ISSN 1067-4136, Russian Journal of Ecology, 2012, Vol. 43, No. 6, pp. 448–453. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © V.N. Bol'shakov, A.G. Vasil'ev, I.A. Vasil'eva, Yu.V. Gorodilova, N.E. Kolcheva, N.M. Lyubashevskii, M.B. Chibiryak, 2012, published in Ekologiya, 2012, No. 6, pp. 427–433.

Technogenic Morphological Variation of the Pygmy Wood Mouse (*Sylvaemus uralensis* Pall.) in the Urals

V. N. Bol'shakov, A. G. Vasil'ev, I. A. Vasil'eva, Yu. V. Gorodilova, N. E. Kolcheva, N. M. Lyubashevskii, and M. B. Chibiryak

Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences,
ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia;
e-mail: vag@ipae.uran.ru

Received May 16, 2012

Abstract—Methods of geometric morphometrics have been used to compare the ranges and directions of geographic and technogenic forms of variation in the morphology of the mandible in Ural populations of pygmy wood mice (*Sylvaemus uralensis* Pall.) exposed to different types of pollutants (radionuclides, fluorides, and toxic petrochemical products). The range of variation in the mandible morphology consequent to chronic radiation exposure in the zone of the Eastern Ural Radioactive Trace is commensurate to the range of geographic variation of the species observed in the Middle and Southern Urals. Unspecific manifestations of variation have been revealed, which apparently result from exposure to technogenic pollutants of different origin. A probable contribution of technogenic variation to rapid morphogenetic rearrangements in populations is discussed.

Keywords: evolutionary ecology, geometric morphometry, technogenic pollutants, pygmy wood mouse

DOI: 10.1134/S1067413612060033

The leading role of ecological factors in evolutionary reorganization of morphogenetic processes (Shvarts, 1980) is gaining increasing recognition worldwide (Debat and David, 2001; Gilbert, 2003; Schlichting, 2003; Klingenberg and Gidaszewski, 2010; etc.). Evolutionary ecological changes are most probable when populations live for a long time in a new, anthropogenically transformed environment, reflecting remote effects of its chronic pollution by certain technogenic substances (Vasil'ev and Vasil'eva, 2005; Bezel', 2006). Directed technogenic transformations of the environment should lead not only to rearrangements in the biota and individual communities (Zherikhin, 2003; Chernov, 2008), but also to adaptive microevolutionary changes in species populations comprising these communities (Vasil'ev and Bol'shakov, 1994; Vasil'ev and Vasil'eva, 2005). The magnitude of anthropogenic impact on the biota is increasing year by year because of numerous catastrophic accidents caused by technological factors or natural causes. Therefore, evolutionary–ecological analysis of the extent and direction of morphogenetic changes in impact populations may become an urgent task already in the nearest future.

One of the most feasible approaches to such studies is to compare the ranges and patterns of intraspecific variation observed in natural and technogenic environments. Since technogenically altered environmental conditions usually have no direct analogs in nature, corresponding areas may serve as test grounds where rapid and, probably, inadaptive (in A.P. Rasnitsyn's terminology) morphogenetic rearrangements in populations can be expected (Vasil'ev and Vasil'eva, 2005). Series of samples from control and impact populations of a model species can be collected so as to reflect situations in natural and technogenic areas within the species range. On this basis, an attempt can be made to compare the magnitudes of natural (geographic and chronographic) intraspecific variation and of anthropogenically conditioned morphogenetic changes in populations that are consequent to the effects of environmental pollution and represent a special form of group variation, namely, *technogenic variation*. This task can be accomplished using methods of geometric morphometrics, which make it possible to analyze variation in the shape of test objects (excluding the effect of their size) on the basis of homologous landmarks in their digital images (Rohlf and Slice, 1990; Zelditch et al., 2004). This approach allows not only visualization of morphological changes but also their morphogenetic interpretation (Klingenberg, 2011).

