

PATTERNS OF CRANIOMETRIC VARIABILITY
OF SIX COMMON SPECIES OF SHREWS
(SORICIDAE: *CROCIDURA*, *NEOMYS*, *SOREX*)

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The morphometric variability of the skull and the lower jaw of 6 species of shrews (*Crocidura suaveolens* s. l., *C. leucodon*, *Neomys anomalus*, *N. fodiens*, *Sorex minutus*, and *S. araneus*) from Bulgaria, Romania, Hungary, and Austria was analyzed. The results from the PCA and the unsupervised model-based clustering showed that morphological geographic intraspecific variation was clearly expressed in *Crocidura suaveolens* and *C. leucodon*, poorly pronounced in *Neomys anomalus* and *N. fodiens*, and almost missing in *Sorex minutus* and *S. araneus*. Our data provide a morphological confirmation of the recent molecular data, which indicate a division of both *Crocidura* species to western and eastern lineages. The geographical location of their boundaries was discussed.

Keywords: morphometrics, geographic variation, *Crocidura*, *Neomys*, *Sorex*

INTRODUCTION

The distinction of species and subspecies is of interest to an array of biological sciences such as conservation biology (registration and management of endangered forms – species and subspecies, identification of priority conservation areas) and evolutionary biology (for describing, quantifying and understanding of the mechanisms of species formation) (AMORI *et al.* 2009). However, the division of the evolutionary units (WIENS 2007), such as subspecies and species, on the basis of the traditional morphological characters, is still far from trivial. According to the formal criteria for subspecies classification proposed by O'BRIEN and MAYR (1991) “members of a subspecies share ... a group of phylogenetically concordant phenotypic characters ...”. In practice, however, it is often difficult to distinguish such features from those that represent the influence of environmental factors within the morphological plasticity of the species. The problem is further complicated by the lack of modern taxonomic revisions. Thus, many names introduced more than 100 years ago, often on the basis of a small number of specimens and characters of uncertain taxonomic significance, remain potentially valid and “available” (AMORI *et al.* 2009). For example, in Europe and neighboring areas, a number of subspecies of soricids with weak or inconsistent morphological distinctiveness and thus with uncertain taxonomic status have been described (NIETHAMMER 1977, CORBET 1978, WOLSAN & HUTTERER 1998, DE JONG *et al.* 2014).

Relatively recently the study of mitochondrial and nuclear genomes has led to the discovery of cryptic lineages and a strong phylogeographic structure across species ranges (FILIPPUCCI & SIMSON 1996, OHDACHI *et al.* 2004, DUBEY *et al.* 2006, 2007a, BANNIKOVA *et al.* 2015). Molecular data of *Crocidura suaveolens* and *Crocidura leucodon* from Europe and Near East show the presence of clearly differentiated clades in both species: a Western European and an eastern clade. According to the inter-SINE PCR and cytochrome b study of BANNIKOVA *et al.* (2006), the former subspecies *C. suaveolens mimula* Miller, 1901 and *C. s. suaveolens* Pallas, 1811 could be treated as separate species: *C. mimula* being restricted to Western Europe and *C. suaveolens* – to Eastern Europe and parts of Asia. Similarly, by using cyt b, VOGEL *et al.* (2003) and OHDACHI *et al.* (2004) found a division between Western European and Asian *C. suaveolens*. According to cyt b analyses of CASTIGLIA *et al.* (2017), their *C. suaveolens* haplotype samples from the Apennine Peninsula belong to a clade distributed also in the western half of the Balkan Peninsula and Central Europe. On the basis of mitochondrial and nuclear gene sequences in *C. leucodon* two clades have been recognized (DUBEY *et al.* 2007b): a European clade (distributed from France eastwards to Turkey) and a Near East one (distributed from Georgia to Romania). The specimens from East Bulgaria (Bourgas) belong to the eastern clade. Two species of water shrews, *Neomys anomalus* Cabrera, 1907 and *N. milleri* Mottaz, 1907, were described on the basis of the overall shape of the skull. Later they were considered as subspecies of *N. anomalus* without any clear argumentation (e.g. ELLERMAN & MORRISON-SCOTT 1966). Nowadays, the genetic analyses reveal that they are independently evolving lineages and confirm their species status (CASTIGLIA *et al.* 2007). Recent DNA analyses reveal that Central European and Western Balkan populations of *Sorex minutus* Linnaeus, 1766, belong to different phylogroups with poorly expressed morphological differences (BERNAL 2010).

