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Analysis of Coupled Geographic Variation of Three Shrew Species from Southern and Northern Ural Taxocenes

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Abstract—Coupled and unidirectional geographical variability of the shape of the mandible in three shrew species of the genus *Sorex* (*araneus, caecutiens*, and *minutus*) has been revealed in a geometric morphometrics-based parallel comparison of cenopopulations forming the Ilmen' (Southern Urals) and Kytlym (Northern Urals) taxocenes. Mandible centroid size (CS) was significantly larger and significantly more varied in all northern cenopopulations as compared to the southern ones. Discriminant analysis of Procrustes coordinates of the mandible shape in shrews of the southern and northern taxocenes without taking the animal species into account resulted in 94.8% correct assignment of the individuals to local taxocenes. Mandible allometry was detected in the northern cenopopulations of Laxmann's (*caecutiens*) and pygmy (*minutus*) shrews, but not in the southern cenopopulations. Parallelism of geographical variation and high reliability of discrimination between individuals from the southern and northern taxocenes was preserved when the allometric effect was eliminated. The absence of association of this reaction to allometry provides direct proof for similar morphogenetic reactions of these species to climate change in the northern latitudes, and high potential for coevolutionary changes common to all sympatric species of the taxocene.

Keywords: geographic variability, shrews, taxocene, cenopopulation, Urals, geometric morphometrics

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The model group of three shrew species of the genus *Sorex* inhabiting different natural areas in the Southern and Northern Urals was selected as the research object to study the patterns of coupled variability in a two-level supraorganism system (Bukvareva and Aleshchenko, 2013) represented by the taxocene (Hutchinson, 1967; Chernov, 2008; Vasil'ev et al., 2010) and its component cenopopulations of taxonomically close sympatric species in the hierarchy. Importantly, the present study was performed at the population-cenotic level, thus allowing for an expansion of the potential of evolutionary ecology due to the transition from the population level to the cenotic level in analyzing manifestations of morphological variability (Violle et al., 2012; Vasil'ev et al., 2013). We previously demonstrated the possibility of assessing the coevolutionary potential of sympatric species occurring in different habitats by means of analyzing coupled morphological variability; parallelism of species variability observed in a broader range of ecological conditions is evidence of a higher coevolutionary potential (Vasil'ev et al., 2013).

Local communities of small mammals confined to specific habitats within a facies or a tract represent *taxocenes*, i.e., taxonomically close species components of cenoses performing similar (mainly trophic) ecological functions in the latter (taxocenes are phylogenetically closely related ecological guilds). Syntopic settlements of each of the sympatric species in a taxocene inhabiting a local biotope are termed *cenopopulations*; they are necessarily involved in ecological interactions with each other due to the common habitat and use of similar food sources.

Small spatially isolated groupings of certain species of animals, which are potentially or actually connected to each other by the flow of migrants and therefore form a single population (=metapopulation) are usually termed micropopulations in zoology (Schwartz, 1969). However, micropopulations characterize only a part (a territorial fragment) of the population of a certain species and are not formally related to a cenosis. The term cenopopulation was proposed by botanists (Rabotnov, 1969; Uranov, 1975; Lyubarskii, 1975) to define an (often temporary) territorial settlement of a particular species confined to a specific biocenosis and, consequently, to a specific biotope. Since this work focused on comparing animals from syntopic groups of sympatric taxonomically related shrew species captured simultaneously in the same biotopes, we deliberately use the term cenopopulation for these local biotopic groups of each of the sympatric species. Comparison of representatives of the same species occupying spatially adjacent areas could also have been performed in terms of micropopulations. Therefore, cenopopula-

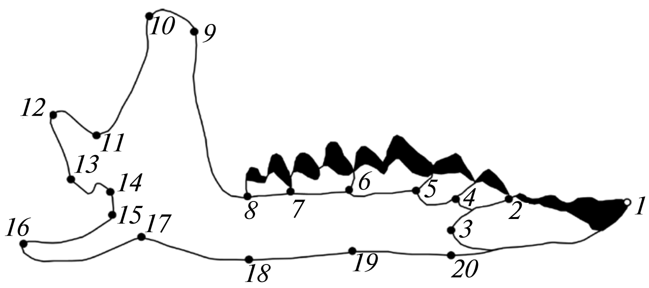

Fig. 1. Location of landmarks (1–20) on buccal side of mandible of common shrew.

tions compared within a local taxocene (with its representatives inhabiting a specific biotope) can be simultaneously considered as micropopulations, provided that the comparison is performed within a single species. Thus, the use of the term cenopopulation is justified for relatively sedentary animals that are closely related to certain local biotopes and should not be limited to plants.

