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Integrative taxonomic revision of the Ethiopian endemic rodent genus *Stenocephalemys* (Muridae: Murinae: Praomyini) with the description of two new species

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Abstract. Ethiopian rats (genus *Stenocephalemys*) represent a monophyletic group of Ethiopian endemic rodents that diverged in the Ethiopian Highlands during the Pleistocene. Because of the frequent occurrence of so-called reticulate evolution (i.e. repeated hybridization of partially diverged populations), their taxonomy has not been adequately resolved, despite the fact that they belong to the most abundant rodent genus in Ethiopia and are important as pests and carriers of pathogens (e.g. hantaviruses). Here we analysed material for 623 *Stenocephalemys* specimens using integrative taxonomy composed of genomic analyses (388 nuclear markers and complete mitogenomes), 2D-geometric morphometry of skulls and classical morphometry of external traits. The genus consists of six clearly defined gene pools (= species), characterized by specific morphology, ecology and distribution. Two of them, described here as new species, live in fragmented populations in Afro-alpine habitats in the north-western part of the Ethiopian Highlands. We also showed that mitochondrial DNA is not applicable as a universal diagnostic tool for species discrimination in *Stenocephalemys*, because of multiple cases of mitochondrial introgression. This finding illustrates the utility of the genus as a suitable model for future studies of mito-nuclear coevolution along an elevational gradient.

Key words: taxonomy, Ethiopian Highlands, mitogenomics, distribution, mammals, Ethiopian rats, morphometry

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Introduction

The Eastern Afromontane biodiversity hotspot (EABH) is one of the most important biodiversity centres on Earth. For example, more than 10% of 510 mammal species in EABH are considered endemic (Mittermeier et al. 2011). The largest block of EABH is formed by the Ethiopian Highlands (EH), an extensive volcanic mountain massif that is split into two main parts (north-western and south-eastern) by the Great Rift Valley (GRV). The EH are characterized by an extremely high proportion of endemic taxa, including mammals (Yalden & Largen 1992, Lavrenchenko & Bekele 2017). For rodents alone, the proportion of species occurring only in Ethiopia reaches 41%, if we consider the whole country, or 90%, if we consider only taxa living in Afromontane ecosystems of EH (Bryja et al. 2019a). Many of the endemic mammal species in the region have originated by allopatric speciation due to the separation of EH from the rest of EABH, and there are also unique deeply divergent evolutionary lineages at the level of separate genera.

One group is the rodent genus *Stenocephalemys* Frick, 1914 (= Ethiopian rats), a monophyletic group within the diverse African murine tribe Praomyini, which diverged from the rest of the tribe during the radiation at the Miocene/Pliocene boundary (Aghová et al. 2018), and currently is found only in EH. Four species, differing by ecological preferences and distribution in EH have been traditionally recognized in most recent lists (Happold 2013, Monadjem et al. 2015, Wilson et al. 2017): *S. albipes* (Rüppel, 1842); *S. albocaudatus* Frick, 1914; *S. griseicauda* Petter, 1972; and *S. ruppi* Van der Straeten & Dieterlen, 1983. The most comprehensive study of genetic diversity and phylogeny of the genus, recently published by Bryja et al. (2018), suggested additional cryptic diversity and a complex evolutionary history of the genus in Pleistocene, including repeated periods of ecological and allopatric diversification followed by hybridization events. A similar evolutionary pattern has been demonstrated in other groups of Ethiopian rodents and is termed "reticulate evolution" (Lavrenchenko et al. 2004, Bryja et al. 2019b). More specifically, the phylogenetic analyses of *Stenocephalemys* based on the sequences of mitochondrial cytochrome *b* gene (cyt*b*) and six nuclear fragments revealed that (i) mitochondrial phylogeny is not concordant with the nuclear phylogeny, and (ii) there is cryptic variability and the number of gene pools (= genetically delimited candidate species) is higher than the four currently recognized species.