These studies, though important to reveal the subtle mechanisms of evolution, cannot be considered as a substitute for taxonomic revisions (WHEELER 2004). Only a classic taxonomic revision based on morphological descriptions and analyzes can provide physical diagnostic features and outline the geographical distribution of taxa (AMORI *et al.* 2009). Until now, there are insufficient data on the relationship between morphological differences that have been commonly used to distinguish subspecies or species in the past, and the new data based on molecular genetics. Much of the discussion in this respect deals with the opposition of classical morphological features versus the molecular ones. An advantage of the first type of characters is that they allow the species to be identified visually and is applicable to both live and museum specimens as well as to fossils. Among the disadvantages can be noted the subjectivity of defining and interpreting the state of the feature, the need of large samples, the continuous but not discrete character of many characters,

and the fact that phylogenetic divergence often occurs without morphological change that determines the existence of morphologically cryptic species. On the other hand the non-critical use of isolated molecular characters (e.g., a single mtDNA locus) can lead to an inflation of species diversity. Reasons could be such as genetic bias in small populations isolated only for a short period of time which may lead to reciprocal monophyletism with respect to a particular character and therefore can be diagnosed. Such situations, however, do not represent the type of diversity of interest for most ecologists and evolutionary biologists, and the question whether they should be recognized as species remains (PADIAL *et al.* 2010). This has led to the importance of evaluating and combining available evidence from a variety of disciplines to support necessary taxonomic revisions, which recently has been termed integrative taxonomy (PADIAL *et al.* 2010, YEATES *et al.* 2011). In this context and considering the above mentioned systematic obscurity with regard to some of the most common soricid species in Europe, we aimed to explore the patterns of morphometric variability of pairs of closely related species of three genera – *Crocidura*, *Neomys*, and *Sorex*, within the context of relevant published molecular data. An explicit goal of this study was to test whether the relatively low-cost method of morphometrics reveals patterns consistent with the available literature data obtained by molecular methods.

MATERIAL AND METHODS

Data were taken from skulls of six species of shrews from two geographic regions – Southeastern Europe, hereinafter referred to as SEE (material from Bulgaria) and Central Europe, hereafter referred to as CE (material from Romania, Hungary, and Austria) (Fig. 1). Only sub-adult specimens with unworn teeth were used in this study in order to preclude distortion of the results because of differences in the age structure within the samples. Additionally, in this way the DEHNEL'S (1949) effect was also eliminated in the samples of red-toothed shrews. In total 830 skulls were studied: *Crocidura suaveolens* s.l. (CE – 50, SEE – 46), *C. leucodon* (CE – 40, SEE – 35), *Neomys anomalus* (CE – 111, SEE – 233), *N. fodiens* (CE – 49, SEE – 130), *Sorex minutus* (CE – 13, SEE – 33), and *S. araneus* (CE – 32, SEE – 57). Only part of this material was sex-determined. The numbers of males and females were as follows: *C. suaveolens* s.l. (CE – 17 / 24, SEE – 9 / 7), *C. leucodon* (CE – 16 / 18, SEE – 4 / 5), *N. anomalus* (CE – 36 / 45, SEE – 46 / 44), *N. fodiens* (CE – 6 / 26, SEE – 37 / 32), *S. minutus* (CE – 5 / 8, SEE – 13 / 20), and *S. araneus* (CE: 16 / 16, SEE – 24 / 33). To determine if these ratios differ from the theoretical ratio of 0.5, the chi-square statistics were calculated. The obtained values ranged from 0 to 2.38 and were smaller than the critical value for 0.05 probability level (3.841), so the sex proportions in individual regional species samples were not significantly different from 0.5. The same can be assumed for the specimens, for which the sex was not determined. These data indicate that as a whole the regional species samples were not sex biased.

The examined material originated from the collections of the Institute of Biodiversity and Ecosystem Research (Bulgarian Academy of Sciences) in Sofia, Natural History Museums in Vienna and Budapest.