The purpose of this study was to apply methods of geometric morphometrics to comparative analysis of the ranges of geographic and technogenic variation in the shape of the mandible in the pygmy wood mouse (*Sylvaemus uralensis* Pall.) in the Urals and Cisural region in order to reveal the remote consequences of

Locations, sizes, and collectors of samples included in the study

Sample, location (geographic coordinates) and year of sampling	Sample size, ind.	Collectors
Eginsai, Sol'-Iletskii raion, Orenburg oblast (55°07′, 50°57′) 1978	28	A.G. Vasil'ev
Chernyi Otrog, Orenburg oblast, (56°02′, 51°53′) 1986	16	A.G. Vasil'ev and O.A. Luk'yanov
Kashkuk (the vicinity of Kuvandyk), Orenburg oblast, (57°17′, 51°28′) 1986	30	V.N. Bol'shakov, A.G. Vasil'ev, I.A. Vasil'eva, and O.A. Luk'yanov
Kuvandyk (SUCP), Orenburg oblast, (57°25′, 51°25′) 2001	17	A.G. Vasil'ev, I.A. Vasil'eva, N.M. Lyubashevskii, M.V. Chibiryak
Kuvandyk (SUCP), Orenburg oblast, (57°25′, 51°25′) 2007	20	A.G. Vasil'ev, I.A. Vasil'eva, Yu.V. Gorodilova, M.V. Chibiryak
Ira, Bashkortostan, (55°56′, 52°52′) 1986	31	A.G. Vasil'ev and O.A. Luk'yanov
Bol'shoi Kuganak, Bashkortostan, (56°09′, 53°48′) 1986	31	A.G. Vasil'ev and O.A. Luk'yanov
Ufa, Bashkortostan, (55°97′, 54°40′) 1986	12	A.G. Vasil'ev and O.A. Luk'yanov
EURT head part, Chelyabinsk oblast, (60°50′, 55°45′) 2005	13	Yu.V. Gorodilova and M.V. Chibiryak
Metlino, Chelyabinsk oblast, (61°01′, 55°49′) 2005	29	Yu.V. Gorodilova and M.V. Chibiryak
Shigaevo, Shalinskii raion, Sverdlovsk oblast, (58°43′, 57°16′) 2006–2008, 2010	27	N.E. Kolcheva

technogenic environmental pollution with radionuclides, fluorides, and toxic petrochemical products.

MATERIAL AND METHODS

Morphological variation in *S. uralensis* young of the year (males and females) was studied using the craniological collection maintained in the Laboratories of Evolutionary Ecology and Population Ecology of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences. Age calibration of samples was performed as described (2009), with some modifications. Juvenile individuals were excluded from the study. The mandible was chosen for analyzing morphological variation because its shape is conditioned by function (food capture and ingestion) and characterizes ecological features of the species; on the other hand, the mandible is a flat object, which is important for correct digitization of images for geometric morphometrics (Zelditch et al., 2004). Manifestations of technogenic variation were studied in samples from *S. uralensis* population groups living for a long time in areas with high levels of pollution with different technogenic substances (below, referred to as impact areas): the head part of the Eastern Ural Radioactive Trace (EURT) heavily contaminated with radionuclides; the zone of impact from the South Ural Cryolite Plant (SUCP) in the vicinities of Kuvandyk, where pollution resulted from accidental emissions of fluorides; and the vicinities of the village of Bol'shoi Kuganak near Sterlitamak (Bashkortostan), where toxic petroleum products were released to the floodplains of the Belaya and Kuganak rivers. All samples consisted of animals representing the eastern European chromosomal form of the European race of the species (Karamysheva et al., 2010).

The material for analysis consisted of 242 right mandibles of *S. uralensis* mice from 11 samples (table), including three allochronous samples from the same Kuvandyk population (Orenburg oblast) taken in different years either in a conditionally clean area (the Sakmara floodplain near the village of Kashkuk, Kuvandukskii raion, 1986) or in the zone of pollution by accidental fluoride emissions from the SUCP (near Kuvandyk, 2001 and 2007). In 2001, the fluorine content in the femur averaged 174.3 ± 16.2 mg/kg in animals from the control area and 1074.3 ± 116.1 mg/kg in animals from the impact zone (the difference is statistically significant); in 2007, the latter value slightly decreased but remained high: 946.3 ± 132.5 mg/kg.

