for a mouse-like stimulus in a behavioural approach.

To understand the striking differences of determined threshold levels we made physiological recordings within the midbrain of rattlesnakes. The Tectum opticum of the midbrain is the most important brain area for the integration of visual and IR sensory cues as well as for representing a topographic map of the visual and IR field of view (Hartline et al., 1978; Newman und Hartline, 1981; 1982). Besides several qualitative analysis (de Cock Buning, 1983b; Hartline, 1971; Kass et al., 1978; Newman and Hartline, 1981; Terashima and Goris, 1976; Wells et al., 1971) the neural tuning characteristics of IR sensitive tectal units are not understood.

We investigated the tuning characteristics of IR sensitive tectal units of pitvipers by means of multi-unit recordings and recordings of evoked potentials. By comparing tuning characteristics of evoked potentials as well as afferent fiber units we found evidence for enhancing mechanisms for IR stimulus processing on the tectal level.

Material and methods

Sixteen captive born *Crotalus atrox* (snout-vent lengths of 40 to 70 cm) were used for the studies. The animals were housed individually in terrariums with a hide box as a shelter at an ambient temperature of 28 ± 1 °C and at a 12-hour light/12-hour dark cycle. The snakes were provided with water *ad libitum* and maintained on a diet of one mouse every 10 to 12 days.

Twelve individuals were blindfolded with a strip of black duct tape. Animals were tested in a circular arena (200 cm in diameter) shielding the snake from unwanted IR stimuli. The experimental setup was as described elsewhere (Ebert and Westhoff, 2006). The snakes had a body temperature of 24 ± 1 °C (room temperature was 23 ± 1 °C), measured with an IR-thermometer (minitemp TM, Raytech, USA).

The IR stimulus was presented to the snakes and set into oscillation (0.5 Hz, amplitude 30 cm) randomly at distances from 10-150 cm (± 0.5 cm) whenever the snakes faced the stimulus. A number of behavioural responses (head jerks, tongue-flicking, freezing in a movement and rattling) were evaluated as "positive response" and pooled together. As these behaviours may also occur spontaneously the spontaneous behaviour was quantified in sham experiments. Controls for hidden cues with blocked pits and/or switched off heat element were carried out as well.

Sixteen individuals were kept under anaesthesia as described elsewhere (e.g., Moiseenkova et al., 2003) and the preparation of the midbrain Tectum opticum was carried out as has been described by Goris and Terashima (1973). A red diode laser (TIM 203/5 mW, ELV Elektronik, Germany) with a wavelength of 640-650 nm focused with a lens into position within the snakes' pits was used as heat stimulus (stimulus duration 1 s). A visual barrier of black plasticine was applied between the loreal pit and the eye. A razor blade attached to a micromanipulator was driven into the laser beam to reduce the power from 5 mW to 3 μ W (measured with Fieldmaster FM, Coherent). Recordings were done in

the Tectum opticum of twelve animals as described elsewhere (Westhoff and Morsch, submitted). Evoked potentials (EP) were recorded in four animals in the middle of the midbrain contralateral to the site of stimulation (for methodology see Westhoff and Morsch, submitted).

Results

Behavioural experiments

The snakes were exposed to 1705 IR stimuli, offered at distances between 10 cm and 150 cm in 130 sessions. Pooled in 5 cm distance intervals positive responses decreased from 72% (shortest source distance 10 cm) to 6% (farthest source distance 150 cm). At distances of less than 35 cm to the IR stimuli the snakes responded the stimulus in 52-72%. About 50% of the snakes responded at distances between 35 and 75 cm and the behavioural responses declined continuously at farther distances. However, there was a sudden drop in responses at about 100 cm and the responses finally dropped to 6%.

The data of the 465 control trials were pooled, 38 of which were scored as "positive response". The control trials were tested against the IR trials which revealed a significant positive response for distances up to 95 cm (Chi²-Test, Chi² = 20.367, df = 1, p < 0.05). There was no significant difference at the distance of 100 cm (Chi² = 0.839, df = 1, p = 0.36).

Physiological experiments

None spontaneously active IR sensitive multi-units (N = 30) were recorded in twelve animals ($1 \leq n \leq 4$) with interspike intervals of less than 2 ms. Individual spikes of the multi-unit responses had a duration of 0.15 – 1.40 ms (N = 5, n = 450, $20 \leq n \leq 370$). Bimodal IR and visual multi unit responses or unimodal or exclusively visual multi units were not found. Testing the neural response for different stimulus intensities the maximal laser intensity was either 1600 μ W (N = 19) or 3200 μ W (N = 11, c.f. Fig. 2A). The spike rate (number of spikes) of the multi-units increased with increasing stimulus intensity. Saturation of spike rate was reached at intensities between 600 μ W and 1000 μ W. The slope of the increasing spike rate differed considerably between units and the maximal numbers of spikes varies from 14 to 100 (examples are given in Fig. 2A). Thus, the multi units reflect variable activity profiles covering a wide range.

Evoked potentials (EP) were recorded in three animals. IR stimuli of 1000 μ W were applied while recording in steps of 100 μ m from the tectal surface to a depth of 2000 μ m. Maximal amplitudes of EPs (peak to peak) were observed at a depth of 800 μ m to 1000 μ m. Recordings were obtained from the centre of the contralateral tectal

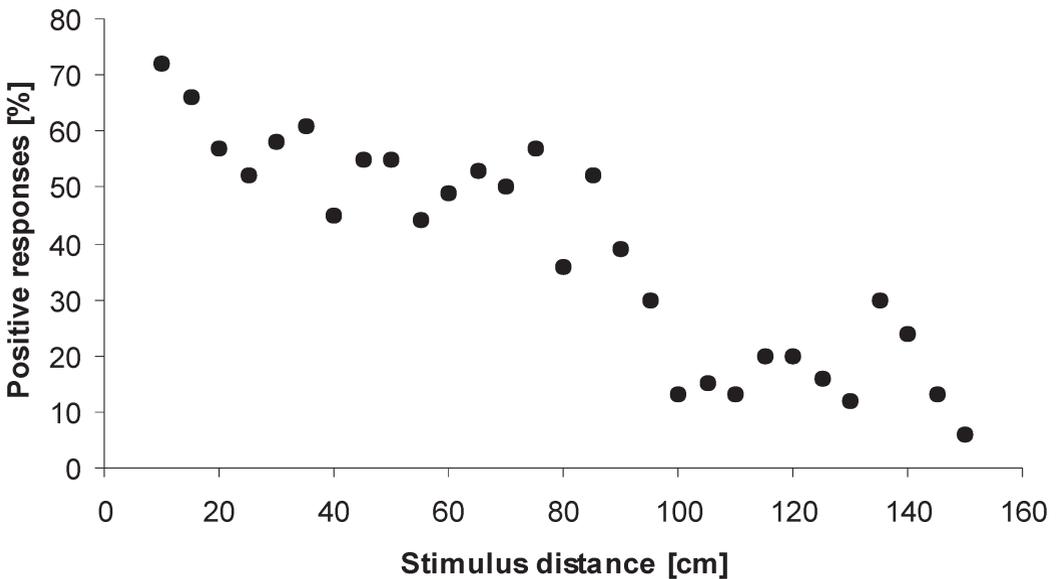


Figure 1. Percentages of positive responses as function of distances of IR stimuli. Note the sudden drop of positive responses at distances of 90–100 cm.

hemisphere whereas the laser stimulus was focused on the centre of the pits membrane. Depth profiles were obtained under same stimulus conditions at a number of slightly different sights in the Tectum opticum. Intensity profiles were recorded at the sight and depth of highest EP amplitudes for intensities of 3 - 2000 μW . The amplitudes of evoked potentials were again measured peak to peak and plotted as function of stimulus intensity (Fig. 2B). Increasing stimulus intensities up to 350 μW resulted in a steep increase of EP amplitudes. In contrast, stimulus intensities higher than 350 μW resulted in almost no increase of EP amplitudes (c.f. Fig. 2B).

Discussion

Behavioural responses

The type of behavioural response (head jerk, tongue flicking, rattling) was irrelevant for this study as the focus laid on a distinct change in behaviour during a 10 s stimulus presentation. The change in behaviour revealed whether the stimulus was perceived by the snake or not. However, conclusions can only be drawn from positive reactions. Thus, a snake that didn't respond might still have perceived the stimulus. In conclusion the actual detection threshold should be considered even more sensitive and the detection distance further than the provided values in this study. Our data support that *C. atrox* detected the IR stimulus at least up to a distance of 95 cm. This is a considerably greater distance than proposed by the calculations of de Cock Buning (1983) (for *Calloselasma*: 66.6 cm) or Jones et al. (2001) (for *Crotalus*: < 5 cm).

Physiological experiments

The width of a spike can be used to distinguish a spike originating from a cell soma from fiber spikes (Zittlau et al., 1986). The spikes of the multi units recorded in the present study had durations of maximally 1.5 ms. In contrast, recordings of single cells within the optic tectum of rattlesnakes revealed a spike duration of 2 to 6 ms (unpublished data). We therefore conclude that the spikes recorded here are from afferent IR processing fibers entering the Tectum opticum rather than the tectal cells. The spike rates of multi units reached a steady state at a stimulus intensity of 600 μW to 1000 μW . In conclusion we consider these values as the saturation levels for IR stimulus intensities of the afferent signals entering the Tectum opticum.

Evoked potentials recorded here can be regarded as the output of IR-sensitive tectal cells. The amplitudes of

EPs increased rapidly with increasing stimulus intensity up to app. 300 μW . This is a 2 to 3 times lower value in comparison to the saturation of spike rates recorded from multi units. Moreover, the increasing of EP amplitudes with increasing stimulus intensity is characterized by a strong slope. In contrast, the dynamic characteristics of spike rates over stimulus intensity are highly variable. Jones et al. (2001) calculated the detection range of a rattlesnake for a mouse like IR stimulus to be less than

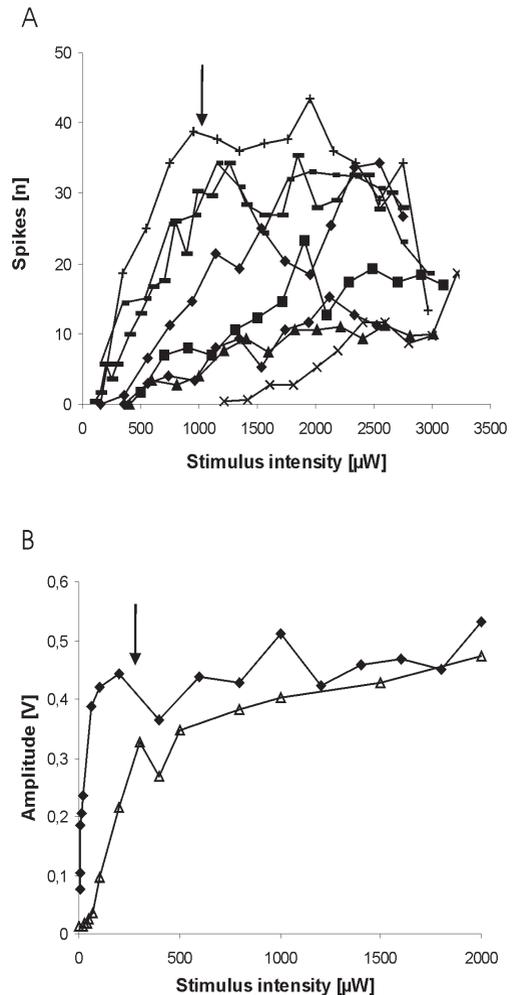


Figure 2. A) Stimulus intensity depending response profile of 8 IR sensitive multi units recorded in the Tectum opticum. The spike rates are plotted as function of stimulus intensity. The arrow indicates the approximate stimulus intensity of spike rate saturation. Note the difference of response profiles. B) Stimulus intensity depending response profiles of evoked potential amplitudes. Arrow indicates approximate stimulus intensity at amplitude saturation of evoked potentials.

5 cm and they were aware that this did not meet the snakes' capabilities. As the calculation was based on an IR threshold determined at the trigeminal nerve, the authors suggested that there might be an enhancing mechanism in the central nervous system. The results of the present study favour the hypothesis that incoming signals of afferent IR sensitive fibers are enhanced by IR sensitive tectal neurons.

The highly variable dynamic range of spike rates in multi units again favours the interpretation that these multi units reflect afferent fibers and thus represent the tectal input. The variability of input units would enable the system to process different stimuli always with the maximum sensitivity and maximum dynamic enhancement. However, further studies are needed to understand the cellular mechanisms responsible for these enhancing processes.

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Auditory atavism and integrated pathways for hearing in snakes

Bruce A. Young

Abstract. Although the auditory sensitivity range of snakes is limited, snake hearing may not be as restricted as previously thought. Three (non-exclusive) hypotheses for the ophidian auditory frequency response are advanced herein: fluid dampening, impedance matching, and neural physiology.

There are now multiple physiological and behavioral studies demonstrating that snakes can respond to vibrational stimuli in the air, ground, or even water (e.g., Westhoff et al., 2005; Young, 2003, 2005). Two earlier physiological studies reported the auditory frequency responses of snakes; while the ranges were similar, the values reported by Hartline (1971a, 1971b) were more restricted than those reported by Wever (1978). These differences may reflect the underlying methodological approaches: Hartline used intracellular recordings while Wever relied on cochlear microphonics, a technique that has been criticized as potentially over-representing auditory ranges (e.g., Manley, 1990).

Young and Harris (ms sub) presented rattlesnakes airborne stimuli of different frequency and amplitude combinations, and reported numerous positive responses beyond the auditory range described by either Hartline (1971a, 1971b) or Wever (1978). These results suggest that snake hearing may not be as restricted as previously thought; nevertheless, the auditory sensitivity range of snakes (from approximately 50 — 1,000 Hertz) is limited, even when compared to other squamate reptiles (Wever, 1978).