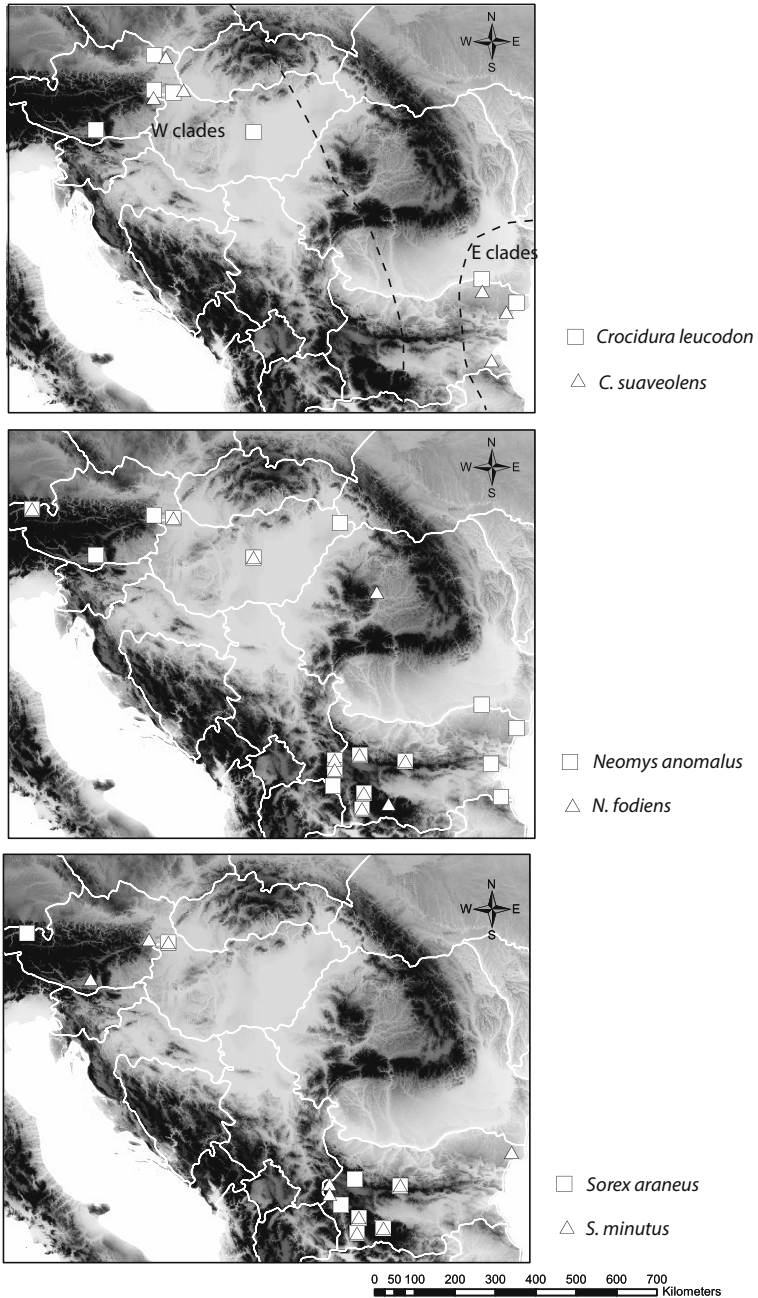


Fig. 1. Geographical origin of the materials examined. Dashed lines denote putative borders between eastern and western clades in the species of genus *Crocidura*, established on the basis of the molecular genetic data (BANNIKOVA *et al.* 2006, DUBEY *et al.* 2007b, CASTIGLIA *et al.* 2017)

For describing the skull morphology as comprehensive as possible 36 morphological measurements from the skull, lower jaw and teeth were obtained (Fig. 2). They were taken with a digital caliper to the nearest 0.01 mm or ocular micrometer to the nearest 0.001 mm by one of us (SZ).

Our analysis was based on an integrated biometrical and methodological approach using principal component analysis (PCA) and model-based clustering. Principal component analyses were conducted within each genus in order to condense the information into several uncorrelated composite variables – Principal Components (PCs) – that represent most of the variation. PCs with eigenvalues more than 1 were retained for further analyses. To improve interpretation of the principal components they were rotated to a simple structure using varimax normalized procedure. Prior to statistical analyses, we tested for normal distribution and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. The tests have shown that all variables do not deviate from the normal distribution and have comparable variances.

Typically, PC1 is considered to be an allometric axis, which is generally considered to represent the overall size; the remaining axes being perpendicular to PC1, i.e., are not correlated with it, reflect variability not related to size. On this basis, PC1 is often interpreted as „size“, while the next major components are viewed as reflecting the „shape“. It should be borne in mind, however, that since the first major component also includes a shape change component due to allometry, the variance of the other major components is not the full variation of the shape (KLINGENBERG 2016). For this reason, further in the text, the size and shape are placed in quotation marks.

In order to classify the specimens from every species into morphological groups, we used unsupervised model-based clustering. For this purpose, a Gaussian finite mixture model fitted by Expectation–Maximisation (EM) algorithm was employed. The optimal number of clusters for classification was determined by likelihood. Having in mind that the likelihood increases with the number of clusters, the final decision should be taken by examining a measure penalized by the number of parameters sought. So, the choice of the number of clusters was made using the Bayesian Information Criterion (BIC). The software packages used for the analyses were STATISTICA 7.0 for Windows (STATSOFT 2004) and R package mclust (FRALEY & RAFTERY 2012).

RESULTS

Genus Crocidura

Three principal components (PC1-3) had eigenvalues larger than 1 and accounted for approximately 30% of the variability of the initial 36 measurements. The first principal component reflected species differences as well as geographical differences within species (Fig. 3a), comprising 18.7% of the variability within the dataset. Almost all variables showed high loadings on the PC1, representing skull and mandibular lengths and skull widths, as well as some dental measurements. The measurements with the highest loadings were the postglenoid width (PGW) and the height of coronoid process (HPC) (Table 1). The second principal component accounted for 10.7% of the variability and reflected some peculiarities of the dentition. Morphometric charac-