The main limitation of the previous phylogenetic study (Bryja et al. 2018) was the relatively low amount of genetic data used. For this reason, for example, the discordance between mitochondrial and nuclear phylogeny can have two alternative explanations. First, it is possible that the cyt*b* phylogeny is affected by convergent evolution (selection) at this gene, as taxa living in similar environments of high-elevation Afroalpine grasslands (ca. about 4000 m a.s.l.) are sisters in the mitochondrial tree, but not in the nuclear tree. An alternative explanation is the introgression of complete mtDNA as a result of past hybridization. The two hypotheses can be tested by a phylogenetic analysis of complete mitogenomes or non-coding parts of mtDNA, i.e. those not affected directly by selection. If the mitogenomic relationships are the same as those observed at cyt*b*, the mitochondrial introgression is more likely than parallel convergent evolution. Further, Bryja et al. (2018) defined six "gene pools" (= potential species) based on six nuclear markers. However, this finding could be affected by ascertainment bias and higher numbers of genomic segments are thus needed to delimit reliable gene pools that might correspond to separate species. In the next step of integrative taxonomic analysis and species delimitation, it is also necessary to analyse phenotypic variability, e.g. external and skull morphology. This work will not only allow diagnosis of particular species based on their phenotype, but also to reconstruct the patterns of morphological evolution as a result of the interplay of allopatric and ecological diversification with occasional hybridization.

In this study, we address the previous limitations by the analysis of significantly more genotypic and phenotypic data (allowed by recent so-called "holistic" collection of new material; see Ferguson 2020 in this issue) for all major *Stenocephalemys* clades. Specifically we aim to: (i) reconstruct the mitochondrial phylogeny by using the complete mitogenomes; (ii) delimit potential species ("gene pools") and construct their species tree by employing significantly larger nuclear data from 388 DNA fragments; (iii) analyse phenotypic variation of genetically delimited species by using the approaches of skull geometric morphometry combined with traditional analysis of external measurements and skull size. Finally, we provide a

formal taxonomic revision of the genus, including the description of two new species.

Material and Methods

Sampling

For this study, we used 623 specimens belonging to the genus *Stenocephalemys* (Table S1 in Supplementary online materials; Fig. 1). Most individuals (except 15 old museum type specimens) were barcoded by cyt*b* and/or nuclear markers according to Bryja et al. (2018) and assigned into one of six candidate species: *S. albipes*, *S. albocaudatus*, *S. griseicauda*, *S. ruppi*, *Stenocephalemys* sp. A., and *S.* "pseudogriseicauda" (see more details in Bryja et al. 2018). The last two species are formally described below as *S. zimai* sp. nov. and *S. sokolovi* sp. nov., respectively, and we use these names for simplicity hereafter. Most material for both genetic and morphological analyses was collected by the Institute of Vertebrate Biology of the Czech Academy of Sciences (Czech Republic), the A. N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (Russia) and the College of Natural and Computational Science, Department of Biology, Mekelle University (Ethiopia) (see Table S1 for more details, including the localization of voucher specimens).

Genomic analyses were based on 27 complete mitogenomes of all *Stenocephalemys* taxa with *Myomyscus* sp. as outgroup. The nuclear phylogeny was analysed on the basis of 388 loci (610965 bp concatenated alignment) in 14 individuals covering the mitochondrial diversity of *Stenocephalemys*. These data were obtained through the so-called anchored phylogenomic approach (Lemmon et al. 2012).