A proximate explanation for the restricted auditory range of snakes has never been developed. Herein I will advance three (non-exclusive) hypotheses for the ophidian auditory frequency response; fluid dampening, impedance matching, and neural physiology. In the mammalian auditory system the displacements of the stapes at the oval window create pressure waves within the perilymphatic fluid that course through the bony labyrinth, around the helicotrema, and ultimately are dissipated through the round window into the middle ear cavity. In the

ophidian auditory system the middle ear cavity is reduced to a small space immediately surrounding the stapes (Wever, 1978) or is lost completely, and there is no round window. As a result, pressure waves within the perilymphatic fluid essentially deflect off of the back of the bony labyrinth, change direction, and radiate back towards the stapedia footplate. Wever (1978) termed this unusual perilymphatic circulation pattern the reentrant fluid circuit.

These perilymphatic pressure waves are ultimately dissipated at the pericapsular sinus, a membranous extension off the bony labyrinth that surrounds the stapedia footplate and the proximal portion of the body of the stapes. Depending on the exact shape and size of the cochlea (see Miller, 1968) some frequencies of perilymphatic pressure waves may reach the pericapsular sinus at significant enough amplitude to effectively dampen the displacements of the stapes, thereby reducing the auditory range of the snake. A model of the stapedia displacements and resulting fluid mechanics within the cochlea (e.g., Ruggero and Temchin, 2002; Gan et al., 2004) could determine what impact, if any, the reentrant fluid circuit has on frequency range.

The most commonly cited model for vibration detection in snakes, what Wever (1978) described as the “substrate hypothesis,” posits that groundborne vibrations are received and transmitted by the lower jaw and its suspensory system. In order for an external vibration to be converted into a perilymphatic pressure wave in this fashion, the displacements would have to travel through the following structures: the scalation of the lower jaw, the underlying connective tissue, the bones (and joints) of the lower jaw, the quadrate/articular joint, the long axis of the quadrate, the quadrate/extrastapes joint, the long axis of the extrastapes, the extrastapes/stapes joint, and finally the long axis of the stapes. The biomechanical properties of each link in this transmission chain could impact the

frequency and/or amplitude of the vibratory wave.

Since impedance matching is frequency sensitive, an impedance mismatch between the ground-borne vibration and any link in this chain could differentially impact certain frequencies resulting in the narrow auditory range of snakes. Wever and Vernon (1960) and Wever (1978) provided some evidence for impedance matching by stimulating different component parts directly, however the details of vibrational transmission through this functional chain have never been explored. It may be particularly profitable to examine the relationship between auditory sensitivity range and the morphology of the contact between the stapes and the quadrate. There is significant morphological variation in this region, including the number of intervening cartilaginous bodies (the extrastapes), the area of contact, and the extent of connective tissue sheathing between the two bones (e.g., de Beer, 1937; Kamal and Hammouda, 1965; Wever, 1978; Rieppel, 1980). The limited data presented by Wever (1978) suggest that species with a more "open" stapes/quadrate joint also have lower auditory sensitivities and more restricted frequency ranges. The impact of impedance matching could also be placed in a broader context by looking at hearing performance within other squamate taxa that include forms with and without tympanic membranes (Mertens, 1971; Wever, 1978) or forms in which the tympanic membrane has been occluded by the scalation (e.g., *Tympanocryptis*).

Hartline (1971a, 1971b) reported that the latency and refractory periods of mid-brain auditory neurons were significantly slower than those of other terrestrial vertebrates. In fact, the cellular performance of these neurons was divergent enough that Hartline questioned whether snakes could process vibrational information in a behaviorally relevant way. Since Hartline was recording from the mid-brain of the snake, the physiological properties he quantified could have arisen at either a peripheral receptor (a cochlear hair cell), a relay neuron (such as the Nucleus Magnocellularis), or within the auditory mid-brain itself. Furthermore, these unusual temporal properties could be produced within a single neuron (presumably by differences in gated channel proteins) or through the interaction of multiple neurons (perhaps by differences in post-synaptic receptors).

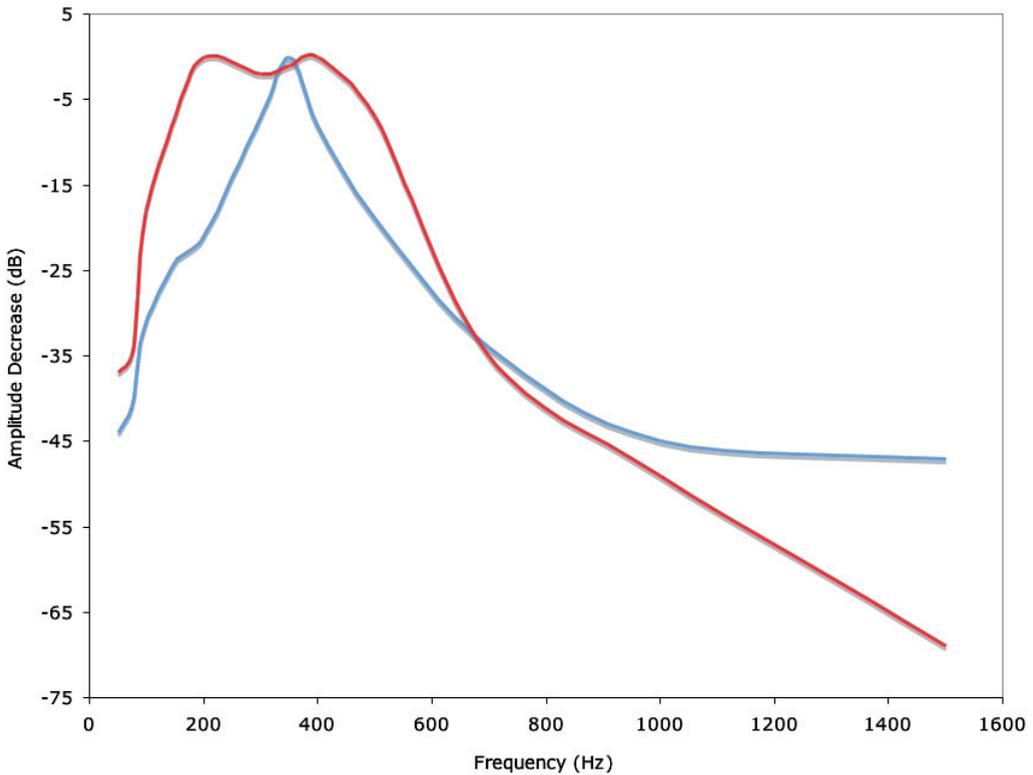
In theory at least, these same functional attributes

could affect frequency response as well as temporal response. The same neural physiology that led Hartline to question the behavioral significance of vibratory stimuli to snakes could also limit the frequency range of the auditory response. Could the narrower frequency range reported by Hartline (1971a, 1971b), as compared to Wever (1978), simply reflect the fact that Hartline was recording from higher up in the auditory system, and thus found additive influences from the additional neurons? It would be informative to obtain intracellular recordings from the basilar papilla and to compare these to both Wever's (1978) cochlear data and Hartline's (1971) mid-brain data.

In addition to these three (non-exclusive) proximate hypotheses, it may be informative to examine the auditory range of snakes from a broader (ultimate) perspective. The phylogeny of snakes remains uncertain. The classic scenario for the evolution of snakes, often referred to as the "fossorial theory," proposes that snakes evolved through a fossorial phase, leading to reduction then loss of the limbs, loss of the external ear, and degeneration off the eye (e.g., Walls, 1942). This scenario has been challenged by workers who have argued for an aquatic ancestral environment (e.g. Lee and Caldwell, 2000). The fossorial theory is relevant to snake hearing not only in accounting for the loss of the external ear, but perhaps also by explaining the restricted auditory range.

Sandy soils propagate vibrations best over a rather narrow range of frequencies centered around 300 Hertz (e.g., Aicher and Tautz, 1990); indeed, the frequency profiles for vibratory propagation in soil and auditory reception in snakes are very similar (Fig. 1). Interpreted this way, the frequency response range of the snake's ear may be a form of auditory atavism, where the modern physiological performance reflects a specialization for ancestral environmental conditions. Some snake species are semi-fossorial in sandy soils (e.g., *Eryx*, *Cerastes*) while other species are found on the surface of the sand (e.g., *Psammophis*). But there are many arboreal, semi-aquatic, and aquatic species — as well as numerous terrestrial forms that routinely adopt postures in which the lower jaws are held off the surface of the substrate (e.g., *Trimorphodon*) — for which this potentially ancestral frequency range may not be optimal for their ecological interactions.

Figure 1. Comparison of the frequency profiles of vibration in damp sand (light trace, data taken from Aicher and Tautz, 1990) and the frequency response of a garter snake (dark trace, data taken from Wever, 1978).



Formulating proximate and/or ultimate explanations for the auditory specializations in snakes is hampered by our incomplete understanding of the basic mechanics of this system. Wever and Vernon (1960) demonstrated conclusively that the ophidian cochlea is capable of responding to both airborne and groundborne vibrations; Hartline (1971b) extended these findings by showing that snakes can also detect vibrations from their body surface. In addition to these auditory and somatic systems, there are several types of cutaneous sense organs in snakes that appear capable of responding to vibrational information (e.g., Proske, 1969; Jackson and Doetsch, 1977; Povel and Kooij, 1997).

Maps of the response field(s) at the level of the auditory mid-brain (Hartline, 1971b) suggest that there are multiple pathways for hearing in snakes, at least up to this level of the auditory processing. When identical vibrational cues were presented to rattlesnakes as either groundborne or airborne stimuli, the rattlesnakes showed significant differences in latency time to response, orientation reaction, and

relative strength of response to different stimuli, suggesting that vibrational stimuli from these two different sources were being processed differently (Young, 2004).

The presence of multiple, at least partially independent, pathways for hearing means that the ophidian auditory system may be one of the most specialized among terrestrial vertebrates. Until we know more about the basic mechanics of these different sensory pathways, and their integration, we can not explore the possible phylogenetic and ecological specializations of this system. For example, what cutaneous receptors are responsible for “somatic” hearing? How are these receptors distributed over the body of the snake, and does this distribution differ between arboreal and aquatic species, or among different phylogenetic groups? Understanding the functional integration of the ophidian auditory system may provide insights into the phylogeny of snakes, as well as their subsequent ecological divergence.

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Herpetological collaboration in Vietnam

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Abstract. This report gives a short overview about herpetological investigations in Vietnam. A list of new amphibian and reptile species described from Vietnam between 1980-2006 is provided.

Introduction

Vietnam has become well known for its remarkable and unique biodiversity, particularly its amphibian and reptile fauna. Numerous surveys of herpetofauna have been carried out in different areas of the country in the last decades. In the early 20th century (1924-1944), Bourret counted 177 species and sub-species of lizards, 245 species and sub-species of snakes, 45 species and sub-species of turtles, and 171 species and sub-species of amphibians for the Indochinese region [Bourret, 1936, 1941a, 1941b, 1942a, 1942b, 1943]. During the period between 1945 and 1954 no remarkable herpetological studies were undertaken, due to the influence of war. From 1954 to 1975, results of several surveys conducted by Vietnamese herpetologists in the north of the country included a total of 68 amphibian and 159 reptile species [Tran et al., 1981]. From the south, Campden-Main (1970) reported on 77 species of snakes in his field guide. From 1976 to 1980, under the collaboration between Vietnamese and Russian institutions, several herpetological surveys were carried out in the north and the centre of Vietnam. The first list and the key to all species of reptiles and amphibians of Vietnam was compiled by Dao Van Tien, including 87 species of amphibians, 77 species of lizards, 165 species of snakes, 32 species of turtles, and two species of crocodiles [Dao, 1977, 1978, 1979, 1981, 1982]. The second checklist was published by Nguyen Van Sang and Ho Thu Cuc (1996) that listed a total of 340 species including 82 amphibian and 258 reptile species. The number of herpetofaunal species increased to 458 in an updated checklist by Nguyen et al. (2005) (i.e., 162 species amphibians and 296 species of reptiles). This checklist includes 200 additional species compared with the previous checklist of

the herpetofauna of Vietnam. This increase in our knowledge about the amphibian and reptile fauna is a result of herpetological cooperations throughout the last over 25 years (1980-2006).

Involved agencies

Vietnam's herpetofauna has been studied with the collaborative involvement of scientists both from Vietnam and from overseas. Institutions and organizations from abroad include the American Museum of Natural History, New York, USA (AMNH); Appalachian State University, North Carolina, USA (ASU); Chelonian Research Institute, Florida, USA (CRI); Natural History Museum of Los Angeles County, California, USA (LACM); Royal Ontario Museum, Ontario, Canada (ROM); Zoological Institute of Saint Petersburg, Russian Academy of Science, Saint Petersburg, Russia (ZISP); Zoological Research Museum Alexander Koenig, Bonn (ZFMK), and the Cologne Zoo, Cologne, Germany; National Museum of Natural Science, Taichung, Taiwan (NMNS). Institutions and organizations within Vietnam comprise Birdlife International (Birdlife); CARE International in Vietnam; Centre for Natural Resources and Environmental Studies (CRES), Fauna and Flora International - Vietnam Programme (FFI); Hanoi National University; Hanoi University of Education (HUE); Institute of Ecology and Biological Resources (IEBR), Vietnamese Academy of Science and Technology (VAST); PLAN International in Vietnam; The World Union for Conservation (IUCN); Traffic Southeast Asia; Wildlife At Risk in Vietnam (WAR); Wildlife Conservation Society (WCS); and World Wide Fund for Nature (WWF - Indochina Program).