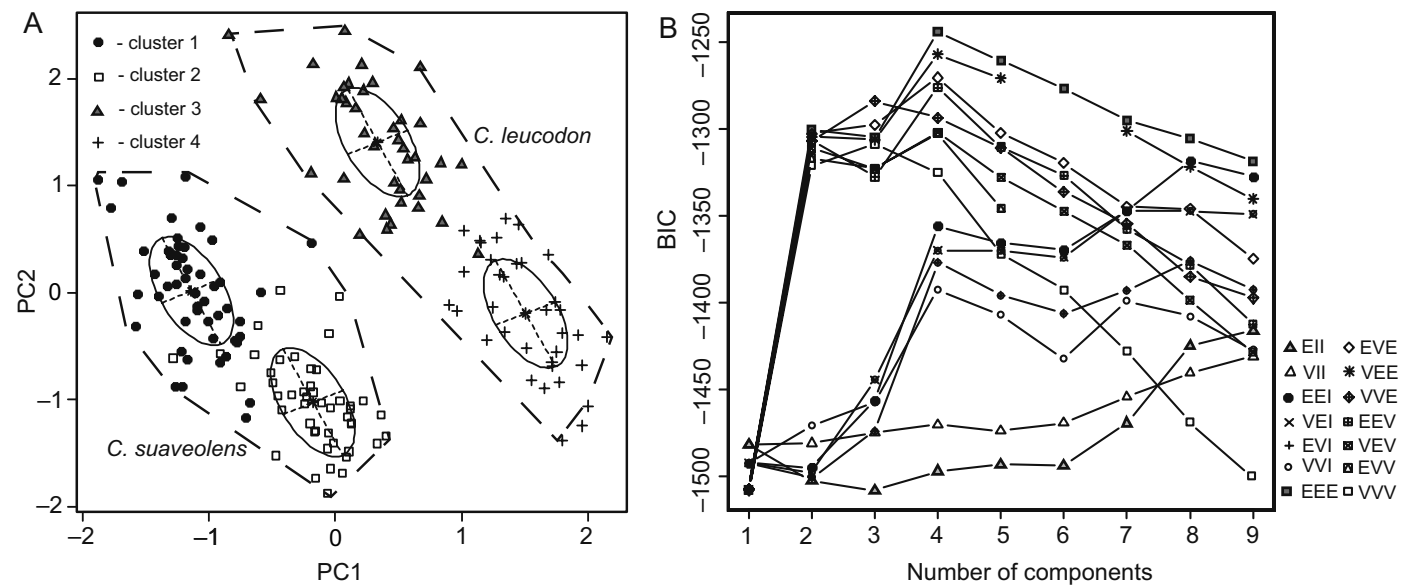


Fig. 3. *Crocidura*: results of PCA and model-based clustering. a: Specimen scores for the first two Principal Components. Ellipses indicate morphological clusters revealed by model-based clustering (see Table 2 for identification of clusters); polygons outline species clouds. b: Bayesian Information Criterion (BIC) of different cluster solutions; different symbols and line types encode different model parameterizations, denoted with three letters: volume, shape, and orientation. E means equal and V means varying across components or clusters; I refers to the identity matrix in specifying shape or orientation and is a special case of E; the 'best' model with the highest BIC is EEE (ellipsoidal, equal volume, shape and orientation) model with 4 components

Table 1. Principal Component (PC) Loadings for Varimax normalized solution in *Crocidura*, *Neomys*, and *Sorex* datasets. Boldface numerals: factorloadings > 0.70

	<i>Crocidura</i>			<i>Neomys</i>			<i>Sorex</i>	
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2
SCW	0.87	0.39	0.21	0.24	0.56	0.46	0.81	0.55
OW	0.80	0.40	0.23	0.10	-0.10	0.77	0.75	0.59
IO	0.87	0.27	0.16	0.23	0.20	0.63	0.69	0.60
CBL	0.87	0.44	0.14	0.41	0.66	0.41	0.80	0.57
BL	0.87	0.42	0.16	0.47	0.62	0.36	0.80	0.57
I1_M3	0.84	0.51	0.08	0.55	0.59	0.30	0.80	0.57
PL	0.79	0.55	0.17	0.66	0.32	0.31	0.72	0.67
RL	0.80	0.54	0.15	0.70	0.29	0.26	0.72	0.68
P4_M3	0.84	0.37	0.00	0.72	0.25	0.22	0.69	0.71
M1_M3	0.56	0.76	0.22	0.83	0.15	0.24	0.54	0.78
A1_A3	0.65	0.67	0.21	0.45	0.53	0.30	0.81	0.54
PGW	0.90	0.27	0.13	0.12	0.44	0.59	0.74	0.62
WP4_P4	0.08	0.12	0.95	0.22	0.28	0.66	0.76	0.55
WM3_M3	0.62	0.59	0.43	0.18	0.34	0.64	0.74	0.60
SCH	0.80	0.21	0.19	-0.08	0.55	0.24	0.86	0.36
PRH	0.84	0.25	0.31	0.32	-0.02	0.72	0.78	0.56
LI1	0.72	0.63	0.10	0.07	0.80	0.09	0.72	0.66
LTI1	0.67	0.64	0.03	0.02	0.79	-0.04	0.72	0.61
HI1	0.65	0.66	0.15	0.35	0.44	0.17	0.72	0.65
LA1	0.61	0.72	0.15	0.22	0.34	0.20	0.79	0.55
WA1	0.56	0.71	0.26	0.35	0.38	0.30	0.78	0.52
BLP4	0.62	0.70	0.19	0.48	0.23	0.26	0.63	0.73
BLM1	0.50	0.77	0.23	0.54	0.20	0.26	0.66	0.72
BLM2	0.39	0.82	0.18	0.73	-0.08	0.12	0.62	0.73
LM3	-0.04	0.51	-0.09	0.74	-0.11	0.07	0.35	0.85
LMD	0.87	0.38	0.11	0.44	0.53	0.47	0.79	0.59
i1_m3	0.86	0.49	0.04	0.58	0.52	0.24	0.79	0.60
p3_m3	0.89	0.41	-0.04	0.60	0.38	0.27	0.74	0.66
m1_m3	0.80	0.52	0.01	0.68	0.32	0.14	0.72	0.67
HPC	0.90	0.34	0.10	0.32	0.49	0.43	0.77	0.62
Li1	0.76	0.59	0.09	0.45	0.37	0.16	0.78	0.61
Lp3	0.66	0.58	0.13	0.24	0.46	0.22	0.75	0.59