Let us emphasize another problem related to the necessity of using the term cenopopulation when investigating coupled variability of sympatric species forming a local taxocene. The use of this term is especially relevant in the case of simultaneous analysis of samples collected in syntopic settlements of shrews ore rodents belonging to several different sympatric species characterized by biotopic and territorial similarity. Let us again emphasize that the use of the term micropopulation in this case makes no sense at all, since the micropopolations of all species are characterized by a complete spatial overlap.

The purpose of our comparative study was to test the hypothesis of the coupled character of morphological variability in cenopopulations of sympatric species in local taxocenes and to search for manifestations of a generalized "cenotic" type of variation in two geographically isolated taxocenes with shrews of the Southern and Northern Urals as the research object. Assessment of morphogenetic reactions of shrews from the northern and southern taxocenes to the different climatic and natural conditions associated with the habitats in different parts of the Urals was of particular interest as well, given the predicted long-term unfavorable changes of the climate (Salamin et al., 2010).

MATERIAL AND METHODS

Geographic variation of the shape of the mandible has been analyzed for three shrew species considered as a model taxocene (Nikolaev, 1977; Nesterenko, 1999): the common shrew (*Sorex araneus* L., 1758), Laxmann's shrew (*S. caecutiens* Laxmann, 1785), and the pygmy shrew (*S. minutus* L., 1766). Samples from the collection of the Zoological Museum of the Institute of Plant and Animal Ecology (Ural Branch of the Russian Academy of Sciences) collected synchronously during the summer months in syntopic shrew cenopopulations of Il'men State Reserve in the southern Urals (1981) and in the environs of the settlement of Kytlym (Sverdlovsk oblast, Northern Urals, collected in 1971) were used. Earlier studies of *S. araneus* by traditional morphometrics showed that geographical morphological variability is substantially higher than the interannual chronographic variability (Bolshakov et al., 1996) and, therefore, comparison of series from craniological collections of different years performed in this work appears acceptable. Notably, the northern and southern populations of common shrew (*Sorex araneus*) compared in this work belong to the same chromosomal race (the Serov race).

The branch of shrew mandible chosen as the object of investigation is relatively flat, and therefore, the variability of its shape can be efficiently analyzed using geometric morphometrics. All 173 specimens used—55 and 51 *Sorex araneus* specimens, 29 and 20 *S. caecutiens* specimens, and 10 and 8 *S. minutus* specimens (for all species, the first number is for the animals collected in the Southern Urals, and the second number is for the animals collected in the Northern Urals)—were obtained from current year's animals of the same age.

Correct comparison of shape variation for morphological structures found in different species is only possible if geometric morphometrics methods are used (Rohlf and Slice, 1990, Pavlinov, 2000; Zelditch et al., 2004; Adams et al., 2013), since these methods allow for independent analysis of variation in object size and shape. Geometric morphometrics methods are potentially the most adequate for the detection of coupled variability of related species within a local taxocene; in addition, they allow visualization of changes in the shape of the morphological objects under investigation. Many researchers believe that geometric morphometry methods allow morphogenetic interpretation of the observed variability (Zelditch et al., 2004; Klingenberg, 2013; Sheets and Zelditch, 2013).