Fields of study and remarkable achievements

Collaboration of Vietnam's herpetology has focused on two fields: discovery of herpetofaunal diversity and conservation of threatened species.

Many surveys of herpetofaunal diversity have been conducted in different areas of the country, resulting in the discovery and description of three new genera, 79 new species and three new sub-species based on voucher collections from 1980 to 2006 (Table 1). Furthermore, at least 90 new records for Vietnam have been reported over this period. Results of these surveys showed that upland areas contain a high diversity of amphibian and reptile species. Several hotspots of the herpetofauna were defined in Vietnam comprising

Table 1. List of new species of reptiles and amphibians described based on voucher specimens from Vietnam (1980-2006).

No	Scientific name	Type locality
	AMPHIBIA	
1.	<i>Tylotriton vietnamensis</i> Böhme, Schöttler, Nguyen and Köhler, 2005	Luc Nam, Bac Giang Province
2.	<i>Leptobranchium banae</i> Lathrop, Murphy, Orlov and Ho, 1998	K Bang, Gia Lai Province
3.	<i>Leptobranchium xanthospilum</i> Lathrop, Murphy, Orlov and Ho, 1998	K Bang, Gia Lai Province
4.	<i>Leptolalax bourreti</i> Dubois, 1980	Sa Pa, Lao Cai Province
5.	<i>Leptolalax nahangensis</i> Lathrop, Murphy, Orlov and Ho, 1998	Na Hang, Tuyen Quang Province
6.	<i>Leptolalax pluvialis</i> Ohler, Marquis, Swan and Grosjean, 2000	Sa Pa, Lao Cai Province
7.	<i>Leptolalax sungi</i> Lathrop, Murphy, Orlov and Ho, 1998	Tam Dao, Vinh Phuc Province
8.	<i>Leptolalax tuberosus</i> Inger, Orlov and Darevsky, 1999	Kon Cha Rang, Gia Lai Province
9.	<i>Ophryophryne gerti</i> Ohler, 2003	Da Lat, Lam Dong Province
10.	<i>Ophryophryne hansii</i> Ohler, 2003	Da Lat, Lam Dong Province
11.	<i>Vibrissaphora echinata</i> Dubois and Ohler, 1998	Sa Pa, Lao Cai Province
12.	<i>Vibrissaphora ngoclinhensis</i> Orlov, 2005	Dak Glei, Kon Tum Province
13.	<i>Microhyla marmorata</i> Bain and Nguyen, 2004	Tra My, Quang Nam Province
14.	<i>Microhyla nanapollexa</i> Bain and Nguyen, 2004	Tra My, Quang Nam Province
15.	<i>Microhyla pulverata</i> Bain and Nguyen, 2004	K Bang, Gia Lai Province
16.	<i>Micryletta erythropoda</i> (Tarkhishvili, 1994)	Ma Da, Dong Nai Province
17.	<i>Amolops spinapectoralis</i> Inger, Orlov and Darevsky, 1999	K Bang, Gia Lai Province
18.	<i>Paa bourreti</i> Dubois, 1987	Sa Pa, Lao Cai Province
19.	<i>Rana attigua</i> Inger, Orlov and Darevsky, 1999	K Bang, Gia Lai Province
20.	<i>Rana bacboensis</i> Bain, Lathrop, Murphy and Ho, 2003	Con Cuong, Nghe An Province
21.	<i>Rana banaorum</i> Bain, Lathrop, Murphy and Ho, 2003	K Bang, Gia Lai Province
22.	<i>Rana cucae</i> Bain, Stuart and Orlov, 2006	Van Ban, Lao Cai Province
23.	<i>Rana daorum</i> Bain, Lathrop, Murphy and Ho, 2003	Sa Pa, Lao Cai Province
24.	<i>Rana hmongorum</i> Bain, Lathrop, Murphy and Ho, 2003	Sa Pa, Lao Cai Province
25.	<i>Rana iriodes</i> Bain and Nguyen, 2004	Vi Xuyen, Ha Giang Province
26.	<i>Rana khalam</i> Stuart, Orlov, and Tanya, 2005	Bach Ma, Thua Thien Hue, Lao Cai Provinces (Paratypes)
27.	<i>Rana megatypanum</i> Bain, Lathrop, Murphy and Ho, 2003	Con Cuong, Nghe An Province
28.	<i>Rana morafkai</i> Bain, Lathrop, Murphy and Ho, 2003	K Bang, Gia Lai Province
29.	<i>Rana orba</i> Stuart and Bain, 2005	Huong Son, Ha Tinh Province (Paratypes)
30.	<i>Rana trunkieni</i> Orlov, Le and Ho, 2003	Phu Yen, Son La Province
31.	<i>Aquixalus (Chirixalus) ananjevae</i> (Matsui and Orlov, 2004)	Huong Khe, Ha Tinh Province
32.	<i>Aquixalus (Rhacophorus) baliogaster</i> (Inger, Orlov and Darevsky, 1999)	Buon Luoi, Tram Lap and Krong Pa, Gia Lai Province
33.	<i>Aquixalus (Philautus) supercornutus</i> (Orlov, Ho and Nguyen, 2004)	Bach Ma, Thua Thien Hue Province
34.	<i>Philautus abditus</i> Inger, Orlov and Darevsky, 1999	K Bang, Gia Lai Province
35.	<i>Philautus truongsongensis</i> Orlov and Ho, 2005	Huong Hoa, Quang Tri Province
36.	<i>Rhacophorus duboisi</i> Orler, Marquis, Swan and Grosjean, 2000	Sa Pa, Lao Cai Province
37.	<i>Rhacophorus exechopygus</i> Inger, Orlov and Darevsky, 1999	K Bang, Gia Lai Province
38.	<i>Rhacophorus hoanglienensis</i> Orlov, Lathrop, Murphy and Ho, 2001	Sa Pa, Lao Cai Province
39.	<i>Rhacophorus orlovi</i> Ziegler and Köhler, 2000	Ky Anh, Ha Tinh Province
	REPTILIA	
	Sauria	
1.	<i>Acanthosaura nataliae</i> Orlov, Nguyen and Nguyen, 2006	K Bang, Gia Lai Province
2.	<i>Bronchocela orlovi</i> Hallermann, 2004	K Bang, Gia Lai Province
3.	<i>Bronchocela vietnamensis</i> Hallermann and Orlov, 2005	K Bang, Gia Lai Province
4.	<i>Leiolepis guentherpetersi</i> Darevsky and Kupriyanova, 1993	Thuy Phu, Thua Thien-Hue Province
5.	<i>Cyrtodactylus paradoxus</i> (Darevsky and Szczerbak, 1997)	Phu Quoc, Kien Giang Province
6.	<i>Cyrtodactylus phongnhakebangensis</i> Ziegler, Rösler, Herrmann, and Vu, 2002	Phong Nha-Ke Bang, Quang Binh Province

Table 1 (continued). List of new species of reptiles and amphibians described based on voucher specimens from Vietnam (1980-2006).

No	Scientific name	Type locality
7.	<i>Dixonius vietnamensis</i> Das, 2004	Nha Trang, Khanh Hoa Province
8.	<i>Gekko grossmanni</i> Gunther, 1994	Khanh Hoa Province
9.	<i>Gekko scientiadventura</i> Rösler, Ziegler, Vu, Herrmann, and Böhme, 2005	Phong Nha - Ke Bang, Quang Binh Province
10.	<i>Gekko ulikovskii</i> Darevsky and Orlov, 1994	Kon Tum Province
11.	<i>Goniurosaurus araneus</i> Grismer, Viets, and Boyle, 1999	Cao Bang Province
12.	<i>Hemidactylus stejnegeri</i> Ota and Hikida, 1989	Ba Mun, Quang Ninh Province
13.	<i>Hemidactylus vietnamensis</i> Darevsky and Kupriyanova, 1984	Cuc Phuong, Ninh Binh Province
14.	<i>Dibamus deharvengi</i> Ineich, 1999	Binh Chau, Ba Ria-Vung Tau Province
15.	<i>Dibamus greeri</i> Darevsky, 1992	Kon Cha Rang, Kon Tum Province
16.	<i>Dibamus kondaoensis</i> Honda, Ota, Hikida and Darevsky, 2001	Con Dao, Ba Ria-Vung Tau Province
17.	<i>Dibamus smithi</i> Greer, 1985	Da Lat, Lam Dong Province
18.	<i>Takydromus hani</i> Chou, Nguyen and Pauwels, 2001	Huong Son, Ha Tinh Province
19.	<i>Takydromus kuehnei vietnamensis</i> Ziegler and Bischoff, 1999	Ke Go, Ha Tinh Province
20.	<i>Leptoseps tetradactylus</i> Darevsky and Orlov, 2005	Phong Nha-Ke Bang, Quang Binh Province
21.	<i>Lygosoma carinatum</i> Darevsky and Orlov, 1996	K Bang, Gia Lai Province
22.	<i>Mabuya darevskii</i> Bobrov, 1992	Cao Pha, Son La Province
	Paralipinia Darevsky and Orlov, 1997	
23.	<i>Paralipinia rara</i> Darevsky and Orlov, 1997	K Bang, Gia Lai Province
24.	<i>Sphenomorphus buenloicus</i> Darevsky and Nguyen, 1983	K Bang, Gia Lai Province
25.	<i>Sphenomorphus cryptotis</i> Darevsky, Orlov and Ho, 2004	Uong Bi, Quang Ninh Province
26.	<i>Sphenomorphus devorator</i> Darevsky, Orlov and Ho, 2004	Uong Bi, Quang Ninh Province
27.	<i>Sphenomorphus rufocaudatus</i> Darevsky and Nguyen, 1983	K Bang, Gia Lai Province
28.	<i>Tropidophorus murphyi</i> Hikida, Orlov, Nabhitabhata and Ota, 2002	Nguyen Binh, Cao Bang Province
29.	<i>Tropidophorus noggei</i> Ziegler, Vu and Bui, 2005	Phong Nha - Ke Bang, Quang Binh Province
	Vietnascincus Darevsky and Orlov, 1994	
30.	<i>Vietnascincus rugosus</i> Darevsky and Orlov, 1994	K Bang, Gia Lai Province
31.	<i>Ophisaurus sokolovi</i> Darevsky and Nguyen, 1983	K Bang, Gia Lai Province
	Serpentes	
32.	<i>Amphiesma andreae</i> Ziegler and Le, 2006	Minh Hoa, Quang Binh Province
33.	<i>Boiga bourreti</i> Tillack, Ziegler and Le, 2004	Phong Nha-Ke Bang, Quang Binh Province
34.	<i>Calamaria lovii ingermarxorum</i> Darevsky and Orlov, 1992	K Bang, Gia Lai Province
35.	<i>Calamaria thanhi</i> Ziegler and Le, 2005	Minh Hoa, Quang Binh Province
36.	<i>Opisthotrophis daovantieni</i> Orlov, Darevsky and Murphy, 1998	K Bang, Gia Lai Province
37.	<i>Bungarus slowinskii</i> Kuch, Kizirian, Nguyen, Lawson, Donnelly, and Mebs, 2005	Van Yen, Yen Bai Province
	Triceratolepidophis Ziegler, Herrmann, David, Orlov and Pauwels, 2000	
38.	<i>Triceratolepidophis sieversorum</i> Ziegler, Herrmann, David, Orlov and Pauwels, 2000	Phong Nha-Ke Bang, Quang Binh Province
39.	<i>Trimeresurus truongsongensis</i> Orlov, Ryabov, Bui and Ho, 2004	Phong Nha-Ke Bang, Quang Binh Province
	TESTUDINES	
40.	<i>Cuora bourreti</i> (Obst and Reimann, 1994)	Linh Cam, Ha Tinh Province
41.	<i>Cuora mouhoti obsti</i> Fritz, Andreas and Lehr, 1998	Central Vietnam
42.	<i>Cuora picturata</i> (Lehr, Fritz and Obst, 1998)	Central Vietnam
43.	<i>Cyclemys pulchriatrata</i> Fritz, Gaulke and Lehr, 1997	Phuoc Son, Quang Nam Province

Fansipan Mountain in the northwestern region; Bac Son and Yen Tu mountains in the northeastern region; Truong Son Range and Tay Nguyen Plateau in the centre of the country; and Me Kong River Basin in the south [Biodiversity Action Plan for Vietnam, 1995]. These areas contain a large expanse of primary vegetation and they still require future research

At present, main threats to the herpetofauna in Vietnam are identified including overexploitation for trade and consumption and habitat loss as a result of forest land alteration. Some specific conservation programs for reptiles and amphibians are being implemented in different protected areas in an effort to mitigate threats to the herpetofauna. Turtles are currently known as the most threatened group because of the high demand of consumption and trade in the Indochinese region. A few conservation projects for both freshwater and marine turtles have been implemented in Vietnam, and the Turtle Conservation and Ecology Project in Cuc Phuong National Park was known as the first conservation centre for reptiles in Vietnam. Moreover, governmental rescue centres for wild animals have been established in Hanoi and Ho Chi Minh cities and in other national parks in Central Vietnam for releasing confiscated wildlife. The population of the Vietnamese salamander *Paramesotriton deloustali*, an endemic species of Vietnam, is recuperating with the efforts of the conservation project at the Tam Dao National Park and buffer zone (Nguyen and Ho, 2004).