The EURT was formed after the accident at the Mayak Radiochemical Plant on September 29, 1957,

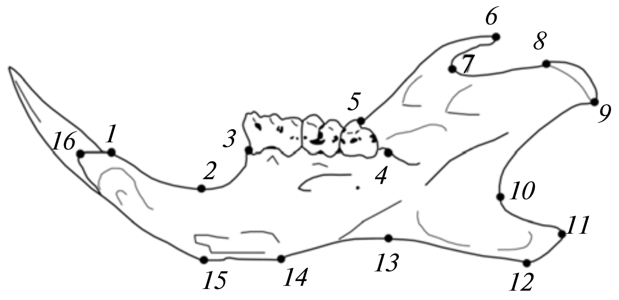

Fig. 1. Locations of 16 landmarks on the lingual side of the mandible in the pygmy wood mouse.

when more than 740 PBq of radioactive material was released into the atmosphere, with 10% of this amount being dispersed over the territory during the passage of the radioactive cloud (*Vostochno-Ural'skii ...,* 1996). Today, ^{90}Sr is still the main contaminant in the EURT zone. The density of soil radioactive contamination with this radionuclide in the EURT head part reaches 23.9–39.8 MBq/m^2, compared to 43.7 kBq/m^2 in the control plot near the village of Metlino (Tarasov, 2000; Pozolotina et al., 2008).

No special studies were performed on the intake of toxic petroleum products by animals from the forest near Bol'shoi Kuganak, in the Belaya and Kuganak floodplains, and the data available to the authors were limited to the results of expert examination of captured animals. Thus, a direct effect of technogenic pollutants on mouse morphogenesis was expectable under conditions of chronic exposure in the EURT and SUCP zones, but such an effect in the area polluted with petroleum products could only be hypothesized.

Images of the right mandible from the lingual side were made with a scanning pad at a standard resolution of 1200 dpi and digitized using the TpsDig2 program (Rohlf, 2010b). Variation in the mandible shape was evaluated by analyzing the pattern of 16 landmarks (Fig. 1). Two images of each mandible were obtained, and each was digitized twice; thus, four landmark patterns were examined in each case. Analysis for possible displacements in this pattern upon repeated imaging and digitization showed that these displacements were small (no more than 2% of the total variance), usually lacked statistical significance, and could be ignored. Nevertheless, average data for all four landmark patterns in each mandible were used in the study.

Procrustes superimposition of landmark patterns was performed with the MorphoJ program (Klingenberg, 2011). Procrustes distances were calculated as the square root of the sum of the squared differences between homologous landmarks (Rohlf and Slice, 1990). Canonical and discriminant analyses of the mandible shape were performed on the basis of Procrustes coordinates. Centroid size (CS), calculated as the square root of the sum of the squared differences from the image center to each landmark, was taken to be proportional to the size of digitized objects. (Rohlf and Slice, 1990).

Statistical data processing was performed using applications program packages TPS (Rohlf, 2010a–2010c), MorphoJ (Klingenberg, 2011), and PAST (Hammer, Harper, and Ryan, 2001).

RESULTS AND DISCUSSION

Preliminary analysis revealed no sex-related differences in the mandible shape between young of the year from different Ural populations of *S. uralensis*: in absolute values, the differences were small and usually lacked statistical significance, which allowed us to use pooled data on males and females in each sample.

Canonical analysis of Procrustes coordinates characterizing variation in the mandible size in 11 control and impact population groups of mice from the Urals and Cisural region provided a basis for estimating the relationship between manifestations of geographic and technogenic forms of variation. It should be noted that comparisons were made using samples from nine population groups, because, as noted above, three allochronous samples (taken in 1986, 2001, and 2007) represented the same Kuvandyk population (Orenburg oblast). On the whole, statistically significant intergroup differences manifested themselves along nine canonical axes, which accounted for 98.97% of total variance. Let us consider these manifestations for the first two canonical variables, which explained the greater part of the variance (64.6%).