Buccal views of right mandibular branches from shrews of all three species were digitized, and 20 homologous landmarks (Fig. 1) were added to the photographs using the tpsUtil software and a tpsDig2 screen digitizer (Rohlf, 2013a, b). An indirect characteristic of the mandible size was inferred from centroid size (CS) of the mandible, calculated as the square root of the sum of squared distances from each landmark to the center of the image (Rohlf and Slice, 1990). The superimposition procedure was performed using generalized orthogonal least-squares Procrustes analysis (GPA) (Rohlf and Slice, 1990), which involves iterative alignment and fitting of landmark configurations from the object under investigation onto the reference configuration, with ultimate construction of a consensus configuration. The resultant Procrustes coordinates characterizing the variability of object shapes were used for further comparison between groups

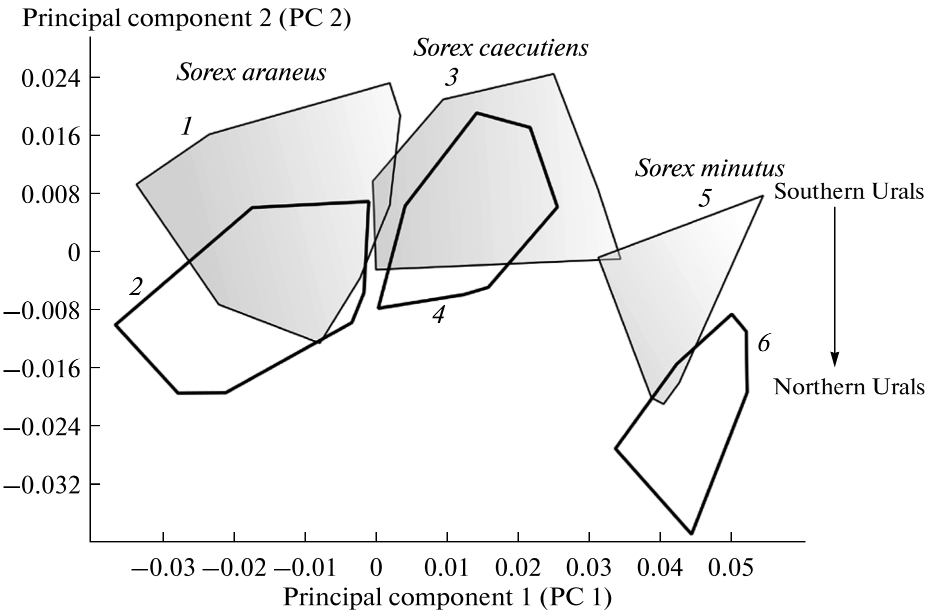

Fig. 2. Parallelism of geographical variation of mandible shape in syntopic populations of three closely related sympatric species of shrews of the genus *Sorex* (*araneus*, *caecutiens*, and *minutus*) in communities (taxocenes) of Southern (*1*, *3*, *5*) and Northern (*2*, *4*, *6*) Urals as revealed by principal component analysis (PC1-PC2).

based on principal component analysis and discriminant analysis. Statistical analysis employed the TPS (Rohlf, 2013a, b), MorphoJ 1.6d (Klingenberg, 2011) and 2.17s PAST (Hammer et al., 2001) software.

Landmark realignment was repeated twice in order to assess the bias related to operator error and the effect of animal sex. The differences were statistically insignificant in both cases and were therefore neglected; data for male and female animals were pooled within the local samples.

RESULTS AND DISCUSSION

Multidimensional ordination of Procrustes coordinates based on principal component analysis made it possible to assess the direction of changes in mandible shape of shrews of the three investigated species; the evaluation was conducted for the sets of samples collected in Northern and Southern Urals. Scatterplots of the cenopopulations from the Northern Urals apparently are shifted downwards relative to those for the respective cenopopulations from the Southern Urals for all studied species (Fig. 2). Therefore it is possible to conclude that the parallelism of geographical variation in the studied shrew species is manifested along the second principal component of shape (PC2), which accounted for 12.50% of the total variance. The variability along the first principal component (PC1) related to species-specific characteristics of mandible shape accounted for 42.60% of the total variance.