Table 1 (continued)

	<i>Crocidura</i>			<i>Neomys</i>			<i>Sorex</i>	
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2
Lp4	0.64	0.64	0.11	0.32	0.07	0.15	0.74	0.62
HMD_m2	0.66	0.55	0.32	-0.09	0.52	0.53	0.80	0.53
HC	0.53	0.61	0.26	0.22	0.38	0.49	0.52	0.74
Wp3	0.58	0.70	0.14	0.31	0.35	0.23	0.80	0.48
Expl.Var.	18.73	10.77	2.05	7.17	6.53	5.27	19.41	14.05
Prp.Totl.	0.52	0.30	0.06	0.20	0.18	0.15	0.54	0.39

Table 2. Species identity and geographical origin of specimens in the clusters identified by model-based clustering. MC = mclust cluster, N = number of specimens, CE = Central Europe, SEE = Southeastern Europe (Bulgaria).

MC	<i>Crocidura</i>						<i>Neomys</i>				<i>Sorex</i>				
	N	<i>C. suaveolens</i>		<i>C. leucodon</i>		N	<i>N. fodiens</i>		<i>N. anomalus</i>		N	<i>S. araneus</i>		<i>S. minutus</i>	
		CE	SEE	CE	SEE		CE	SEE	CE	SEE		CE	SEE	CE	SEE
1	51	49	2	-	-	40	38	1	-	1	89	32	57	-	-
2	47	2	45	-	-	224	-	3	5	216	46	-	-	13	33
3	38	-	-	38	-	140	9	123	1	7	-	-	-	-	-
4	36	-	-	1	35	119	2	2	105	10	-	-	-	-	-

ters that consistently accounted for the most variation in this component were M1–M3, the measurements of particular teeth and especially BLM2 (Table 1). The distribution of specimen scores along this axis indicated that it represents the geographical variability within each species (Fig. 3a). PC3 represented only WP4–P4 (Table 1). It explained a very small percentage of the variability and did not show a particular trend that can be interpreted from a biological point of view. Within the morphospace defined by the retained PCs, four clearly differentiated morphogeographic groups were identified by model-based clustering (Figs 3a,b): cluster 1 consisted mainly of specimens of *C. suaveolens* from CE, cluster 2 was composed mainly of specimens of *C. suaveolens* from SEE, cluster 3 contained only specimens of *C. leucodon* from CE, and cluster 4 included mainly specimens of *C. leucodon* from SEE (Table 2). The univariate statistics of the characters with the highest loadings on the first two principal components showed that in both species the Bulgarian (SEE) forms have larger skull dimensions and smaller teeth (Table 3).

Table 3. Descriptive statistics of the measurements with the highest loadings on the first two PCs by morphogeographic groups, identified by model-based clustering of *Crocidura*.

Morphogeographic group	N	PC1						PC2					
		PGW		HPC		CBL		BLM2		BLM1		M1_M3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>C. suaveolens</i> (s.l.) – CE	49	5.72	0.16	4.06	0.162	16.26	0.370	1.21	0.050	1.41	0.049	3.00	0.093
<i>C. suaveolens</i> (s.l.) – SEE	45	5.91	0.20	4.25	0.185	16.61	0.525	1.16	0.045	1.33	0.051	2.88	0.072
<i>C. leucodon</i> – CE	38	6.47	0.20	4.85	0.187	18.83	0.520	1.39	0.055	1.64	0.066	3.44	0.092
<i>C. leucodon</i> – SEE	35	6.76	0.17	5.10	0.159	19.16	0.488	1.29	0.048	1.54	0.044	3.27	0.101

Table 4. Descriptive statistics of the measurements with the highest loadings on the first two PCs by morphogeographic groups identified by model-based clustering of *Neomys*.