Supposedly, latitudinal geographic variation should have manifested itself along the first canonical axis, since comparisons were made between the series of samples taken in the direction from the southernmost localities in the Southern Urals (Orenburg oblast) to the northern boundary of *S. uralensis* range in the Middle Urals (Sverdlovsk oblast). However, distinct latitudinal variation was observed only along the second canonical axis (17.1% of the total intergroup variance); the distribution of the samples along the first axis (47.5% of the variance) was indicative of unidirectional and apparently unspecific changes in the mandible shape associated with chronic exposure to technogenic pollutants of different origin (Fig. 2). Geographic variation along the second canonical variable axis (CV2) was manifested in a sequential distribution of the centroids of Orenburg, Bashkir, Chelyabinsk, and Sverdlovsk populations in the direction from negative to positive values.

In the total morphological space (Fig. 2), the control and impact samples form two distinct subspaces (delimited by different lines), with their centroids concentrating on the left and on the right, respectively. The highest deviation from this pattern is apparently explained by the remote morphological consequences of chronic irradiation in the EURT zone, which has affected more than 100 generations of mice since the radiation accident at the Mayak plant. This concerns one of the control samples (from Metlino, Chelyabinsk oblast), which is in the right part of the plot, near the impact sample from the southern part of the EURT. The Metlino sample can only conditionally be regarded as control, and only with respect to the sample from the EURT, because the corresponding population group lives at a distance of only 10–12 km from the impact group in the area that adjoins the narrow EURT zone. It is known that rodent communities of these areas have been exchanging individuals during more than 100 generations born after the radiation accident (Il'enko and Krapivko, 1989; Grigorkina and Olenev, 2006), and differences in mandible morphogenesis between them could have leveled off as a result of intercrossing. Thus, relative to other control groups, the Metlino sample can be regarded as a conditional impact group.

A similar displacement to the right is also observed for the centroids of two *S. uralensis* samples from the vicinities of Kuvandyk, which were taken near the SUCP in 2001 and 2007. The control sample from the same region was taken in the Sakmara floodplain in 1986 (its centroid in the plot is within the subspace of other control samples). As noted above, the fluorine contents in the bones of mice caught near the SUCP were almost an order of magnitude higher than in animals from the Sakmara floodplain. The impact sample from Bol'shoi Kuganak (located in the subspace of other impact samples) was taken in 1986 in the forest growing in the Belaya River floodplain, which was exposed to long-term pollution with toxic petroleum products. Thus, the rightward displacement of the centroids of all impact samples along the first canonical axis appears to reflect unspecific unidirectional changes in the shape of *S. uralensis* mandible shape in response to different kinds of chronic technogenic impact. It should be noted that all generalized Mahalanobis and Procrustes distances between the pairs of samples proved to be statistically significant ($p < 0.001$).

It was interesting to find out whether the common (unspecific) features of mandible shape manifest themselves in animals from all impact samples, irrespective of the type of technogenic impact. To this end, we performed discriminant analysis of the mandible shape in the pooled series of control and impact samples and revealed consistent differences between them ($D^2 = 7.72$; $T^2 = 464.84$; $p < 0.0001$) (Fig. 3). This result was confirmed by a cross validation test with 10 000 iterations ($p < 0.0001$). The correctness of discrimination between the control and impact animals was 90.3% after discriminant analysis and 87% after the cross-validation test.