Geographic variation was less expressed in Laxmann's shrew, a species showing less sympatry with common and pygmy shrews in the Urals and in other parts of the geographical range as compared to the sympatry of the two latter species (Bolshakov et al., 1996). This feature may be due to species specificity of habitat requirements apparent from the structure of geographical ranges of the studied species. The geographical ranges of common and pygmy shrews largely coincide and are located in Europe; i.e., the species have similar requirements for the environment, while the geographical range of Laxmann's shrew only overlaps with those of the two former species in the northern regions, this species being allopatric in the eastern part of the geographical range (the Far East and most of Siberia). The evident and similarly directed geographical variability of common and pygmy shrews may be due to long-term formation of a set of similar morphogenetic responses in adaptation to a similar range of environmental conditions within almost the same geographical range.

Conventional elimination of the interspecies borders to construct generalized scatterplots for each taxocene caused them to assume a slightly different appearance. Results of conventional combination of scatterplots for the three species within each of the two taxocenes (the Il'men taxocene in the south and the Kytlym taxocene in the north) are shown in Fig. 3.

It is rather difficult to describe the morphological variability for the entire community, but investigation of a taxocene (a fragment of the biotic community used as a cenotic model) is completely feasible. Consolidation of the population scatterplots for all three species of shrews from a specific taxocene into a common morphological space revealed bias of the generalized scatterplot of the northern taxocene relative to

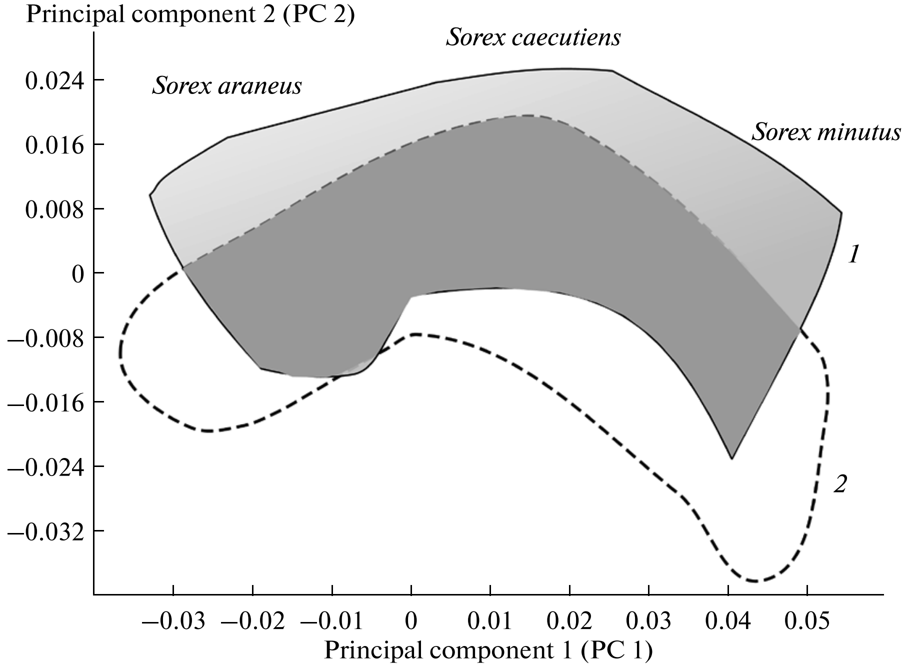

Fig. 3. Combined scatterplots for cenopopulations of three species of shrews of the genus *Sorex* for the southern (*1*) and northern (*2*) taxocenes along the first two principal components (PC1–PC2) in a common morphological space.

that of the southern. The transgression zones obtained by superposition of scatterplots for the two taxocenes along the first and second principal components were rather narrow. Notably, the morphological spaces of the two taxocenes hardly approached each other due to deviation along the third axis; therefore, the subspaces corresponding to both taxocenes almost never overlapped in the common morphological space along the three principal components. This agrees well with the results of further discriminant analysis of the southern and northern taxocenes. In this case the comparison was performed after removal of the interspecies borders and consolidation of samples of different species into single samples for each taxocene.