Many results have been published as a result of this successful research collaboration (see extended references section). Several field guides (e.g., Photographic guide to the turtles of Thailand, Laos, Vietnam, and Cambodia [Stuart et al., 2001]; Reptiles and amphibians of Cuc Phuong National Park [Nguyen et al., 2003]; A field guide to reptiles and amphibians of Vietnam [Nguyen et al., 2005]; Amphibians and reptiles of Ba Be National Park and Na Hang Nature Reserve [Le et al., 2004]) and checklists (e.g., Herpetofauna of Vietnam. A checklist. Part 1. Amphibia [Orlov et al., 2002]; A checklist of amphibians and reptiles of Vietnam [Nguyen et al., 2005]) have appeared both in English and Vietnamese. Furthermore, a hundred articles of herpetofaunal diversity and descriptions of new taxa have been published in international and national journals during period from 1980 to 2006.

Further opportunities for collaboration

Vietnam's herpetofauna is still imperfectly known, and many areas remain either un-explored or under-

surveyed, as indicated by the numerous new species being described in such a short period of time. In order to ensure that Vietnam's herpetofauna can be more closely understood and better protected, efforts should be focused in three important fields: 1) Diversity studies, which include field surveys for presence and novel natural history information, detailed study of natural history museum specimens, and the combination of both of these in morphological and genetic studies; 2) conservation, including monitoring at population level and evaluation of threats to herpetofauna and concomitant assignment of priority areas for herpetofaunal conservation; and 3) training for workers in the fields of science and conservation: training for scientists from institutions and universities, training for natural resource managers, training for staff from protected areas, and improvement of scientific capacity (equipment, collection management, information exchange and literatures). Perhaps most important to the overall success of this enterprise are the ongoing efforts to strengthen the collaboration between scientists and conservation managers so that information can be collected, disseminated, and ultimately used to guide decision-making in a systematic and timely manner.

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On the taxonomy of the *Xenochrophis piscator* complex (Serpentes, Natricidae)

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Abstract. In this paper, we present the preliminary results of our revision of the Asian natricid snakes currently known as *Xenochrophis piscator* / *Xenochrophis flavipunctatus*. We divide this complex into eight taxa, of which seven are given a specific status; the status of eighth one is still provisional. *Xenochrophis flavipunctatus* is shown to be clearly distinct from *Xenochrophis piscator* auctorum. The taxa *melanzostus*, *schnurrenbergeri* and *asperrimus* are also shown to be distinct species. The population of the Andaman Islands belongs to a distinct species, for which the combination *Xenochrophis tytleri* is available. Specimens of “*X. piscator*” from Sri Lanka (others than *X. asperrimus*) and extreme southern India belong to an undescribed species. The distribution, based on materials examined by us and basic ecology of all these taxa are summarized.

Introduction

Malnate (1960) split the genus *Natrix* into five genera and choose the genus name *Fowlea* Theobald, 1868 for the species treated here. Later the name was changed to *Xenochrophis* Günther, 1864 to include the species *Psammophis cerasogaster* Cantor, 1839 (Malnate & Minton, 1965). The genus description was mainly based on the structure of the hemipenes and the number and form of the maxillary teeth as well as on the position of the nostrils (Malnate, 1960, Malnate & Minton, 1965).

The taxonomy of the Asian natricid species related to *Xenochrophis piscator* (Schneider, 1799), widespread throughout tropical Asia, has long been controversial. Whereas Boulenger (1896) recognized (in the genus *Tropidonotus*) the validity of three species, namely *Xenochrophis piscator* (Schneider, 1799), *Xenochrophis asperrimus* (Boulenger, 1891) and *Xenochrophis sanctijohannis* (Boulenger, 1890), Smith (1943) admitted, in the genus *Natrix*, the validity of a single species, *Natrix piscator*, with four subspecies (*piscator*, *flavipunctata*, *melanzostus* and *asperrimus*). This taxonomy was accepted until Taylor (1965) showed that two of the subspecies recognized by Smith, *piscator* and *flavipunctata* respectively were living in sympatry in northern Thailand. For the eastern species, Taylor

used the name *Natrix flavipunctata* (Hallowell, 1860), whereas the specific nomen *piscator* was retained for the western species.

This position has been variously accepted (Cox, 1991, Manthey and Grossmann, 1997, Pauwels et al., 2003) or refuted (Tweedy, 1983, Karsen et al., 1986, Zhao and Adler, 1993, Cox et al., 1998) by recent authors. Population from Indonesia were treated as a subspecies and named *X. piscator melanzostus* (Gravenhorst, 1807) in most publications (Welch, 1988; David and Vogel, 1996), but also regarded as full species in some (Iskandar and Colijn, 2001, Whitaker and Captain, 2004). In order to clarify these taxonomical problems, we investigated the morphological variation in the *X. piscator* complex on the basis of about 450 specimens from the whole range of the complex.

Materials and methods

In this first step, only univariate analyses on main morphological characters, scalation and pattern, were conducted. Measurements on bodies were made to the nearest millimeter. The number of ventral scales was counted according to Dowling (1951). The terminal scute, present, is not included in the number of subcaudals.

Abbreviations: Sc: number of subcaudal plates. - TaL: tail length. - TL: total length. - TaL / TL: ratio tail length / total length. - Ven: number of ventral plates.

Results

Results of the preliminary analyses are presented in Table 1. In a quite unexpected way, these preliminary data revealed eight clearly defined groups. Seven of these groups, of which the distribution overlap but for which morphological characters are well separated without or with little overlapping, are here recognized as distinct species. The status of the last

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Data	n	Ve	Sc	TA/TL	TL max	Nuchal mark
Males						
<i>flavipunctatus</i>	34	122-130	78-91	0,289-0,348	784	V like
" <i>sanctijohannis</i> "	6	139-140	88-94	0,276-0,325	862	None
<i>schnurrenbergeri</i>	10	132-139	71-80	0,257-0,283	700	Crossbar
<i>piscator</i>	10	132-143	85-99	0,293-0,329	947	Inverted V
<i>Xenochrophis</i> sp.	9	125-132	88-93	0,303-0,353	629	Inverted V
<i>melanzostus</i>	6	128-134	79-83	0,286-0,302	549	Wide V
<i>asperrimus</i>	2	127-132	77-89	0,290-0,314	612	Crossbar
<i>tytleri</i>	6	135-138	---	---	---	Wide V
females						
<i>flavipunctatus</i>	35	131-143	70-87	0,256-0,306	974	V like
" <i>sanctijohannis</i> "	2	148-154	84-87	0,266-0,270	637	None
<i>schnurrenbergeri</i>	20	141-152	61-70	0,215-0,236	895	Crossbar
<i>piscator</i>	22	136-151	68-87	0,264-0,290	1020	Inverted V
<i>Xenochrophis</i> sp.	6	131-138	79-91	0,284-0,305	714	Inverted V
<i>melanzostus</i>	11	136-142	66-77	0,233-0,254	975	Wide V
<i>asperrimus</i>	7	132-140	75-83	0,257-0,283	890	Crossbar
<i>tytleri</i>	5	144-145	77-79	0,279	920	Wide V

Table 1. Main morphological characters of the *Xenochrophis piscator* complex.

one, *X. sanctijohannis* auctorum, is still doubtful and requires further research.

The seven species defined here are easily distinguishable by a combination of meristic characters, if sexes are considered separately, and of their pattern. The pattern is especially important in this complex. Some characters, very easy to identify, are absolutely constant among several tens, if not hundred of specimens.

One character that has seemingly been overlooked by all previous herpetologists is the shape of the nuchal marking. Either it is absent, a common case in *X. piscator* and *X. "sanctijohannis"*, or only four shapes are present, as follows:

- 1) An inverted V, namely the marking opens posteriorly when seen from above.
- 2) A direct V, namely the marking opens anteriorly on the nape.
- 3) A straight, broad, often subrectangular crossbar.
- 4) A widely open U or even a double YY, present in populations from Indonesia and the Andaman Islands.

This character may seem to be trivial, but we could not identify any exception but two specimens of *X. flavipunctatus* among our 450 examined specimens. Beside the differences in pholidosis, there are also differences in biology especially in the mainland species. For example, *Xenochrophis piscator* often occurs close to water, but is not strongly aquatic. In contrast, *X. flavipunctatus* and *X. schnurrenbergeri* are more strongly aquatic, spending a large part of their time in water to which they retreat swiftly when threatened (Taylor, 1965, Kramer, 1977, our unpublished observations).

From our results, we recognize the species listed above. In this preliminary paper, we put emphasis on readily visible characters, especially the pattern.

Xenochrophis piscator (Schneider, 1799)

As we define it, this species occurs in the western part of the complex and is distributed throughout India, Pakistan, Nepal, Bangladesh, much of Myanmar, northern and northwestern Thailand and northwestern Laos. It barely enters China in



Figure 1. Distribution of the species of the *X. piscator* complex. Blue: *Xenochrophis piscator*; Red: *X. flavipunctatus*; Yellow: *X. tyleri*; Green: *X. melanzostus*; Violet: *X. schnurrenbergeri*; Pink: *X. asperrimus* and *Xenochrophis* sp.

southwestern Yunnan Province and in western Guangxi Province.

This is the largest of all species of this complex, with a total length up to 947 mm in males and 1020 mm in females. It is characterized by (1) a chess-like pattern on the body, sometimes with broad blotches and very conspicuous (northeastern India), sometimes with smaller blotches; (2) large, more or less diffuse (“cloudy”) cream or pale grey dorsolateral blotches; (3) a nuchal marking often absent, if not always present as an inverted V; (4) subocular streaks faint, reduced to a blotch or absent; (5) a venter uniform, with the ventrals darker only on the outermost edges; (6) a high number of ventral and subcaudal scales in males.

It is mostly a lowland species.

Xenochrophis asperrimus (Boulenger, 1891)

This form was accepted as a valid species by most recent authors (de Silva, 1990, Das, 2005). Nevertheless Wall (1921) regarded it as a colour variation of *X. piscator*, and Smith (1943) as a subspecies of this species. Our data suggest that this taxon deserves a distinct specific status.

It is living on Sri Lanka sympatrically with *Xenochrophis* sp. (see below).

This species is characterized by (1) a peculiar dorsal pattern made of broad, dark, confluent blotches on a paler background anteriorly, separated blotches posteriorly; (2) a broad, subrectangular crossbar on the nape, as in *X. schnurrenbergeri* but even wider; (3) two narrow subocular streaks; (4) a venter uniform, with the ventrals darker only on the outermost edges or their tips; (5) a high number of ventrals in both sexes.

Furthermore it differs from *Xenochrophis* sp. by a higher number of ventrals in males and by a lower number of subcaudals and a shorter tail in females.

Xenochrophis flavipunctatus (Hallowell, 1860)

This species inhabits the eastern and northern part of the range of this complex. It is sympatric with *X. piscator* in northern Thailand, southern Myanmar, northwestern Laos and extreme southern China. It is the sole species present in Cambodia, Vietnam and West-Malaysia. It is also found throughout Thailand, Laos and southern China.

This species is characterized by (1) a dorsal pattern made of small dark blotches and streaks, larger on the sides; (2) small, white or yellow dorsolateral dots; (3) a well-defined nuchal marking, always appearing as a direct V (at the exception of two specimens from North Thailand); (4) two well-defined subocular streaks, the posterior one extending from the eye to the corner of the mouth then meeting the V-marking; (5) ventral and subcaudal scales all with entire, broad, dark margins; (6) a rather low number of ventral scales in males. The number of ventrals in males is lower than in *X. piscator*, but there is a wide overlap in females.

X. flavipunctatus is very variable in colouration and in dorsal pattern, but this variability is not geographically correlated. The colouration of a specimen found in Singapore may have the same colour than one from South China. There are often some red or yellow hues on the forepart of the body.

It is mostly a lowland species.

Xenochrophis melanzostus (Gravenhorst, 1807)

This species has mostly been regarded as a subspecies of *X. piscator* in the literature. According to our data, there is little doubt about the distinct specific status of this form. It is more similar to *X. flavipunctatus* than to *X. piscator*.

X. melanzostus is probably endemic to Java. It has been mentioned for Sulawesi and Borneo, but these records are erroneous (Stuebing and Inger, 1999, Lang and Vogel, 2005). Its occurrence in Sumatra has yet to be confirmed. The population from the Andaman Islands is here referred to a distinct species (see below).

This species is characterized by (1) two different dorsal patterns: (a) a blotched form, characterized by elongated blotches, and (b) a striped form, with

broad dark longitudinal stripes; (2) a well-defined nuchal marking, always appearing as a direct, widely open V or U; (3) two well-defined subocular streaks; (4) ventral and subcaudal scales all with entire, broad, dark margins; (5) a high number of ventral scales in males; (6) a tail shorter in females than in other species of the complex; (7) a lower number of subcaudal scales than in other species of the complex.

The number of ventrals in males is higher than in *X. flavipunctatus*, with some overlap. *X. melanzostus* also differs from *X. flavipunctatus* by its colouration. It differs from *Xenochrophis schnurrenbergeri* by a longer tail.

It is a lowland species, but little is known on its ecology.

Xenochrophis schnurrenbergeri Kramer, 1977
new combination

This taxon has obviously been overlooked by most herpetologists. It occurs at the northern limits of the range of *X. piscator*, with which it lives in sympatry over most of its range. Smith (1943) misidentified a specimen from Assam as *X. flavomaculatus*. This species was first known as *Xenochrophis flavipunctatus schnurrenbergeri* Kramer, 1977, described from Nepal. Beside Nepal, we identified as such many specimens from Pakistan and northern India, close to the Himalayas.

This species is characterized by (1) a dorsal pattern chess-like, but made of small dark blotches; (2) a broad, straight crossbar on the nape; (3) two well-defined subocular streaks, the posterior one extending from the eye to the corner of the mouth but not meeting the nuchal crossbar; (4) ventral and subcaudal scales all with entire, broad, dark margins; (5) a low number of subcaudals in both sexes, lower than in the other species; (6) a lower relative tail length than in *X. piscator* or *X. flavipunctatus*. There is no overlap if the sexes are considered separately. Lastly, this species is a little bit shorter than *X. piscator* or *X. flavipunctatus*.