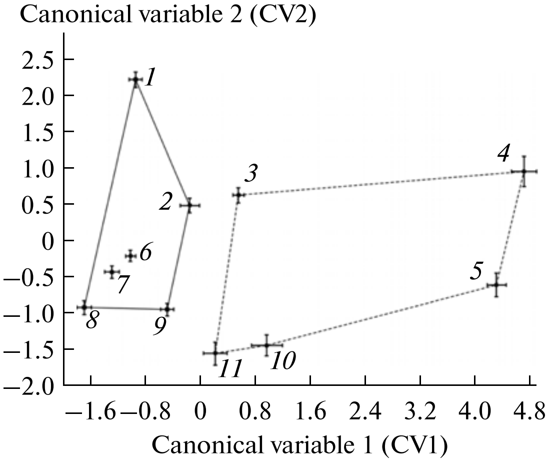

Fig. 2. Results of canonical analysis for the shape of the mandible in 11 control and impact samples of pygmy wood mice from population groups of the Urals and Cisural region: (*1*) Shigaevo, (*2*) Ufa, (*3*) Bol'shoi Kuganak, (*4*) EURT, (*5*) Metlino, (*6*) Ira, (*7*) Eginsai, (*8*) Chernyi Otrog, (*9*) Kashkuk (1986), (*10*) Kuvandyk (2001), (*11*) Kuvanduk (2007). Centroids and standard errors along the corresponding axes of canonical variables are shown; solid and broken lines connect control and impact samples, respectively.

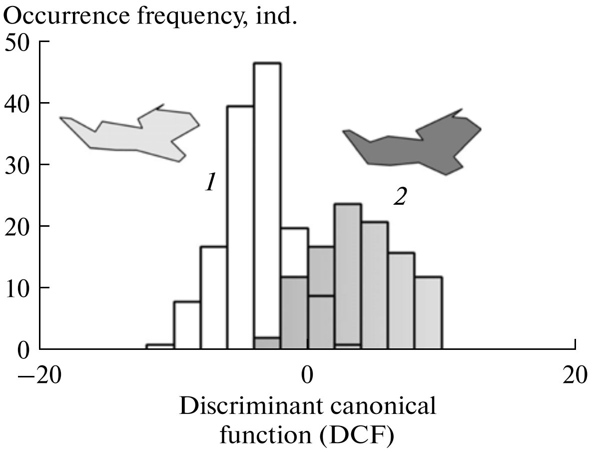

Fig. 3. Results of generalized discriminant analysis for the shape of the mandible in (*1*) control and (*2*) impact samples of pygmy wood mice from the Southern and Middle Urals.

As follows from Fig. 3, the mandibles of mice from the impact samples markedly differed from those of control mice in having the coronoid–symphyseal region more elongated dorsally, with the angular process being relatively short. It appears that exactly these phenotypic features reflect the unspecific morphogenetic response to different types of chronic technogenic pollution. Therefore, the general morphogenetic response to living in a technogenically polluted environment manifests itself in the disturbance of normal allometric relationships during the growth of different morphogenetic modules of the mandible in young of the year: the growth of the angular process module is inhibited, while the growth of the coronoid module in the dorsal direction is enhanced.

In addition, the same two pooled series of samples were also used to analyze variation in mandible size as estimated by its centroid size (CS). The CS in animals

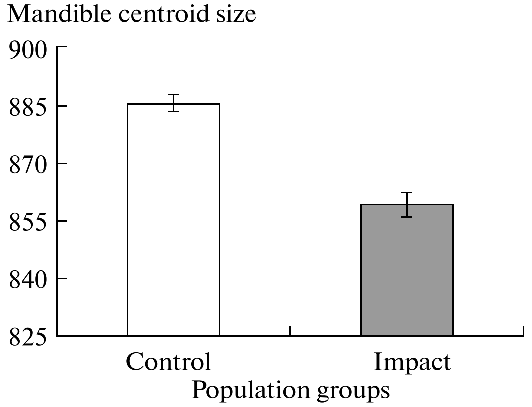

Fig. 4. Comparison of the mandible centroid sizes (CS ± SE_{CS}) between pooled control and impact samples of pygmy wood mice from the Southern Urals and Cisural region.