Morphogeographic group	N	PC1						PC2					
		P4_M3		M1_M3		BLM2		LM3		LI1		LTI1	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>N. anomalus</i> – CE	105	5.30	0.13	3.75	0.10	1.47	0.05	0.82	0.05	1.84	0.08	0.84	0.06
<i>N. anomalus</i> – BG	216	5.51	0.14	3.88	0.10	1.52	0.05	0.86	0.04	1.77	0.07	0.78	0.05
<i>N. fodiens</i> – CE	38	5.61	0.11	3.89	0.08	1.47	0.05	0.84	0.04	2.15	0.08	0.95	0.06
<i>N. fodiens</i> – BG	123	5.74	0.15	3.98	0.11	1.56	0.05	0.86	0.04	2.03	0.07	0.87	0.05

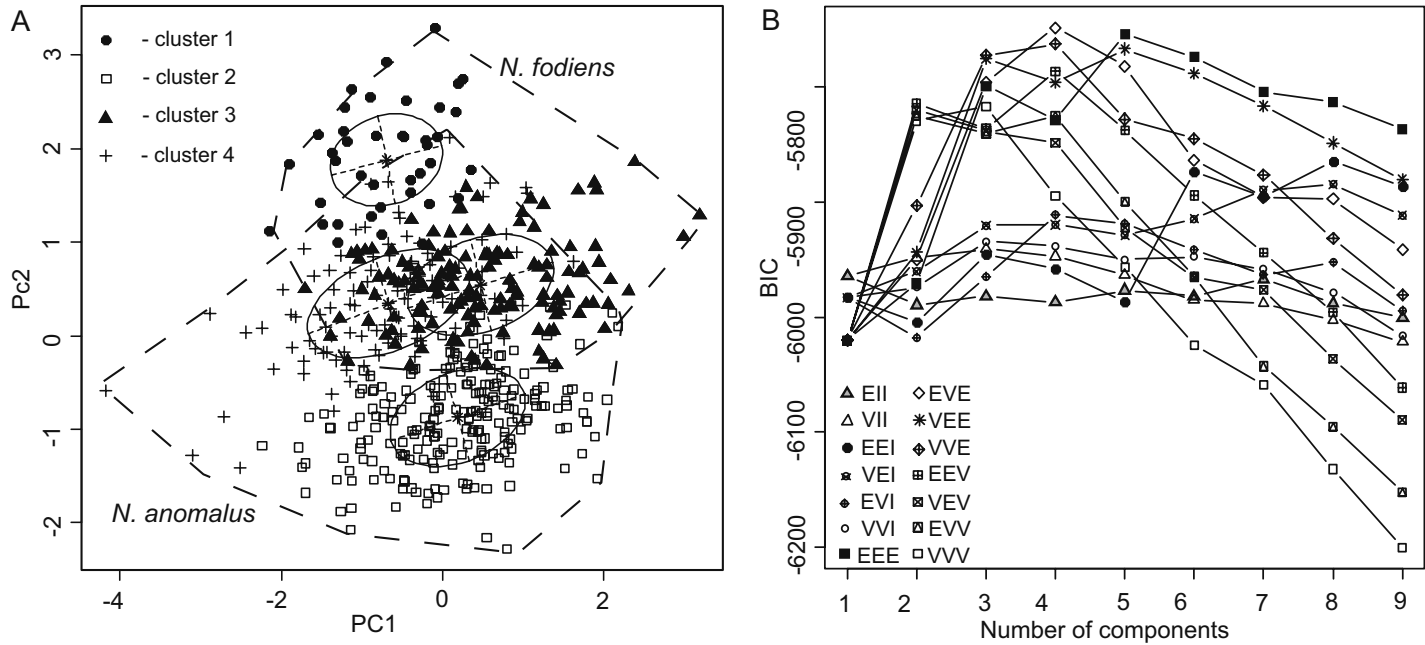


Fig. 4. *Neomys*: results of PCA and model-based clustering. a: Specimen scores for the first two Principal Components. Ellipses indicate morphological clusters revealed by model-based clustering (see Table 2 for identification of clusters); polygons outline species clouds; b: Bayesian Information Criterion (BIC) of different cluster solutions (for identification of symbols see Fig. 3b); the 'best' model with the highest BIC is EVE (ellipsoidal, equal volume and orientation) model with 4 components