Discriminant analysis of individual values of Procrustes coordinates revealed significant differences in the shape of the mandible between animals from the southern and northern taxocenes. Images of mandible branches shown in Fig. 4 represent generalized consensus images derived from data on all three species—in other words, the average configurations of the mandible and lower dentition for a specific taxocene. A consensus image for the Kytlym taxocene is shown on the left, and that for Il'men taxocene is shown on the right. Superposition of mandible outlines (image on the upper right) reveals a difference in shapes between samples from the southern and northern taxocenes. Gracile mandibles with the coronoid process shifted anteriorly are characteristic of all shrew species of the southern (Il'men) taxocene (shape shown in a darker color).

Representatives of every species could be correctly attributed to a specific (northern or southern) taxocene in the overwhelming majority (94.8%) of all cases; i.e., almost every individual could be reliably assigned to a local taxocene. Interestingly, such high reliability of discrimination of individuals cannot always be attained during comparison of animals from related species, whereas we attempted to discriminate between representatives of the southern and northern taxocenes regardless of their species using information on shape of the mandible. This is evidence of advanced evolutionary and ecological integration of species forming a specific shrew taxocene, revealing the manifestation of similar morphogenetic reactions of the species to a wide range of environmental conditions (coevolutionary potential) (Vasil'ev et al., 2013).

The centroid size (CS) of the mandible, providing an indirect characteristic of its size, is significantly higher in animals of the northern populations than in those in the southern populations of all three species (Fig. 5a). The variance is significantly higher for the parameters of the northern populations as well, except for those of the pygmy shrew, which only exhibited a trend towards an increase (Fig. 5b). Mandible size and its taxocenotic variability within the group were generally higher for shrews of the Kytlym taxocene exposed to harsh environmental conditions of the northern habitat than for the animals of the southern Il'men taxocene. Two-way ANOVA performed for the centroid size (CS) revealed statistically significant effects of the species (factor *S*), taxocene (factor *T*), and species-taxocene interaction ($S \times T$) (table). Notably, the

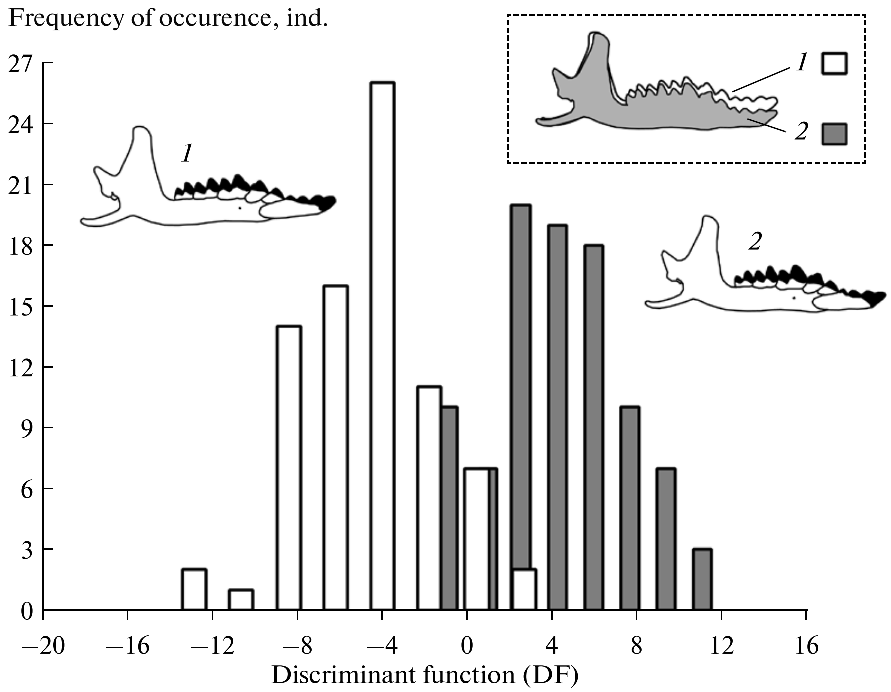

Fig. 4. Results of discriminant analysis of Procrustes coordinates characterizing variability of mandible and lower dentition shapes in shrews of northern Kytlym (*1*) and southern Il'men (*2*) taxocenes. Consensus images of mandibular branches for each taxocene were constructed using pooled samples of all three species (the superposition of generalized consensus contours of mandibles from representatives of southern and northern taxocenes is shown separately).

variance corresponding to the interaction of the parameters was one order of magnitude lower than the variance corresponding to the assignment of an individual to a specific taxocene.