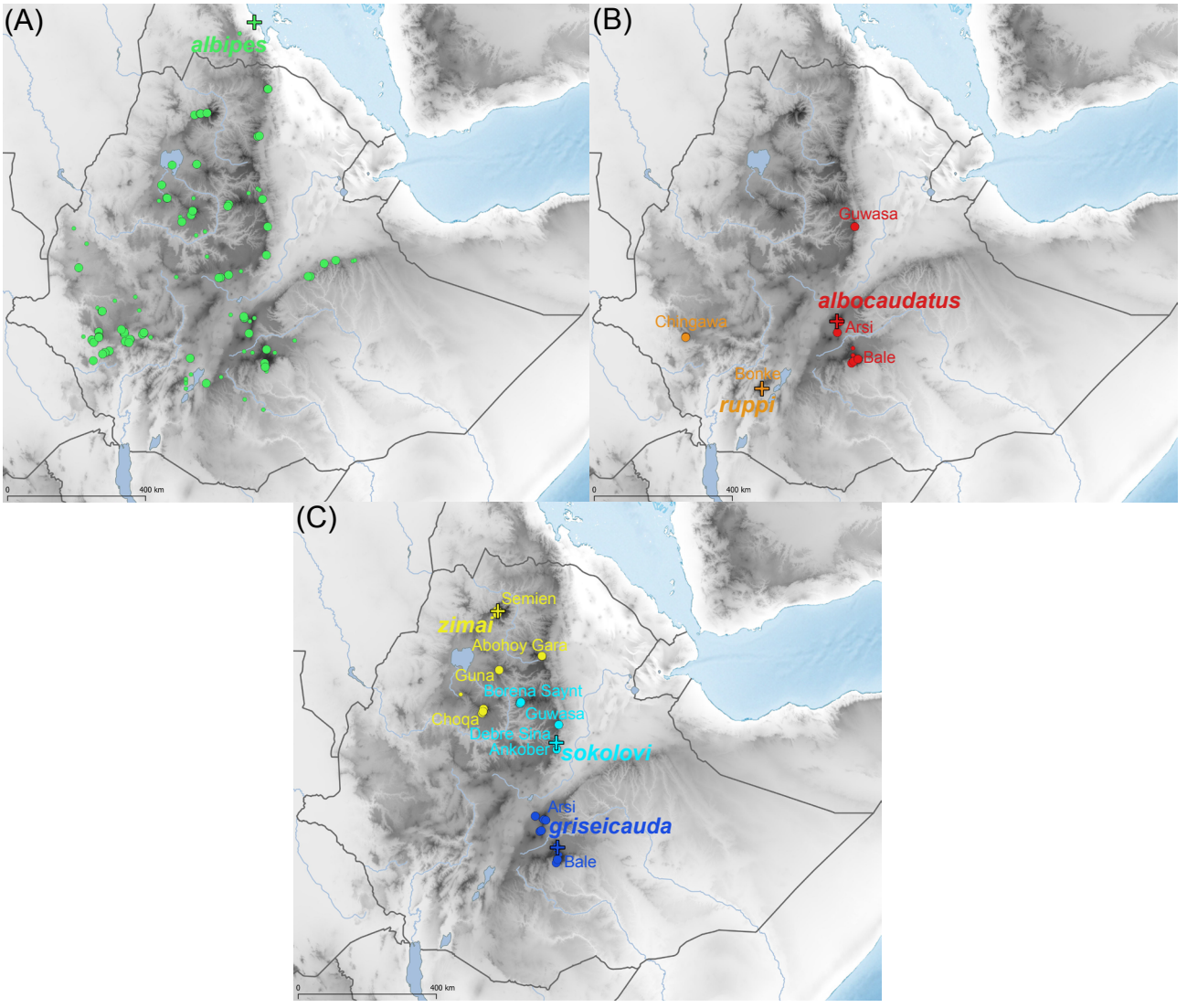

Fig. 1. Distribution of the six species of *Stenocephalemys*. Large symbols show localities of genetically confirmed individuals (based on Table S1), small symbols are those taken from Yalden et al. (1976, 1996) and crosses indicate the type localities. (A) *S. albipes*; (B) *S. albocaudatus* (red), *S. ruppi* (orange); (C) *S. griseicauda* (dark blue), *S. sokolovi* sp. nov. (light blue), *S. zimai* sp. nov. (yellow).