It is both a lowland and hill species. In ecology it is close to *X. flavipunctatus* with which it is parapatric.

Xenochrophis tytleri (Blyth, 1863)

This species has variously been cited as *X. piscator melanzostus* (Das 1999) or *X. melanzostus* (Whitaker and Captain, 2004) in the literature. Our data

suggest that this taxon deserves a specific status, for which the binomen *Tropidonotus tytleri* Blyth, 1863 is available. It is endemic to Andaman Islands and possibly Nicobar Islands. It is not sympatric with any other species.

This species is characterized by (1) a dorsal pattern made of much elongated, dark blotches, sometimes producing stripes; (2) a well-defined nuchal marking, appearing as a broad, direct, widely open U, connected to the dorsolateral stripes; (3) two broad subocular streaks (broader than in any other species of the complex), the posterior one extending from the eye to the corner of the mouth then meeting the nuchal U; (4) ventral and subcaudal scales entirely uniform or only darkened on their tips; (5) a higher number of ventrals in both sexes than *X. melanzostus*, the sole species sharing a similar pattern.

It is a lowland species, but nothing is known on its ecology.

Xenochrophis sp.

This form has been called *X. piscator* until now. We regard it as a new, undescribed species, sharing characters of both *X. piscator* and *X. flavipunctatus*, but not of *X. asperrimus* with which it is sympatric in Sri Lanka.

This species is characterized by (1) a pattern similar to *X. piscator*, with small blotches; (2) an inverted V like pattern; (3) two narrow subocular streaks; (4) a venter uniform, with the ventrals darker only on the outermost edges or their tips; (5) a lower number of ventrals than *X. piscator* in males, but about the same as in *X. flavipunctatus*. The females have more subcaudals than other species of this complex and thus are easy to tell apart.

Xenochrophis “*sanctijohannis*” (Boulenger, 1890) We cannot yet conclude about the status of this taxon. Although typical *X. sanctijohannis* differ from typical *X. piscator*, there are several intermediate morphs. This taxon is known from higher elevations in Nepal, northern India and northern Myanmar.

It differs from *X. piscator* especially by a patternless body and a slightly higher number of ventral scales. However, the dark patternless body may be an adaptation to the ecological conditions of high mountains rather a taxonomical difference.

This paper is a preliminary step towards a revision

of the *Xenochrophis piscator* complex. Some specimens could not be assigned to any species yet, for example animals from Indonesia and South India. These populations will be discussed in a subsequent paper.

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Review of the amphibian and reptile diversity of Phong Nha – Ke Bang National Park and adjacent areas, central Truong Son, Vietnam

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Abstract. We provide an updated review of the herpetofauna of the Phong Nha – Ke Bang National Park including adjacent areas within the Quang Binh province, central Vietnam. Beside confirmation of species and specific allocation of previously undetermined taxa, we herein add 19 species (2 Megophryidae, 1 Dicoglossidae, 2 Ranidae, 2 Rhacophoridae, 2 Geomydidae, 1 Gekkonidae, 1 Lacertidae, 1 Scincidae, and 7 Colubridae) which were not known from the region before. For each of these we provide information about distribution, natural history and characteristic features. Meanwhile, 140 amphibian and reptile species are known from the karst forests of the Phong Nha – Ke Bang National Park, representing more than 30 percent of the herpetofauna currently known for the country.

Introduction

Since 1999 the Zoological Garden Cologne engages in a German-Vietnamese cooperation project concerning nature conservation and biodiversity research in a unique karst forest area in central Vietnam, the Phong Nha Nature Reserve. In the meantime this protected area has been extended to the Phong Nha - Ke Bang National Park, which was recently declared by the UNESCO as world heritage site. This region is part of the central Indochina limestone, which stretches along the Lao-Vietnamese border and is one of the largest ranges of contiguous limestone karst in Indochina and certainly the largest within protected areas (Baltzer et al., 2001). With respect to diversity research, we have focused on

the amphibian and reptile fauna of this wet evergreen and semi-evergreen forest on limestone for nine years. Six years ago, we published a first, preliminary list of the area's herpetodiversity, based on own fieldwork and first Vietnamese reports, comprising 96 amphibian and reptile species (Ziegler & Herrmann, 2000). Some years later, we could bring the total number of amphibian and reptile species known for the area as a result of further field work to 128 (Ziegler et al., 2004), of which approximately 20 % were listed in the Red Data Book of Vietnam. Herein, we present our recent findings and additions to the herpetofauna of the Phong Nha – Ke Bang National Park including adjacent regions in the northwest of the Quang Binh province.

Materials and Methods

Specimens collected by Le Khac Quyet, Vu Ngoc Thanh and Thomas Ziegler are deposited in the following collections: Collection of the Science Research Centre, Phong Nha – Ke Bang National Park, Quang Binh province, Vietnam; Muséum National d'Histoire Naturelle (MNHN), Paris, France; Collection of the Zoological Museum of the Vietnam National University, Hanoi (VNUH), Vietnam; Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany.

Systematics followed Campden-Main (1970), Zhao & Adler (1993), Ota et al. (1995), David & Vogel (1997), Inger et al. (1999), Chou et al. (2001), Ziegler (2002), Ohler (2003), Bain & Nguyen (2004), Orlov et al. (2004), Rösler et al. (2004), Stuart & Platt (2004), Tillack et al. (2004), Ziegler & Le (2005), Ziegler et al. (2004; 2005), Ohler & Delorme (2006), as well as the online reference "Amphibian species of the world, 3.0" by the American Museum of Natural History (see <http://research.amnh.org/herpetology/amphibia/index.php>) and the most recent overview by Frost et al. (2006). Common English and Vietnamese names generally follow Nguyen et al. (2004). Abbreviations are as follows: SVL: snout-vent length (from snout tip to end of urostyle in amphibians, and from snout tip to cloaca in reptiles); TaL: tail length (from cloaca to tail tip).

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Figure 1. Impressed tortoise (*Manouria impressa*).

Specific allocation of previously undetermined taxa listed for the Phong Nha – Ke Bang National Park

Amphibia
Anura
Microhylidae

Microhyla marmorata Bain & Nguyen, 2004: Marble pigmy frog / Nhai bau hoa cuong
(= *Microhyla* cf. *annamensis* / *Microhyla* sp. n. in Ziegler *et al.*, 2004)

Distribution. Bain & Nguyen (2004) described the species based on specimens from Ha Tinh, Quang Binh, and Quang Nam (see also Nguyen *et al.*, 2005; Orlov, 2005), including one paratype from Phong Nha - Ke Bang.

Natural history notes. The paratype ZFMK 76192, an adult female, was collected in the forest of Phong Nha - Ke Bang in about 800 m altitude above sea level. Six additional female specimens from Phong Nha - Ke Bang have been collected in the dry season (May-June) 2004: ZFMK 82893-82898 (SVL 15.7-21.0 mm, mean 18.3 mm).

Characteristic features. This species is characterized by a marbled belly, two metatarsal tubercles, expanded discs at the tip of the digits with dorsal median longitudinal grooves producing the appearance of two scutes, finger I less than one-half the length of finger II, and extensive webbing on feet: on toe IV web full to distal subarticular tubercle (determination followed Bain & Nguyen, 2004).

Rhacophoridae

Polypedates mutus (Smith, 1940): Burmese whipping frog / Chau chang Myanmar

(= *Polypedates* sp. in Ziegler, 2002; 2004; Ziegler & Herrmann, 2000; Ziegler *et al.*, 2004)

Distribution. According to Orlov *et al.* (2001; 2002) the species occurs throughout northern Vietnam, from the Chinese border to Nghe An province of the northern Annam mountains (see also Nguyen *et al.*, 2005). It has also been observed in Ben En National Park (Thanh Hoa Province) in summer 1997 (Ohler, unpublished data) and in the Ke Go area (as *Polypedates* sp. in Ziegler, 2002) in Ha Tinh province. Thus, our records represent not only the first definite records for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province, but also the southernmost locality known for the species in Vietnam.

Natural history notes. For detailed information about the ecology of this forest dwelling species including tadpoles, mating calls, morphology, coloration, pattern and body proportions see Ziegler (2002); voucher specimens from Phong Nha - Ke Bang are listed in Ziegler & Herrmann (2000) and Ziegler *et al.* (2004); two additional specimens have been collected in early July 2004 (MNHN 2005.0236-0237).

Characteristic features. *Polypedates mutus* is a member of the *leucomystax* group as it has co-ossified skin on the head (Dubois, 1987). It has a larger body size than *P. leucomystax* and *P. megacephalus* and relatively longer tibia than these species. It shows only one kind of dorsal pattern, a series of longitudinal stripes whereas the two other species show stripes and hourglass patterns. The posterior surface of the thigh is dark brown and exhibits large sized round spots. These spots are of small size forming a reticulum in *P. leucomystax* and *P. megacephalus*. *P. mutus* males have no vocal pouches which does not render them mute: their call is quite distinct from *P. leucomystax*. *P. mutus* can sympatrically occur with *P. leucomystax* and *P. megacephalus* whereas sympatry of the latter two species has not been observed.

Reptilia
Squamata: Sauria
Gekkonidae

Gekko scientiadvventura Rösler, Ziegler, Vu, Herrmann & Böhme, 2004

(= *Gekko* sp. / *Gekko* sp. n. in Ziegler *et al.*, 2004; Ziegler, 2004)

Distribution. Known only from its type locality in Phong Nha - Ke Bang (see Rösler *et al.*, 2004; Ziegler *et al.*, 2004; Ziegler, 2004).



Figure 2. Annam spadefoot toad (*Brachytarsophrys intermedia*).

Natural history notes. All records were made at night in primary forest areas directly or in the immediate neighbourhood of the karst limestone outcrops that were partly overgrown with vegetation. The geckos were mostly seen on the vegetation in low height rather than on the bare rocks or on the ground. Eggs have been found from the dry season (June) until the beginning rainy season (September) on mass egg-laying sites or as single eggs or pairs of eggs in karst rock crevices (Rösler et al., 2004; Ziegler, 2004).

Characteristic features. The small-bodied species is the only known Vietnamese *Gekko* without dorsal tubercles. Furthermore, *G. scientiaventura* is characterized by its slender habitus, a distinctly depressed body in combination with considerably enlarged dorsal scales, and a depressed head that is distinctly broader than neck; unregenerated tail always longer than head-body length, slightly depressed, not constricted at its base and not thickened; lateral fold weak; digits and toes slightly webbed at the base, only interspace between toes 4 and 5 not webbed, all digits and toes, except the inner ones, clawed; 14-17 subdigital lamellae below fourth toe; 5-8 preanal pores, nostril touches rostral, no internasals, and posterior ciliaries spiny; dorsum yellowish to brownish in life, seven large light spots dorsally that may be expanded to lateral narrow wavy bands, and tail with 7-10 light crossbands; gular region as well as parts of venter marbled.

Squamata: Serpentes
Viperidae

Trimeresurus truongsoneis Orlov, Ryabov, Bui & Ho, 2004: Truong Son pitviper / Ran luc Truong son (= *Trimeresurus* sp. in Ziegler et al., 2004)

Distribution. Known only from its type locality in



Figure 3. Hansi's narrow mouthed horned toad (*Ophryophryne hansii*).



Figure 4. Johns' frog (*Rana johnsi*).



Figure 5. Annam flying frog (*Rhacophorus annamensis*).



Figure 6. Giant Asian pond turtle (*Heosemys grandis*).

Phong Nha - Ke Bang (see Orlov *et al.*, 2004; Ziegler *et al.*, 2004; Nguyen *et al.*, 2005). We recorded further specimens (photographs only) adjacent to the northern border of the Phong Nha - Ke Bang National Park from Thuong Hoa to Dan Hoa communes.

Natural history notes. We collected one adult female (ZFMK 82914: SVL 488 mm, TaL 94 mm) in the dry season (May-June 2004). The specimen was found at daytime on a branch in primary limestone forest at an altitude of 600 m above sea level. This record represents the first collected female specimen, as the type series consisted of males only. The folded oviducts indicate that reproduction already took place; maximum egg diameter measured 4.4 mm in the partly dissected specimen.

Characteristic features. Slender, small pitviper, with small, triangular head; 11 supralabials, with first supralabial clearly divided from large nasal; 13 infralabials, 167 ventrals, anal scale entire, 61 divided subcaudals, and 21 (dorsally keeled) scales across the midbody; about 72 dark bands on dorsum of neck, body and tail (determination after Malhotra & Thorpe, 2004b; Orlov *et al.*, 2004).

Remarks. Nguyen *et al.* (2005) list *Trimeresurus kanburiensis* (= *Cryptelytrops kanburiensis* sensu Malhotra & Thorpe, 2004a) for Phong Nha - Ke Bang, Quang Binh province. This is most probably due to the paper by Orlov *et al.* (2003) in which *Trimeresurus kanburiensis* (cover, page 237) and *T. cf. kanburiensis* (pp. 219, 224) are listed for Phong Nha - Ke Bang; however, these specimens were described as *T. truongsongensis* by Orlov *et al.* (2004). Thus, *Cryptelytrops kanburiensis*, which can easily be distinguished from *Trimeresurus truongsongensis* e.g. by its lower (19) midbody scale count (Malhotra & Thorpe, 2004b), must be deleted from the herpetofaunal list of Phong Nha - Ke Bang as well as from Vietnam in general. Orlov *et al.* (2004) found it difficult to refer the new taxon to any group proposed by Malhotra & Thorpe (2004a) and therefore described *truongsongensis* still in the genus *Trimeresurus*.