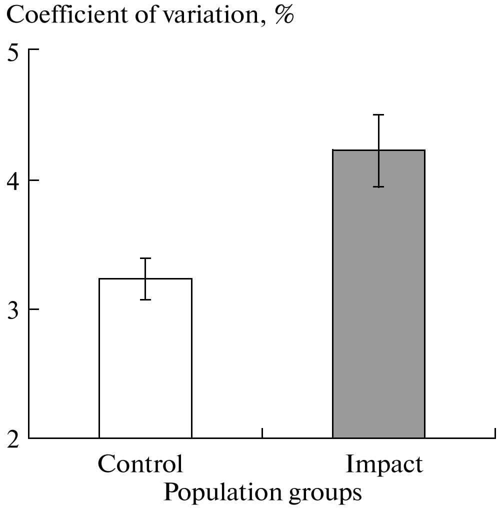

Fig. 5. Coefficients of variation ($CV \pm SE_{CV}$) in the mandible centroid size in pooled control and impact samples of pygmy wood mice.

from the impact population groups proved to be significantly smaller (Fig. 4), while its variation coefficient was significantly higher than in the control ($p < 0.001$) (Fig. 5). These facts may be regarded as evidence for inhibition of mandible growth and relative instability of its morphogenesis in animals from technogenically polluted areas.

The data presented above show that *S. uralensis* is characterized by a high morphogenetic responsiveness to the chronic impact of different technogenic pollutants and is more sensitive to them than to changes in natural environmental factors along the latitudinal gradient. In the Urals, geographic variation in this species is manifested only upon transition between different natural zones.

Thus, technogenic variation in the *S. uralensis* mandible shape caused by the remote consequences of environmental pollution with radionuclides, fluorides, and toxic waste from the petrochemical industry is comparable to or exceeds the level of natural geographic (latitudinal) variation observed in this species in the Urals and Cisural region. In other words, the extent of morphogenetic rearrangements under chronic exposure to different technogenic pollutants, including the 50-year-long effect of radioactive contamination in the EURT, can reach the level required for natural intraspecific geographic differentiation, which is a long-term evolutionary–ecological process. Nevertheless the observed morphological rearrangements cannot be regarded as microevolutionary phenomena, because the impact population have not differentiated to the subspecies level (Gorodilova, 2011). It appears, however, that technogenic variation can potentially play the main role in rapid microevolutionary modifications of morphogenesis related to the disturbance or alteration of allometric relationships in the ontogeny of individuals from impact groups.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 11-04-00720), Scientific School Support Program (project no. NSh-5325.2012.4), and Basic Research Program of the Presidium of the Ural Branch of the Russian Academy of Sciences (projects nos. 12-S-4-1031 and 11-4-NP-443).

REFERENCES

Bezel', V.S., *Ekologicheskaya toksikologiya: populyatsionnyi i biotsenoticheskii aspekty* (Ecological Toxicology: Population and Biocenotic Aspects), Yekaterinburg: Goshchitskii, 2006.

Chernov, Yu.I., *Ekologiya i biogeografiya: izbrannye raboty* (Ecology and Biogeography: Selected Works), Moscow: KMK, 2008.

Debat, V. and David, P., Mapping Phenotypes: Canalization, Plasticity, and Developmental Stability, *Trends Ecol. Evol.,* 2001, vol. 16, pp. 555–561.

Gilbert, S.F., Evo-Devo, Devo-Evo, and Devgen-Popgen, *Biol. Philos.*, 2003, vol. 18, pp. 347–352.

Gorodilova, Yu.V., Morphological Variation in Chromosomal Races of the Pygmy Wood Mouse: Geometric Morphology of the Mandible, *Ekologiya: skvoz' vremya i rasstoyanie: Mat-ly konf. molodykh uchenykh* (Ecology: Through Time and Space. Proc. Young Sci. Conf.), Yekaterinburg: Goshchitskii, 2011, pp. 34–42.

Grigorkina, E.B. and Olenev, G.V., Reproductive Strategy of Murine Rodents in a Radioactively Contaminated Biocenosis, *Izv. Chelyabinsk. Nauch. Tsentra,* 2006, no. 4 (34), pp. 101–105.

Hammer, O., Harper, D.A.T. and Ryan, P.D., PAST: Paleontological Statistics Software Package for Education and Data Analysis, *Palaeontol. Electronica*, 2001, vol. 4, no. 1.

Il'enko, A.I. and Krapivko, T.P., *Ekologiya zhivotnykh v radiatsionnom biogeotsenoze* (Ecology of Animals in a Radioactive Biogeocenosis), Moscow: Nauka, 1989.