Morphometric analyses were based on external measurements (373 individuals) and on skulls (2D geometric morphometry of 141 skulls) of adult specimens. Both datasets represent the most comprehensive material of all *Stenocephalemys* taxa covering majority of their known distribution area in Ethiopia (Fig. 1; see more details in Table S1).

Phylogenomic analyses

(i) Anchored phylogenomics (ASTRAL)

Anchored hybrid enrichment (AHE) data collection was performed by the Center for Anchored Phylogenomics (www.anchoredphylogeny.com). The *Stenocephalemys* dataset was filtered out from a larger dataset collected within another study (Nicolas et al., unpublished data) and consists of 14 specimens (Table S1) from all major mitochondrial lineages. As outgroup we used the sister genus *Myomyscus* (Nicolas et al., unpublished data). In final analyses we used 388 nuclear loci genotyped in a majority of samples (Data S1 in Supplementary online materials).

Gene tree conflict as a result of incomplete lineage sorting (ILS) poses a problem for estimation of species trees. For this reason we used the ASTRAL (Accurate Species Tree Algorithm) method for estimating species trees from unrooted gene trees, under the multi-species coalescent model (Mirarab & Warnow 2014). The gene trees were calculated in a Bayesian setting using MrBayes v. 3.2.6 (Ronquist et al. 2012) and are provided as Data S2 in Supplementary online materials. ASTRAL accepts just a single tree per gene and it was calculated as a maximum bipartition credibility tree (MBCT) in the package "phangorn" (Schliep 2011) for R.

(ii) Mitogenomic phylogeny

Phylogenetic relationships of mtDNA of *Stenocephalemys* have been inferred recently using the cyt*b* marker (Bryja et al. 2018). Observed discordance between phylogenetic trees based on cyt*b* and six nuclear sequences can be explained by convergent evolution of cyt*b* in different elevations or by mitochondrial introgression. To test these hypotheses we produced 27 complete mitogenomes of all major *Stenocephalemys* clades (Table S1, Fig. 2) and performed robust analysis of the mitochondrial phylogeny. Complete mitogenomes were assembled as a by-product of anchored phylogenomic genotyping or by the sequencing of long-range PCR products (as described in Nicolas et al. 2020). We used PartitionFinder v. 2.1.1 (Lanfear et al. 2016) to detect partitions and the most suitable substitution models for different parts of mtDNA using the corrected Akaike Information Criterion (AICc, Burnham & Anderson 2002). Bayesian analysis was carried out by Markov Chain Monte Carlo (MCMC) simulations of posterior probabilities using MrBayes 3.2.6 (Ronquist et al. 2012) with three heated and one cold chain. Two independent runs were conducted with 20 million generations each with trees and parameters sampled every 1000 generations initiated from random trees. Convergence was checked using TRACER v1.5 (Rambaut et al. 2018). For each run, the first 25% of sampled trees were discarded as burn-in. Bayesian posterior probabilities were used to evaluate branch support of the tree. The final tree was visualized in FigTree 1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/). All phylogenetic analyses of mitogenomes were run on CIPRES Science Gateway (Miller et al. 2012).

Phenotypic variability

Four standard body measurements (lengths of head + body, tail, hind foot and ear), one body form index (relative tail length = tail/head + body) as well as skull size and shape were compared among six species (*S. albipes*, *S. albocaudatus*, *S. griseicauda*, *S. ruppi*, *S. sokolovi* sp. nov. and *S. zimai* sp. nov.). To avoid taxonomic confusion, we analysed only genetically identified individuals. The analysis was also confined to adult specimens, as identified by tooth wear (cusps at least partly fused) and reproductive condition. In total, we analysed 373 sets of body measurements and 141 skulls. Sample sizes of species *albipes*, *albocaudatus*, *griseicauda*, *ruppi*, *sokolovi* sp. nov. and *zimai* sp. nov. (in that order) were 181, 61, 35, 18, 50 and 28 for body measurements and 67, 15, 7, 14, 19 and 19 for skull size and shape data (Table S1).