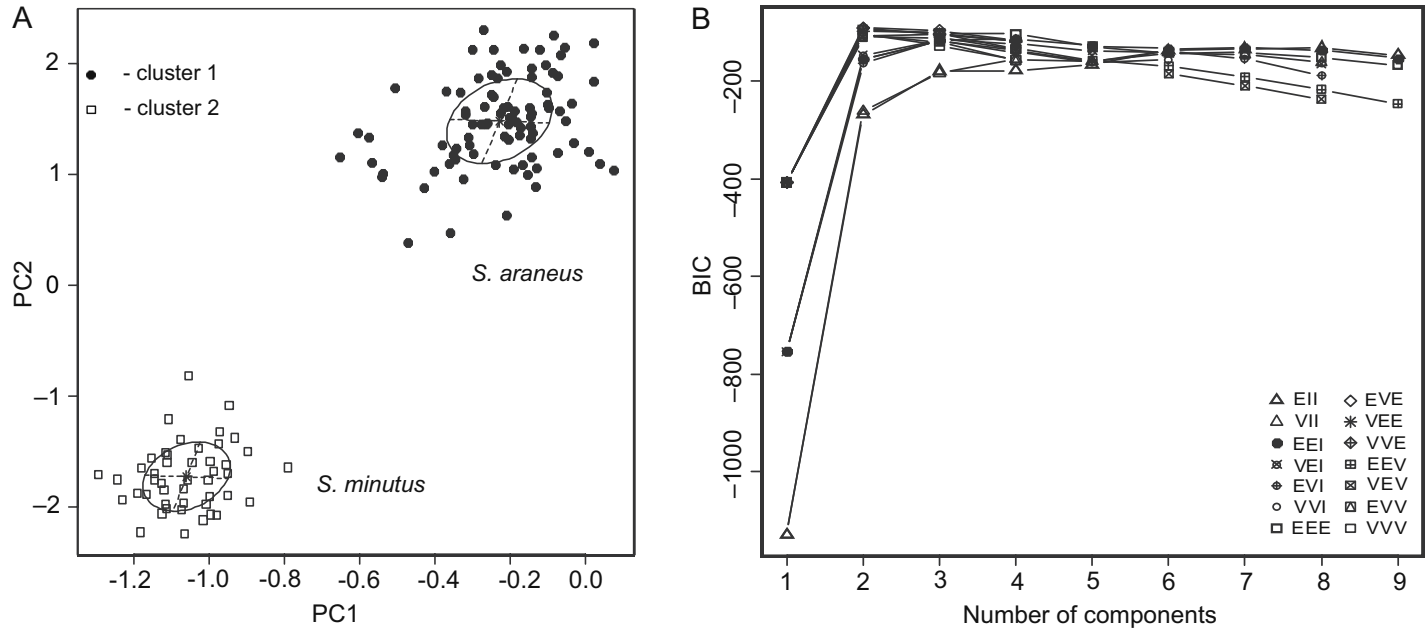


Fig. 5. *Sorex*: results of PCA and model-based clustering. a: Specimen scores for the first two Principal Components. Ellipses indicate morphological clusters revealed by model-based clustering (see Table 2 for identification of clusters); b: Bayesian Information Criterion (BIC) of different cluster solutions (for identification of symbols see Fig. 3b); the 'best' model with the highest BIC is VEV (ellipsoidal, equal shape) with 2 components

Genus *Neomys*

The first three PCs with eigenvalues larger than one accounted for 19% of variation in the dataset (Table 1). This low percentage compared with *Crocidura*, shows that in *Neomys* the inter- and intraspecific variability is less pronounced. Indeed, although the model based clustering revealed the presence of four morphological groups, they were tightly packed showing a substantial overlapping (Table 2, Figs 4a,b). PC1, accounting for 7.2% of the variation, represented some poorly expressed species differences in the lengths of the rostrum, the upper and the lower teeth rows (Table 1). PC2 explained 6.5% of the variation; it had significant positive loadings for morphometric characters associated with the overall skull size, but mainly with the size of the first upper incisive. PC1 described some poorly pronounced differences between the smallest form – *N. anomalus* from Central Europe and the largest form within the dataset – *N. fodiens* from Bulgaria (SEE). To some extent, PC1 can be interpreted as an axis representing species differences. PC2 reflected the geographic variability of *N. fodiens* and *N. anomalus*. Along this axis, the Bulgarian forms of both species are larger. According to the specimen scores (Fig. 4a) and univariate statistics of characters with great loadings on this axis (Table 4), this component registered variability opposite to that represented by PC1 – the forms from Bulgaria are smaller than those from the Central Europe. This axis reflects mostly the differences between *N. fodiens* from Central Europe and *N. anomalus* from Bulgaria. Interestingly, the differences between the two species were poorly pronounced along PC1 (dimensions of tooth rows) and better along PC2, representing the size of the upper incisor, with *N. fodiens* having bigger dimensions. It is noteworthy that the intra-specific differences were also better expressed along PC2. In this respect, it should be mentioned that along this axis the overlap between species from one geographical area was least expressed.

Genus *Sorex*

Since the differences in the sizes between the two species are too large, PCA gave only one component with eigenvalue larger than 1. The PC1 had eigenvalue equal to 32, while in PC2 it was only 0.5 (unrotated solution). Predictably, model – based clustering revealed only 2 groups (Fig. 5). When the analyses were applied separately for each species, model – based clustering showed no grouping within species, displaying homogeneity of both species samples and lack of a clear intra-specific morphological geographic variability in the studied region.