Confirmation of species previously recorded only by Le *et al.* (1997) and Nguyen *et al.* (1997) for the Phong Nha – Ke Bang area

Reptilia
Testudines
Testudinidae

Manouria impressa (Günther, 1882): Impressed tortoise / Rua nui vien

Natural history notes. We found one specimen in montane forest (Fig. 1). It was discovered in April 2004 at noon on the forest floor.

Characteristic features. The species is characterized according to Stuart *et al.* (2001) by its distinct coloration and pattern, in combination with the carapace flattened on top, rounded legs with large scales, and by having solid, elephant-like feet.

Squamata: Serpentes
Typhlopidae

Ramphotyphlops braminus (Daudin, 1803) Common blind snake / Ran giun thuong

Natural history notes. In July 2004, a juvenile specimen was found at night below a stone in the vicinity of a rice field. The specimen was caught but could escape afterwards into a small hole in the dry ground below a shrub.

Characteristic features. Although scalation features could not have been recorded, the specimen was very similar to the species description provided by Ziegler (2002).

New herpetofaunal records for the Phong Nha – Ke Bang National Park

Amphibia
Anura
Megophryidae

Brachytarsophrys intermedia (Smith, 1921): Annam spadefoot toad / Coc mat trung gian

Distribution. The species was known only from the southern Vietnamese provinces Lam Dong, Dac Lac, Gia Lai and Kon Tum (Orlov *et al.*, 2002; Nguyen *et al.*, 2005); Orlov (2005) lists it in general as endemic for the plateaus of the central highland in Vietnam. Thus, our new record represents not only the first record for the

Phong Nha - Ke Bang National Park as well as for the Quang Binh province, but also the northernmost locality known for *B. intermedia*, more than 300 km air distance from the northernmost border of Kon Tum province.

Natural history notes. A single specimen was discovered on the ground of the primary karst forest in the cold season (early November) 2004.

Characteristic features. Although the specimen was not collected and only photographed (Fig. 2), the genus is morphologically well discernible and the specific allocation of that endemic species for Vietnam seems to be justified by the following features: a single projection on the upper eyelid whereas *B. carinensis* (Boulenger, 1889) has several projections on each eyelid, the anterior back and head of the specimen from Quang Binh is light grey, almost whitish, and the warts which form longitudinal lines on the back are well developed skinny flaps.

Ophryophryne hansii Ohler, 2003: Hansi's narrow mouthed horned toad

Distribution. Known only from the type locality, Buon Luoi in Gia Lai province (Ohler, 2003). According to Orlov (2005) an endemic species of the plateaus of Vietnam's central highlands. Our finding represents the first record for the Phong Nha - Ke Bang National Park, the first record for the Quang Binh province and in general the northernmost species record known.

Natural history notes. We found two specimens: one adult female (ZFMK 83674: SVL 43.5 mm), collected on 14 June 2005 during the dry season at night in primary karst forest. The frog (Fig. 3) sat between some branches on the forest ground near a small pond (ca. 3 m diameter). The second specimen was an adult male (ZFMK 82916: SVL 38.6 mm) that was collected in June 2003 during the dry season in the northwestern border area of the National Park.

Characteristic features. The male is of dark coloration and its dorsal skin is densely covered with tubercles and spines; the female is much lighter in aspect and the tubercles and spines are less developed. The general dorsal pattern as well as the spots on the rear part of the thigh is similar in both specimens and corresponds to the coloration of the type series (Ohler, 2003).

Ranidae

Rana johnsi SMITH, 1921: Johns' frog / Hiu hiu

Distribution. Known from numerous localities in southern, central and northern Vietnam (Nguyen & Ho, 1996; Orlov et al., 2002; Ziegler, 2002). Former records

of *R. sauteri* from Phong Nha - Ke Bang most probably were confused with *R. johnsi* (Ziegler et al., 2004; Nguyen et al., 2005).

Natural history notes. An adult female specimen (ZFMK 82887, SVL 53.0 mm) was found at the beginning of July 2004, throughout dry season. The specimen (Fig. 4) jumped at night in the leaf litter near a small primary karst forest stream. Further three specimens (SVL 22.8-25.3 mm) had been collected at daytime in the dry season (May-June 2004) on the forest ground and were subsequently deposited in the collection of the Science Research Centre of the Phong Nha - Ke Bang National Park.

Characteristic features. This species can easily be recognized among the brown hylaranas by its yellowish to reddish back and its distinct dark brown temporal triangle; on the dorsum, distinct and continuous, narrow dorso-lateral glandular ridges and a v-shaped and anteriorly pointed fold between shoulders is present; finger tips are slightly enlarged but without distinct lateral grooves; hind legs are long, shanks are 5-6 times longer than broad and adpressed hind limbs reach far beyond snout tip (determination followed Bourret, 1942; Inger et al., 1999).

Rhacophoridae

Rhacophorus annamensis Smith, 1924: Annam flying frog / Ech cay Trung bo

Distribution. Known only from the South of Vietnam northwards to Thua Thien - Hue province (Inger et al., 1999; Orlov et al., 2002; Nguyen et al., 2005; Orlov, 2005). Thus, our new record represents not only the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province, but also the northernmost locality known for the species.

Natural history notes. Two adult males (SVL 59.2-62.2 mm) were found in the dry season (May-June 2004); ZFMK 82900 and the other specimen being deposited in the collection of the Phong Nha - Ke Bang National Park Science Research Centre (Fig. 5). Both frogs were collected during daytime on the ground of primary limestone forest in elevations of about 400 m above sea level.

Characteristic features. Habitus stocky, snout pointed, nostrils slightly closer to tip of snout than to eye, and tympanum distinct, less than half diameter of eye; discs of fingers rounded, those of outer fingers wider than tympanum; dark webbing, web reaching edge of subarticular tubercle of first finger, to disc of three outer fingers; subarticular tubercle conspicuous, toes webbed

to base of discs, and low inner, but no outer metatarsal tubercle; heel with small, bluntly pointed projection, and infra-anal area with long tubercles; color in life brown dorsally and laterally, with irregular light spots on the back and faint dark crossbars on limbs; lower half of sides white with dark marbling, and venter white with small dark spots on throat and chest (determination according to Inger *et al.*, 1999).

Remarks. This species is one of the brown colored *Rhacophorus* with full webbing on hands as have *R. pardalis* Günther, 1859 and *R. robinsoni* Boulenger, 1903. *R. annamensis* is larger than *R. pardalis*, and *R. annamensis* has dark grey webbing, which is orange in *R. pardalis*. The poorly known *R. robinsoni* can be distinguished from the two species by its shorter snout (Bourret, 1942).

Reptilia
Testudines
Geoemydidae

Heosemys grandis (Gray, 1860): Giant Asian pond turtle / Rua dat lon

Distribution. The species was known from some lowland and hill areas of central and southern Vietnam (Stuart *et al.*, 2001). Our finding represents not only the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province, but also the northernmost record known for Vietnam (Nguyen & Ho, 1996; Nguyen *et al.*, 2005).

Natural history notes. A single specimen (carapace length about 26 cm), that was caught by local people, was recorded on 3 July 2004 in the local market (Nguyen, 2005: 30, 34).

Characteristic features. Although the specimen was not collected and only photographed (Fig. 6), determination was possible due to the well discernible characteristic features: carapace with spikes on back edge, and a pale vertebral keel on the midline of carapace; plastron yellow with black lines radiating outward from a black blotch on each scute, and underside of marginals yellow with radiating black lines, head pale orange with faint black spots and streaks; straight seam between femoral and anal scutes, and lack of a plastron hinge (determination followed Stuart *et al.*, 2001).

Ocadia sinensis (Gray, 1834): Chinese striped-neck turtle / Rua co soc

Distribution. The species was known from some provinces in central and northern Vietnam (Nguyen

& Ho, 1996; Ziegler, 2002; Nguyen *et al.*, 2005). Our finding represents the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province.

Natural history notes. A single, subadult specimen (carapace length about 14 cm) was recorded on 3 July 2004 in the local market (see also Nguyen, 2005: 31-32, 34).

Characteristic features. Although the specimen was not collected and only photographed (Fig. 7), determination was possible due to the well discernible characteristic features: top of head black, with many thin black and yellow-green lines on the sides of the head and throat, and legs striped same as head; carapace dark brown to blackish, and plastron pale with dark blotch on each scute (determination followed Stuart *et al.*, 2001).

Squamata: Sauria
Gekkonidae

Gekko palmatus Boulenger, 1907: Palmated gecko / Tac ke chan vit

Distribution. Known only from the northern provinces Lang Son and Vinh Phuc as well as from the offshore island Cu Lao Phon Vong (Ota *et al.*, 1995; Nguyen *et al.*, 2005). Thus, our new record represents not only the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province, but also the southernmost locality known for the species.

Natural history notes. The adult female (ZFMK 82888, SVL 60.5, Tal 64.5 mm) was found at night of 3 July 2004 in a karst forest area. The specimen (Fig. 8) climbed on a big stone near a forest path.

Characteristic features. Characteristic are the nostril in contact with rostral, two internasals, smaller than nasorostrals, and tubercles present on dorsum of body, lacking on forelimb and thigh; body scales in 145 rows around midbody, webs well developed, and cloacal spur single; one pair of dark, roundish or somewhat elongated spots in occipital region, smaller but more distinct dark spot in nuchal region, and light broken middorsal stripe evident on body (determination followed Ota *et al.*, 1995).

Remarks. In contrast to Ota *et al.* (1995: their fig. 3), our specimen bears two small supranasals each, that are in addition not distinctly larger than the internasals. Furthermore, the rostral scale is posteriorly notched in the middle.

Scincidae

Tropidophorus noggei Ziegler, Vu & Bui, 2005

Distribution. Known only from its type locality in Phong Nha - Ke Bang (see Ziegler et al., 2005).

Natural history notes. The type series (one adult male, ZFMK 83668; two adult females, ZFMK 83669, VNUH 18.6.'05-1) was found throughout June 2005, at the end of the dry season, in a steep primary karst forest area. The skinks were discovered at night at the base of karst rock outcrops where they sat in front of narrow rock crevices near the forest floor into which they subsequently tried to escape. This depressed-bodied skink species seems to be adapted to karst rock crevices (Ziegler et al., 2005). *Characteristic features.* The species is characterized by its distinctly depressed body in combination with considerably enlarged dorsal scales resulting in a very low (22) midbody scale count.

Squamata: Serpentes
Colubridae

Boiga guangxiensis Wen, 1998: Guangxi cat snake /
Ran rao Quang Tay

Distribution. According to Orlov et al. (2003) very common in North Vietnam ("Tonkin and Annam mountains"), but rarer in South Vietnam (see also Orlov, 2005; Nguyen et al.; 2005). Our finding represents the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province.

Natural history notes. We found one specimen, ZFMK 83673: an adult male (SVL 1240, TaL 421 mm), collected on 16 June 2005 during the dry season at night in primary karst forest. The snake (Fig. 9) was discovered when crawling in the branches about 0.3 m above a small stream.

Characteristic features. One loreal, two postoculars, and 2-3 anterior and three posterior temporals; eight supralabials (3-5 in contact with the eye), 11-12 infralabials, and 21 (dorsally smooth) scales across the midbody; 265 ventral scales and 144 divided subcaudal scales; remarkable are the anal plate, that is entire but with a median fold, and 1-2 preoculars (on the left side, two preoculars are present; on the right side the suture between "both scales" is not complete), with uppermost reaching top of head, but not touching frontal (determination followed Orlov et al., 2003; Tillack et al., 2004).

Remarks. Le et al. (1997) listed *Boiga cynodon* for Phong Nha - Ke Bang; as *B. guangxiensis* has often been



Figure 7. Chinese striped-necked turtle (*Ocadia sinensis*).



Figure 8. Palmated gecko (*Gekko palmatus*).



Figure 9. Guangxi cat snake (*Boiga guangxiensis*).



Figure 10. South China green snake (*Cyclophiops major*).

confused with *B. cynodon* or *B. siamensis* (formerly *B. ocellata*, see Pauwels *et al.* 2005) in the older literature and records of true *B. cynodon* from Vietnam are still missing (Orlov *et al.*, 2003; Tillack *et al.*, 2004), *B. cynodon* should be deleted from the National Park's herpetofaunal list.

Cyclophiops major (Günther, 1858): South China green snake / Ran dai lon

Distribution. Known only from some provinces in northern Vietnam (Bourret, 1936b; Nguyen & Ho, 1996; Zhao & Adler, 1993; Nguyen *et al.*, 2005). Thus, our finding represents not only the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province, but also the southernmost record in Vietnam.

Natural history notes. We found one adult specimen (SVL 635, TaL 200 mm), deposited in the collection of the Phong Nha - Ke Bang National Park Science Research Centre: the specimen (Fig. 10) was collected throughout daytime on the ground of primary limestone forest at an altitude of about 400 m above sea level.

Characteristic features. One loreal, eight supralabials (4-5 in contact with the eye), 15 scales across the midbody, 169 ventral scales, anal divided, and 76 divided subcaudal scales (determination followed Bourret, 1936b, who lists the species as *Liopeltis m. major*).

Pareas carinatus Wagler, 1830: Keeled slug snake / Ran ho may go

Distribution. Nguyen & Ho (1996) and Nguyen *et al.* (2005) mention records of *P. carinatus* (listed in Nguyen & Ho, l. c. in the genus *Dipsas*) from northern, central and southern Vietnam. Our findings represent the first record for the Phong Nha - Ke Bang National Park as well as for the Quang Binh province.

Natural history notes. We found two adult male specimens: ZFMK 82890 (SVL 365, TaL 123 mm), collected on 5 July 2004; VNUH 15.6.'05-1 (SVL 457, TaL 144 mm), collected on 15 June 2005. Both specimens were discovered during the dry season at night in primary karst forest near rock outcrops: the snake ZFMK 82890 (Fig. 11) was seen when crawling on the leaf litter and the specimen VNUH 15.6.'05-1 was found in the branches about 1.5 m above a small stream.