Karamysheva, T.V., Bogdanov, A.S., Kartavtseva, I.V., Likhoshvay, T.V., Bochkarev, M.N., Kolcheva, N.E., Marochkina, V.V., and Rubtsov, N.B., Comparative FISH Analysis of C-Positive Blocks of Centromeric Chromosomal Regions of Pygmy Wood Mice *Sylvaemus uralensis* (Rodentia, Muridae), *Russ. J. Genet.*, 2010, vol. 46, no. 6, pp. 712-724.

Klingenberg, C.P., MorphoJ: An Integrated Software Package for Geometric Morphometrics, *Mol. Ecol. Resources*, 2011, vol. 11, pp. 353–357.

Klingenberg, C.P. and Gidaszewski, N.A., Testing and Quantifying Phylogenetic Signals and Homoplasy in Morphometric Data, *Syst. Biol.*, 2010, vol. 59, no. 3, pp. 245–261.

Kolcheva, N.E., The Degree of Tooth Wear As a Criterion of Animal Age for Analyzing the Age Population Structure of the Pygmy Wood Mouse, *Vestn. Orenburg. Gos. Univ.*, 2009, special issue, part 1, pp. 77–80.

Pozolotina, V.N., Molchanova, I.V., Karavaeva, E.N., Mikhailovskaya, L.N., and Antonova, E.V., *Sovremennoe sostoyanie nazemnykh ekosistem zony Vostochno-Ural'skogo radioaktivnogo sleda: urovni zagryazneniya, biologicheskie effekty* (Current State of Terrestrial Ecosystems in the Zone of the Eastern Ural Radioactive Trace: Contamination Levels and Biological Effects), Yekaterinburg: Goshchitskii, 2008.

Rohlf, F.J., *TpsRelw, Relative Warps Analysis, Version 1.49*, Stony Brook, NY: Department of Ecology and Evolution, State University of New York, 2010a.

Rohlf, F.J., *TpsDig, Digitize Landmarks and Outlines, Version 2.16*, Stony Brook, NY: Department of Ecology and Evolution, State University of New York, 2010b.

Rohlf, F.J., *TpsUtil, File Utility Program, Version 1.47.9*, Stony Brook, NY: Department of Ecology and Evolution, State University of New York, 2010c.

Rohlf, F.J. and Slice, D., Extension of the Procrustes Method for the Optimal Superimposition of Landmarks, *Syst. Zool.*, 1990, vol. 39, no. 1, pp. 40–59.

Schlichting, C.D., Origins of Differentiation via Phenotypic Plasticity, *Evol. Dev.*, 2003, vol. 5, no. 1, pp. 98–105.

Shvarts, S.S., *Ekologicheskie zakonomernosti evolyutsii* (Ecological Patterns in Evolution), Moscow: Nauka, 1980.

Tarasov, O.V., Radioecology of Terrestrial Vertebrates in the Head Part of the Eastern Ural Radioactive Trace, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Ozersk, 2000.

Vasil'ev, A.G. and Bol'shakov, V.N., A View on Evolutionary Ecology Yesterday and Today, *Ekologiya*, 1994, no. 3, pp. 4–15.

Vasil'ev, A.G. and Vasil'eva, I.A., Epigenetic Rearrangements in Populations As a Probable Mechanism of Biocenotic Crisis, *Vestn. Nizhegorod. Gos. Univ. im. N.M. Lobachevskogo, Ser. biol.*, 2005, no. 1 (9), pp. 27–38.

Vostochno-Ural'skii radioaktivnyi sled (Sverdlovskaya oblast') (The Eastern Ural Radioactive Trace, Sverdlovsk Oblast) Chukanov, V.N., Ed., Yekaterinburg: Ural. Otd. Ross. Akad. Nauk, 1996.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., et al., *Geometric Morphometrics for Biologists: A Primer,* Elsevier, 2004.

Zherikhin, V.V., *Izbrannye trudy po paleoekologii i filotsenogenetike* (Selected Works in Paleoecology and Phylocenogenetics), Moscow: KMK, 2003.