Significant size differences were detected upon the comparison of northern and southern populations of the studied species; therefore, it was possible to expect an allometric effect determining the geographical variability of the mandible. Pearson correlation coefficients between the values of the first principal component (PC1) and centroid size (CS) were evaluated for each geographic population in order to test this assumption. The correlation of mandible size and shape was only significant for the northern cenopopulations of Laxmann's shrew *S. caecutiens* ($r = 0.51$; $z = 2.32$; $p = 0.021$) and pygmy shrew *S. minutus* ($r = 0.87$; $z = 2.98$; $p = 0.003$), this being a manifestation of allometry.

The parallelism of geographical variability directions in the representatives of the southern and northern taxocenes was conserved when the first principal component was excluded to eliminate the allometric effect and the data were compared along the second and third principal components. Attribution of representatives of all species to the southern or northern taxocene remained reliable (correct) in the case of discriminant analysis of the values of principal components other than PC1. The results (not shown) are directly indicative of similar morphogenetic responses of shrews to changes in environmental conditions in northern latitudes; these responses are not related to allometry and demonstrate a high potential for coevo-

Results of two-way ANOVA of mandible centroid size (CS) in shrews of three sympatric species of the genus *Sorex* (*araneus, caecutiens*, and *minutus*) in taxocenes of the the Southern and Northern Urals

Source of variability (factor)	Sum of squares	d.f.	Mean square	d.f.	Significance level (p)
Species (S)	1269000	2	634500	4484.00	≪0.0001
Taxocene (T)	57620	1	5620	407.20	≪0.0001
Interaction ($S \times T$)	6274	2	3137	22.17	≪0.0001
Within group	23630	167	141.5		
Total	1327000	172			