Characteristic features. Prefrontals not in contact with the eye, two preoculars, in contact with the single loreal, 1-2 suboculars between supralabials and eye, and 1-2

postoculars; 6-7 supralabials, and 15 (dorsally keeled) scales across the midbody; 176-177 ventral scales, 78-80 divided subcaudal scales, and anal plate entire (determination after Bourret, 1936a; b).

New herpetofaunal records from adjacent areas in Quang Binh province

Amphibia
Anura
Dicroglossidae

Limnionectes poilani (Bourret, 1942)

Distribution. Known only from Dong Tam Ve, Quang Tri province. The discovery of this species from Thuong Hoa commune, Minh Hoa district extends its range about 100 km to the north.

Natural history notes. We collected a male specimen (ZFMK 82904: SVL 37.9 mm) in the dry season (May-June 2004). The specimen (Fig. 12) was found at daytime, on the ground of a limestone valley nearby a stream at an altitude of 450 m above sea level.

Characteristic features. The specimen is a member of the genus *Limnionectes* (*Elachyglossa*) as it has feet with moderate webbing and distinctly enlarged toe tips. As stated by Taylor (1962) specific allocation of females and juveniles of this group of frogs is very difficult. Nevertheless reliable allocation of adult male specimen lead to a discontinuous distribution pattern. Thus geographic origin can be used as a hint for species allocation. The specimen from Thuong Hoa lacks secondary sexual characters (head not strongly enlarged, tooth-like projection on lower jaw absent, no foldings and no dark coloration on throat) so we have to consider it being a subadult. The geographically closest species is *Limnionectes* (*Elachyglossa*) *poilani* (Bourret, 1942) (see Ohler *et al.*, 2002) which was described based on a specimen from Quang Tri province. The specimen ZFMK 82904 shares with the holotype MNHN 1948.0127 the color pattern of the back and the coloration of the tympanum which is dark in its upper part and light in the lower part. Both specimens show warts and spinules on the posterior back and elongated rather than dense glandular warts on the flanks. The web reaches toe IV near the distal subarticular tubercle and continues as a narrow fringe to the toe pad. The holotype of *Rana toumanoffi* Bourret, 1941 has quite a large sized tympanum. The rugosity of the dorsal skin is similar but

the flanks are much smoother and the color pattern of the back is quite uniform. *Limnonectes dabanus* Smith, 1922 has a tympanum of uniform color and the webbing of the feet extends to the distal phalange. The back of adult males is much more spiny in *L. dabanus* than in the specimen from Thuong Hoa.

Remarks. Both geographical pattern and morphology indicate this specimen being *Limnonectes poilani*. Thus new material can be allocated to another of the species discovered by Bourret. Ultimately only study of adult males would allow confirmation of this discovery.

Ranidae

Sylvirana maosonensis (BOURRET, 1937): Mao-son frog / Chang Mau Son

Distribution. Known from northern Vietnam, southwards to Ha Tinh province (Nguyen & Ho, 1996; Orlov et al., 2002; Ziegler, 2002; Nguyen et al., 2005). Thus, our new records from adjacent to Phong Nha - Ke Bang, Thuong Hoa commune, Minh Hoa district represent not only the first record for Quang Binh province, but also the southernmost locality known for *S. maosonensis*.

Natural history notes. We collected eight adult male specimens (SVL 35.1-43.7 mm) between May and June 2004 (Fig. 13): ZFMK 82905-82910 and two specimens deposited in the collection of the Science Research Centre of the Phong Nha – Ke Bang National Park. All specimens were discovered during the dry season at daytime on the ground of a large limestone valley nearby a stream at an altitude of about 450 m above sea level.

Characteristic features. Distinct but interrupted dorso-lateral glandular ridges, and larger glandular fields behind tympanum and above arm insertion; dorsum rough, warty, flanks with wart-like structures; legs dorsally with tubercles, finger and toe tips somewhat broadened, with marginal grooves, and first finger longer than second; external metatarsal tubercle, toes 3/4 webbed, fourth toe webbed somewhat beyond the median subarticular tubercle, and adpressed hind limbs reach snout tip (determination after Bourret, 1942; Inger et al., 1999).

Rhacophoridae

Philautus cf. *jinxuensis* Hu, 1978

Distribution. This species was originally described from Guangxi (China). Ohler et al. (2000) and Orlov et al. (2004) reported on this species from northern Vietnam.



Figure 11. Keeled slug snake (*Pareas carinatus*).



Figure 12. *Limnonectes poilani*.



Figure 13. Mao-son frog (*Sylvirana maosonensis*).



Figure 14. *Philautus* cf. *jinxuensis*.

The single specimen from adjacent to Phong Nha - Ke Bang, Dan Hoa commune, Minh Hoa district, Quang Binh province extends the range far to the south.

Natural history notes. The single specimen, ZFMK 82899, sat at night on a branch near a small stream (Fig. 14). The adult female (SVL 34.6 mm) was collected during the dry season (May-June 2004) in montane forest (750 m above sea level).

Characteristic features. It is a moderate sized *Philautus* with a triangular spot on the head which continues as band from the central shoulder region to the groin. The specimen is rather dark and densely mottled. It shows a slight web between fingers III and IV. The web of the feet is moderately developed. Dorsal skin shows numerous flat tubercles with whitish top. It has no tubercles on the external border of finger IV, toe V, and tarsus.

Remarks. Taxonomic allocation of this single female specimen is very difficult as male sexual characters cannot be used. The dorsal color pattern is present in other members of the genera *Philautus* and *Aquixalus*, but never in *Rhacophorus* (in species like *R. annamensis* the triangle on the head is followed by a more or less complete middorsal band). The species allocated to *Aquixalus* according to Delorme *et al.* (2005, but compare systematics in Frost *et al.* 2006) have rather complete webbing. However, the specific allocation of the present specimen is difficult and lack of data refrain us from describing it as a new species. Due to its general phenotype we allocate it to *Philautus jinxiuensis* but studies on larger samples are needed to confirm this. Furthermore, Orlov & Ho (2005) list their new species *P. truongsongensis* as occurring as well in Phong Nha - Ke Bang National Park. However, as this record by Orlov & Ho (2005) was based on a photograph only (that in addition differed somewhat from the *P. truongsongensis* type series), we refrained from listing this species in Table 1 until reference specimens are available for closer analyses.

Reptilia
Squamata: Sauria
Lacertidae

Takydromus hani Chou, Nguyen & Pauwels, 2001:
Green grass lizard / Liu diu xanh

Distribution. Known only from Ha Tinh, Thua Thien-Hue, Da Nang and Quang Nam provinces in central and southern Vietnam (Chou *et al.*, 2001; Nguyen *et al.*, 2005). Our specimen from adjacent to the border of the Phong Nha - Ke Bang National Park, Dan Hoa

commune, Minh Hoa district represents the first record for the Quang Binh province.

Natural history notes. The adult male (ZFMK 82919, SVL 78, Tal 255 mm) was collected at daytime in June 2003. The specimen (Fig. 15) was found on a large stream bank in about 250 m above sea level. In the original description it was stated that the species occurs in undergrowth by streams in primary subtropical forests; Kizirian (2004) reports the species being primarily a tree-canopy inhabitant with preference for riparian forest.

Characteristic features. Characteristic are the single postnasal scale, four pairs of chin shields, and six longitudinal rows of large dorsale scales between the hind legs, enlarged lateral scales on body absent, eight longitudinal rows of ventral scales, ventrals keeled, and seven femoral pores on each side (determination after Chou *et al.*, 2001).

Squamata: Serpentes
Colubridae

Amphiesma andreae Ziegler & Le, 2006: Andrea's keelback / Ran sai Andrea

Distribution. Known only from a single specimen from adjacent to Phong Nha - Ke Bang, Thuong Hoa commune, Minh Hoa district, Quang Binh province.

Natural history notes. The male specimen (ZFMK 83747, SVL 420, TaL 188 mm) was found during the late afternoon on the forest ground of a large limestone valley nearby a stream at an altitude of 450 m above sea level (Fig. 17).

Characteristic features. The recently described species (Ziegler & Le, 2006), is characterized by its distinct coloration and pattern, in combination with a slender body and tail (tail/total length ratio 0.31), large eyes, a single loreal and preocular, three postoculars, a single anterior and posterior temporal, nine supralabials (4-6 in contact with eye), nine infralabials, 179 ventrals (plus two prefrontals according to Dowling, 1951), anal plate divided, 99 divided subcaudals, dorsal scales in 19-19-17 keeled rows, 34 maxillary teeth (the two posteriormost enlarged), and a simple spinose hemipenis, with undivided sperm groove.

Boiga bourreti Tillack, Ziegler & Le, 2004: Bourret's cat snake / Ran rao Bourret

Distribution. Known only from a single specimen from north-west to Phong Nha - Ke Bang, Minh Hoa district, Quang Binh province (see Tillack *et al.*, 2004).

Natural history notes. The adult female (ZFMK 82921: SVL 921, TaL 234 mm) was found in April 2004 in evergreen primary forest 550 m above sea level. The specimen was discovered at night on the forest ground between leaves.

Characteristic features. The species is characterized by its dorsal scales in 21:19:15 bent rows, 236 ventrals, 106 divided subcaudals, and a divided anal scute; 8 supralabials (3-5 in contact with eye), and 11/12 infralabials; one large loreal, 2/3 preoculars, with uppermost reaching top of head, but not touching frontal, two postoculars, and 3/4 anterior temporals with 3/3 posterior ones following; a blackish brownish, light-margined postocular stripe runs past the corner of the mouth towards the wide dark neck band; the body pattern consists of the dark band on the neck, two subsequent V-shaped bands and a chequered pattern of light and dark flecks that extends along the body and only dissolves on the dorsal side of the tail.

Calamaria thanhi Ziegler & Le, 2005: Thanh's reed snake / Ran mai gam Thanh

Distribution. Known only from a single specimen from adjacent to Phong Nha - Ke Bang, Dan Hoa commune, Minh Hoa district, Quang Binh province (see Ziegler & Le, 2005).

Natural history notes. The adult female (ZFMK 82920, SVL 424, TaL 31 mm) was found in June 2003 in a limestone cave of primary forest.

Characteristic features. The species is characterized by the following combination of characters: dark, iridescent body with four yellowish to beige zigzag shaped bands, light dorsal markings on base and tip of tail, and light venter; modified maxillary teeth; large size, tail tapering gradually to a point, reduction to five dorsal scale rows on tail; 198 ventral scales, 21 divided subcaudals, four supralabials (second and third entering orbit), and five infralabials; mental not touching anterior chin shields, and three gular scales in midline between posterior chin shields and first ventral; rostral wider than high, paraparietal surrounded by six shields and scales, and absence of a preocular scale.

Dryocalamus davisonii (Blanford, 1878): Bridle snake/ Ran de

Distribution. Known only from southern and central Vietnam northwards up to Quang Tri province (Campden-Main, 1970; Nguyen & Ho, 1996; Nguyen et al., 2005). Thus, our specimen represents not only the first record for Phong Nha - Ke Bang and Quang Binh



Figure 15. Green grass lizard (*Takydromus hani*).



Figure 16. Bridle snake (*Dryocalamus davisonii*).



Figure 17. Andrea's keelback (*Amphiesma andreae*)

province, but also the northernmost record in Vietnam. *Natural history notes.* The subadult specimen (ZFMK 82913, SVL 211, TaL 65 mm) was found adjacent to the border of the Phong Nha - Ke Bang National Park in Ca Tap valley, Thuong Hoa commune, Minh Hoa district, Quang Binh province. The snake (Fig. 16) was discovered at the afternoon of 8 May 2004 in a secondary limestone forest valley in about 350 m above sea level. The specimen was in a hole between the roots of a big tree nearby a large but dry stream bed with dense vegetation and canopy, respectively. *Characteristic features.* Seven supralabials (3-4 in contact with eye), one loreal, lacking preocular, two postoculars, 13 dorsal midbody scale rows, 239 ventrals, 111 divided subcaudals, and anal scute entire (determination after Campden-Main, 1970; Bourret, 1936a; b).