DISCUSSION

The results show that the species of *Neomys* and *Crocidura* exhibit well-established morphological differences between the regions, while they were poorly expressed in *Sorex*. Considering that the samples were composed of individuals from one age group, as well as the unbiased sex ratio of the regional samples to which the obtained morphological groups correspond nearly completely (Table 2), it can be concluded that for the first two genera these results reflect geographic variability and are not the result of random differences in the age structure or sex ratio of the samples. Regarding the possible influence of the sex ratio, it can also be mentioned that a previous study has shown that, although there are sexual size differences, they are of much smaller magnitude than the geographic variability (ZIDAROVA 2015).

In *Crocidura*, PC1 having high loadings for nearly all measurements can be interpreted as representing the general "size", while PC2 with high loadings for some teeth measurements emphasizes the "shape" differences (Table 1). The forms of *Crocidura* from Bulgaria (SEE) have larger dimensions, but smaller teeth. The registered geographic variability with respect to the "overall size" represented by the first principal component does not correspond to the Bergman's rule. Literature data on the nature of geographic variability in *Crocidura* are limited and contradictory. VESMANIS (1976) reported for an opposite to Bergman's rule tendency in *C. suaveolens*, but according to YOMTOV and GEFFEN (2006) there is a negative correlation between body size and temperature in this species. It can be supposed that the differences in teeth dimensions, presented by the second principal component, reflect some genetic differences between geographical forms of both species. With regard to *C. suaveolens*, this assumption is in accordance with the recent molecular data (BANNIKOVA *et al.* 2006), which indicate that the former subspecies *C. s. mimula* (Central Europe) and *C. s. suaveolens* (SE Europe) should be treated as separate species. Taking into consideration our results, as well as the geographical proximity of our sample localities and those from Crimea (Fig. 1), the type area for *C. suaveolens*, it is highly probable that shrews inhabiting eastern parts of Bulgaria belong to this species.

As for *C. leucodon*, the situation is similar to that of *C. suaveolens* (s. l.). The mitochondrial analyses of DUBEY *et al.* (2007b) revealed two clades – a western one recorded from France eastward to Greece and northwestern Anatolia, Turkey; and an eastern clade recorded in eastern parts of Bulgaria and Romania, Georgia and the rest of Anatolia. Our results generally agree with the molecular data, showing that the populations from Eastern Bulgaria, which geographically belong to the eastern clade, are morphologically distinct from the Central European ones, belonging to the western clade. As in *C. suaveolens*, it could be suggested that the boundary between western and eastern clade of

C. leucodon extends meridionally throughout Bulgaria (Fig. 1). Further genetic and morphologic studies are required to test these hypotheses.

In *Neomys*, the intraspecific differences are better expressed along PC2. In this respect, it should be mentioned that along this PC the overlap between species from one geographical area is least expressed. Given that the upper incisor is of a great importance for the capture of prey (DÖTSCH 1985), it can be assumed that these relatively well-pronounced differences in sympatry result from selection in the direction of avoiding competition. In general, these results correspond to previous analyzes that indicate that in *N. fodiens* the geographical variability is opposite to that of the Bergman's rule (BALČIAUSKAS *et al.* 2014) and that part of variation of both species may be explained by character displacement (POPOV & ZIDAROVA 2008). In contrast with the situation in *Crocidura*, the tight packing in the morphological space is probably the main reason that character displacement or convergence due to competition in sympatry (RYCHLIK *et al.* 2006, KRYŠTUFEK & QUADRACCI 2008, POPOV & ZIDAROVA 2008) have a very pronounced effect and may embarrass the identification of unequivocal diagnostic morphological characters (IGEA *et al.* 2015).

In comparison with *Crocidura* and *Neomys* species, *S. araneus* and *S. minutus* don't reveal any clear intraspecific variation by means of the chosen analytical approach. Our results correspond to the data presented by other authors that there is no clear relation between morphology and genetics. WÓJCIK *et al.* (2000) and MISHTA (2007) did not find larger morphological differences between races of *S. araneus* from Western European and those from Eastern European chromosomal groups than amongst different races from one chromosomal group. A number of researchers found no correlation between the karyotype differentiation and the phenotypic variability, and the craniometrical variability in particular (SEARLE & THORPE 1987, MEYER & SEARLE 1994, ZIMA & KRÁL 1995, WÓJCIK *et al.* 2000). According to VEGA *et al.* (2016), morphological variation does not reflect genetic subdivision in *S. minutus*. BERNAL (2010) found only poorly expressed morphological differences between CE and Balkan phylogroups with the exception of the mandible size, which is larger in the Balkans.

The above comparisons of the phenotypic variation of shrews with the available data from mitochondrial and nuclear markers show that the application of morphometric methods in taxonomy could be highly informative. The use of a wide range of morphological traits and their subsequent synthesis in terms of "size" and "shape" by applying various methods of multivariate analysis would help to interpret the patterns of morphological geographic variability and allow to some extent to distinguish the impact of environmental factors from that of genetic factors. Of course, best results could be expected by using both, morphometric and molecular, methods in parallel (HILLIS 1987).

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