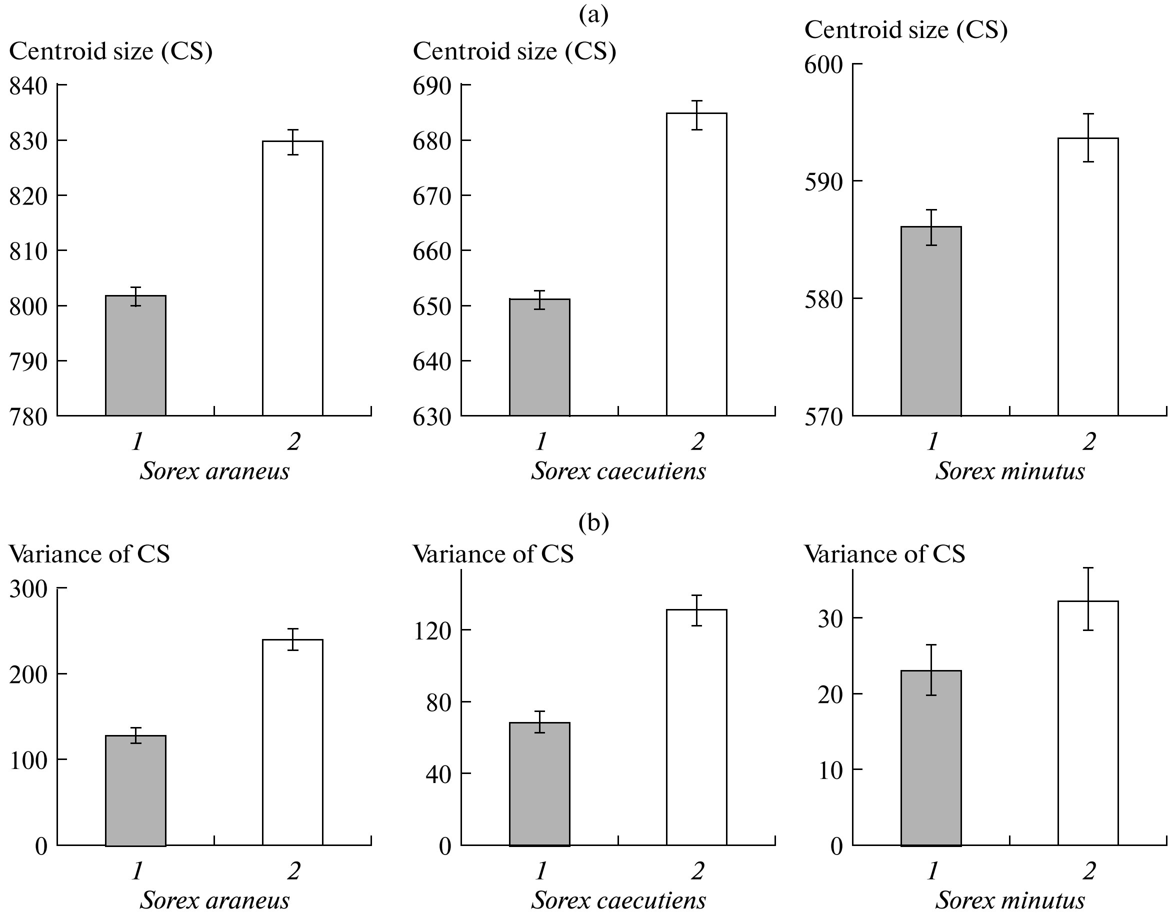

Fig. 5. Comparison of centroid size CS (a) and CS variance (b) of mandibular branches of shrews belonging to Southern Ural (*1*) and Northern Ural (*2*) cenopopulations of three sympatric *Sorex* species. Standard error (S.E.) of values is shown in graphs.

lutionary changes common to the sympatric species within a taxocene.

CONCLUSIONS

Finally, we should emphasize the presence of common morphogenetic features in syntopic cenopopulations of sympatric and taxonomically similar shrew species forming local taxocenes and the resulting possibility of accurate geographical assignment of populations of each species and even individual animals. This phenomenon is indicative of the evolutionary-ecological emergence of a taxocene as a historically formed integral supraorganism biosystem; similar growth trends and morphogenetic reactions related to developmental regulation (such as the general trend toward increased variability within groups in representatives of the northern taxocene) provide further indirect proof of this statement.

The mandible structure in all three species forming the local taxocene is supposedly related to the trophic specificity of shrews determined by features of the habitat. More massive jaws in the northern animals versus gracile jaws of the southern animals likely reflect the size and regional variation in populations of invertebrates consumed by the shrews of the Southern and Northern Urals. The existence of common morphogenetic characteristics making it possible to assign an animal to a specific taxocene regardless of species demonstrates the importance of cenosis for the formation of coevolutionary morphogenetic changes in communities, as well as for manifestation of targeted geographic variability of individual sympatric species forming a taxocene.

Since unfavorable long-term climate changes are predicted for the Urals and other regions (Salamin et al., 2010; Sutherland et al., 2013), the comparative analysis performed in this study and encompassing the geographic populations of three species of the genus *Sorex* inhabiting different natural zones can be considered a model that makes it possible to compare the morphogenetic reactions of shrews from northern and southern taxocenes to varying environmental conditions. Models of this type, employing spatial climate changes (e.g., in terms of comparing representatives of northern and southern biota) to simulate a temporary climate change (cooling), and vice versa, were termed processual reconstructions (Meien, 1984). Analysis of manifestations of coupled geographical variability of shrews based on these concepts makes it possible to a predict an increase in variability of animals concomitantly with size increase evoked by long-term cooling of the climate; morphogenetic transformations of a similar direction can be expected of all representatives of the taxocene in this case. Allometric effects can be expected to occur in shape variability upon an increase of lower jaw size. High coevolutionary potential, characteristic of Laxmann's and pygmy shrews at least, can be expected to maintain the stability and integrity of the shrew taxocene even in case of substantial climate change.

The aspect of morphological comparison of populations within cenoses addressed in this work represents a novel procedure for taxocene monitoring that makes it possible to interpret morphological differences detected by geometric morphometrics in terms of evolutionary ecology.

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