Discussion

As already discussed in Ziegler *et al.* (2004), not all of the 128 amphibian and reptile records listed therein will consist in the future, as there are some doubtful records in the lists by Le *et al.* (1997), Nguyen *et al.* (1997) and Vassiliev (1999), which still have to be proven. One example is “*Naja naja*”, which was mentioned according to the old Asian cobra concept so that it still has to be clarified, which species was implied to occur. Thus, we herein recommend to delete *Naja naja* from the actual species list, as well as *Boiga cynodon*, as was pointed out before. Adding our eleven new herpetofaunal records (2 Megophryidae, 1 Ranidae, 1 Rhacophoridae, 2 Geoemydidae, 1 Gekkonidae, 1 Scincidae, and 3 Colubridae) to the remaining 126 species, we currently know about 137 amphibian and reptile species for Phong Nha – Ke Bang (Fig. 18). Herein, we do not consider

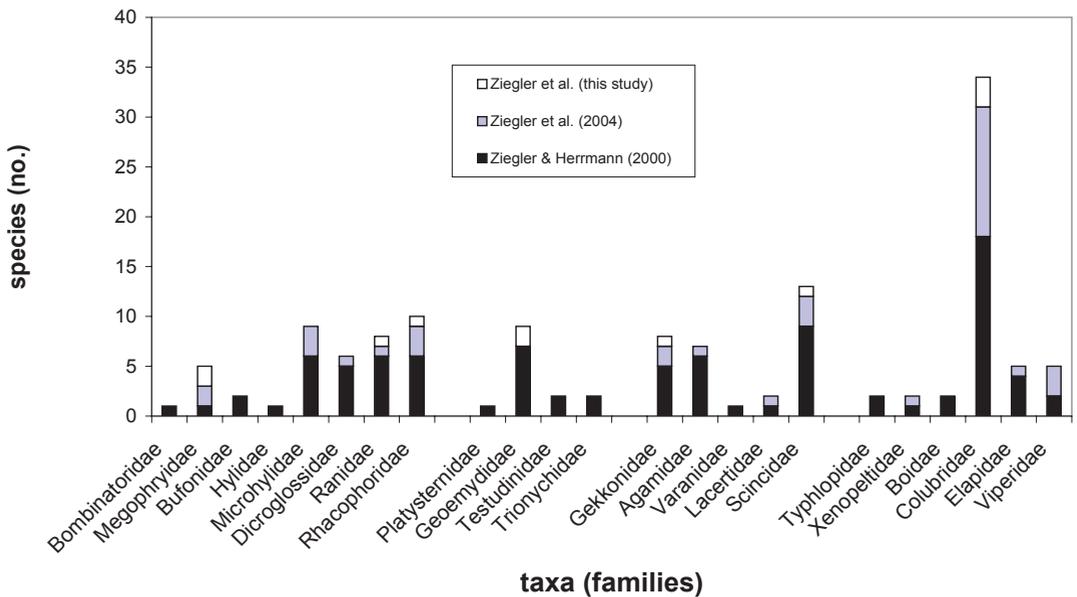


Figure 18. Amphibian and reptile species recorded for Phong Nha - Ke Bang based on the lists by Ziegler & Herrmann (2000) (black bars), Ziegler *et al.* (2004) (grey bars) as well as the findings of our recent surveys (this paper) (white bars).

Because the results (determinations) of several authors are treated together in Ziegler & Herrmann (2000) and Ziegler *et al.* (2004), double- or misidentifications cannot be excluded. The four species (*Occidozyga laevis*, *Rana sauteri*, *Cyrtodactylus pulchellus*, and *Takydromus wolteri*) that were recommended to delete from the herpetofaunal list of the National Park by Ziegler *et al.* (2004) are not included in this diagram, as well as *Boiga cynodon* and *Naja naja*, which we recommend for deletion from the list in this paper.

In this diagram we did not consider the eight species (1 Dicroglossidae, 1 Ranidae, 1 Rhacophoridae, 1 Lacertidae, and 4 Colubridae), which were recorded by us adjacent from the National Park’s border, although it can be expected, that they will be recorded also from inside the National Park in the near future; also the recent scincid records listed by Darevsky & Orlov (2005) are not included in the diagram (see discussion).

the eight species (1 Dicroglossidae, 1 Ranidae, 1 Rhacophoridae, 1 Lacertidae, and 4 Colubridae), which were recorded by us adjacent from the National Park's border, although it can be expected, that they will be recorded also from inside the National Park in the near future. In addition, Darevsky & Orlov (2005) recently listed as further herpetological records for the Phong Nha – Ke Bang National Park the following scincid species: *Eumeces elegans*, *Leptoseps tetradactylus*, “*Scincela cf. rupicolum*” (sic), and *Tropidophorus cf. baviensis*. Because the latter species most probably is conspecific with the recently described *Tropidophorus noggei*, this brings the total number of amphibians and reptiles known for the Phong Nha - Ke Bang National Park to 140 (see Table 1), representing more than 30 percent of the 458 amphibian and reptile species listed in the updated checklist for Vietnam by Nguyen et al. (2005).

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Appendix. List of amphibians and reptiles known for Phong Nha – Ke Bang according to Ziegler *et al.* (2004) and the results of this study (without the eight species being recorded by us adjacent from the National Park's border). Species whose presence was confirmed by the authors are marked with an asterisk, the remaining listed species are based on project reports and lists by Le *et al.* (1997), Nguyen *et al.* (1997), Vassiliev (1999), and Darevsky & Orlov (2005). Species, previously recorded by the latter authors that remain doubtful are marked with a question mark; undetermined records by Vassiliev (1999) were not considered (e.g., *Amolops* sp., *Sphenomorphus* sp.). *Bombina maxima* was replaced by *B. microdeladigitora*, *Megophrys lateralis* by *Xenophrys major*, *Microhyla* cf. *annamensis* / *Microhyla* sp. n. by *M. marmorata*, *Hoplobatrachus rugulosus* by *H. chinensis*, *Polypedates* sp. by *P. mutus*, *Rhacophorus reinwardtii* by *R. kio*, *Gekko* sp. / *Gekko* sp. n. by *G. scientiadventura*, *Tropidophorus* cf. *baviensis* by *T. noggei*, and *Trimeresurus* sp. by *T. truongsonensis*; nomenclature follows Ohler *et al.* (2000), Bain & Nguyen (2004), Malhotra & Thorpe (2004a), Orlov

et al. (2004), Rösler et al. (2004), Stuart & Platt (2004), Ziegler et al. (2004), Darevsky & Orlov (2005), Delorme et al. (2005), Utiger et al. (2005), Vogel & David (2005), Ohler & Delorme (2006), as well as the online reference «Amphibian species of the world, 3.0» by the American Museum of Natural History (see <http://research.amnh.org/herpetology/amphibia/index.php>) and the review by Frost et al. (2006). The most recent record of *Philautus truongsongensis* for Phong Nha - Ke Bang National Park by Orlov & Ho (2005) is not considered here, because it was based on a photograph only and further examinations of *Philautus* from that area are still outstanding (refer to the *P. cf. jinxiuensis* chapter within this paper).

AMPHIBIA

ANURA

Bombinatoridae

Bombina microdeladigitata? Liu, Hu & Yang, 1960

Megophryidae

*Brachytarsophrys intermedia** (Smith, 1921)

*Leptobrachium chapaense** (Bourret, 1937)

*Leptolalax cf. pelodytoides** (Boulenger, 1893)

*Ophryophryne hansi** Ohler, 2003

*Xenophrys major** (Boulenger, 1908)

Bufoinidae

*Duttaphrynus melanostictus** (Schneider, 1799)

*Ingerophrynus galeatus** (Günther, 1864)

Hylidae

*Hyla simplex** Boettger, 1901

Microhylidae

*Kalophrynus interlineatus** (Blyth, 1854)

*Kaloula pulchra** Gray, 1831

*Microhyla berdmorei** (Blyth, 1856)

*M. butleri** Boulenger, 1900

*M. heymonsi** Vogt, 1911

*M. marmorata** Bain & Nguyen, 2004

*M. ornata** (Duméril & Bibron, 1841)

*M. pulchra** (Hallowell, 1861)

*Micryletta inornata** (Boulenger, 1890)

Dicroglossidae

*Fejervarya limnocharis** (Gravenhorst, 1829)

*Hoplobatrachus chinensis** (Osbeck, 1765)

*Limnonectes hascheanus** (Stoliczka, 1870)

*L. kuhlii** (Tschudi, 1838)

Occidozyga lima (Gravenhorst, 1829)

*O. martensii** (Peters, 1867)

Ranidae

Amolops ricketti (Boulenger, 1899)

Huia andersonii (Boulenger, 1882)

*H. chloronota** (Günther, 1876)

*Hylarana guentheri** (Boulenger, 1882)

H. macrodactyla Günther, 1858

H. taipehensis (Van Denburgh, 1909)

*Rana johnsi** Smith, 1921

*Sylvirana nigrovittata** (Blyth, 1856)

Rhacophoridae

*Chiromantis vittatus** (Boulenger, 1887)

*Kurixalus verrucosus** (Boulenger, 1893)

*Polypedates leucomystax** (Gravenhorst, 1829)

*P. mutus** (Smith, 1940)

*Rhacophorus annamensis** Smith, 1924

*R. bipunctatus** Ahl, 1927

*R. demysi** Blandford, 1881

*R. kio** Ohler & Delorme, 2006

*R. orlovi** Ziegler & Köhler, 2001

*Theloderma asperum** (Boulenger, 1886)

REPTILIA

TESTUDINES

Platysternidae

*Platysternon megacephalum** Gray, 1831

Geoemydidae

*Cuora galbinifrons** Bourret, 1939

*C. mouhotii** (Gray, 1862)

*C. trifasciata** (Bell, 1825)

*Cyclemys tcheponensis** (Bourret, 1939)

*Heosemys grandis** (Gray, 1860)

Malayemys subtrijuga? (Schlegel & Müller, 1844)

*Mauremys mutica** (Cantor, 1842)

*Ocadia sinensis** (Gray, 1834)

*Sacalia quadriocellata** (Siebenrock, 1903)

Testudinidae

Indotestudo elongata (Blyth, 1853)

*Manouria impressa** (Günther, 1882)

Trionychidae

*Palea steindachneri** (Siebenrock, 1906)

*Pelodiscus sinensis** (Wiegmann, 1834)

SQUAMATA: SAURIA

Gekkonidae

*Cyrtodactylus phongnhakebangensis** Ziegler, Rösler, Herrmann & Vu, 2003

*Gehyra mutilata** (Wiegmann, 1834)

*Gekko gecko** (Linnaeus, 1758)
*G. palmatus** Boulenger, 1907
*G. scientiadventura** Rösler, Ziegler, Vu, Herrmann & Böhme, 2004
*Hemidactylus frenatus** Duméril & Bibron, 1836
H. garnotii Duméril & Bibron, 1836
H. karenorum? (Theobald, 1868)

Agamidae

Acanthosaura crucigera? Boulenger, 1885
*A. lepidogaster** (Cuvier, 1829)
*Calotes emma** Gray, 1845
*C. versicolor** (Daudin, 1802)
Draco maculatus (Gray, 1845)
Leiolepis belliana? (Gray, 1827)
*Physignathus cocincinus** Cuvier, 1829

Varanidae

*Varanus salvator** (Laurenti, 1768)

Lacertidae

Takydromus sexlineatus Daudin, 1802
*T. kuehnei** van Denburgh, 1909

Scincidae

Eumeces elegans Boulenger, 1887
E. quadrilineatus (Blyth, 1853)
Eutropis chapaense (Bourret, 1937)
*E. longicaudata** (Hallowell, 1856)
*E. macularia** (Blyth, 1853)
*E. multifasciata** (Kuhl, 1820)
Leptoseps tetradactylus Darevsky & Orlov, 2005
Lygosoma quadrupes (Linnaeus, 1766)
*Scincella melanosticta** (Boulenger, 1887)
*S. reevesii** (Gray, 1838)
S. rupicola (Smith, 1916)
Scincella sp.*
Sphenomorphus buenloicus Darevsky & Nguyen, 1983
*S. indicus** (Gray, 1853)
*Tropidophorus cocincinensis** Duméril & Bibron, 1839
*T. noggeri** Ziegler, Vu & Bui, 2005

SQUAMATA: SERPENTES

Typhlopidae

*Ramphotyphlops braminus** (Daudin, 1803)
Typhlops diardi Schlegel, 1839

Xenopeltidae

*Xenopeltis hainanensis** Hu & Zhao, 1972
*X. unicolor** Boie, 1827

Boidae

*Python molurus** (Linnaeus, 1758)
*P. reticulatus** (Schneider, 1801)

Colubridae

*Ahaetulla prasina** (Boie, 1827)
Amphiesma khasiense (Boulenger, 1890)
Amphiesma sp. n.*
*A. stolatum** (Linnaeus, 1758)
*Boiga guangxiensis** Wen, 1998
*B. multomaculata** (Boie, 1827)
Calamaria pavementata Duméril, Bibron & Duméril, 1854
C. septentrionalis Boulenger, 1890
Chrysopelea ornata (Shaw, 1802)
*Coelognathus radiatus** (Boie, 1827)
*Cyclophiops major** (Günther, 1858)
*C. multicinctus** (Roux, 1907)
*Dendrelaphis ngansonensis** (Bourret, 1935)
D. pictus (Gmelin, 1789)
Dinodon cf. *rufozonatum** (Cantor, 1842)
D. septentrionalis (Günther, 1875)
*Enhydryis plumbea** (Boie, 1827)
*Lycodon fasciatus** (Anderson, 1897)
*L. paucifasciatus** Rendahl, 1943
*Oligodon chinensis** (Günther, 1888)
O. taeniatus (Günther, 1861)
*Oreocryptophis porphyraceus** (Cantor, 1839)
Orthriophis moellendorffi (Boettger, 1886)
*O. taeniurus** Cope, 1861
*Pareas carinatus** Wagler, 1830
*P. margaritophorus** (Jan, 1866)
*Psammodynastes pulverulentus** (Boie, 1827)
*Ptyas korros** (Schlegel, 1837)
*P. mucosus** (Linnaeus, 1758)
*Rhabdophis chrysargos** (Schlegel, 1837)
*R. subminiatus** (Schlegel, 1837)
Sibynophis collaris (Gray, 1853)
*Simonatrix percarinata** (Boulenger, 1899)
*Xenochrophis flavipunctatus** (Hallowell, 1860)

Elapidae

*Bungarus candidus** (Linnaeus, 1758)
*B. fasciatus** (Schneider, 1801)
Naja cf. *atra** Cantor, 1842
*Ophiophagus hannah** (Cantor, 1836)
Sinomicrurus maccllellandi (Reinhardt, 1844)

Viperidae

Cryptelytrops albolabris (Gray, 1842)
*Protobothrops cornutus** (Smith, 1930)
*Triceratolepidophis sieversorum** Ziegler, Herrmann, David, Orlov & Pauwels, 2000
*Trimeresurus truongsonensis** Orlov, Ryabov, Bui & Ho, 2004
*Viridovipera vogeli** (David, Vidal & Pauwels, 2001)