Skull size and shape were studied by two-dimensional geometric morphometry (Dryden & Mardia 2016). The skull was photographed from three sides and size and shape of each was described by a set of anatomically homologous landmark points. The landmark sets are shown in Fig. S1 in Supplementary online materials where it is also indicated which points were treated as semi-landmarks, whose position was computationally optimized by sliding between their immediate neighbours. Side-specific configurations were subjected to the Generalized Procrustes Alignment (GPA, Rohlf & Slice 1990) with Procrustes distance as the semi-landmark sliding criterion (Bookstein

1997). Centroid size was used as the measure of skull size and Procrustes shape (i.e. GPA-standardized) coordinates as descriptors of skull shape. Centroid sizes were highly correlated (r > 0.98) between three sides and thus we used only dorsal centroid size. Side-specific skull shapes were analysed jointly after concatenation of all Procrustes shape coordinates into a single matrix. The landmark points were digitized by one of us (O. Mikula) in tpsDIG2 software (Rohlf 2009) and all analyses were performed in the R using the package geomorph (Adams et al. 2019).

Variation in body measurements and skull size was visualized using violin plots (using the R package vioplot, Adler & Kelly 2018) and the basic descriptive statistics (mean, standard deviation, range) were calculated for each of six species. Variation in skull shapes was visualized by their ordination in the shape space of between-group principal components (bgPCA; see Cardini et al. 2019 and references therein). Skull shapes of type specimens were projected into this space to show the fit with their respective species. The skull of *S. albipes* holotype (SMF_4320) was severely damaged and its position in bgPCA space was, therefore, assessed by repeating the analysis only with landmarks located on the intact part of the skull. Every trait (body measurement, skull size or skull shape) was also subjected to cluster analysis, which estimated how many morphological types (morphotypes) can account for the variation observed within species and between them. The cluster analysis started with a single morphotype and proceeded in stepwise manner. It always assessed all possible pair-wise mergers of the current morphotypes and accepted the best one, until there was no merger improving the specific support measure. Finally, it compared the result with all alternative solutions created either by moving one species between morphotypes or by moving one species into its own morphotype. For the body measurements and skull size we used the Gaussian mixture model fit in the R package mclust (Scrucca et al. 2016) and assessed by AICc. Every morphotype was assumed to correspond to a single Gaussian component differing by its mean and/or variance. The clustering accepted mergers maximally decreasing AICc and also retained an alternative solution whose AICc was at most one point higher than in the original solution. For the skull shape we used bgPCA, whose performance was assessed by one hundred times repeated five-fold cross-validation. In every cross-validation round, the specimens were assigned to the nearest morphotype centroids and a confusion matrix was created. The classification success was quantified from the average confusion matrix using the chance corrected index:

$$\tau = \frac{n_0 - \sum_{i=1}^{k=1} n_i/k}{n - \sum_{i=1}^{k=1} n_i/k'}$$

where n is the total sample size, n_0 is the number of correctly classified individuals and n_i is the sample size of the ith out of k morphotypes (Klecka 1980, p. 51). The alternative solutions were retained, if their τ was lower by 0.01 (one per cent) or less. In highly multivariate settings, bgPCA is preferred over more common canonical variate analysis as it is less biased when the number of individuals is low compared to dimensionality of data (Mitteroecker & Bookstein 2011, Cardini et al. 2019).

Finally, we examined morphological variation at a finer phylogenetic scale. Violin plots and bgPCA ordination was created for ten phylogeographic lineages rather than six species. In this classification *S. albipes* was split into lineages 1 and 3, *S. albocaudatus* into the lineages found east and west of the Rift Valley, *S. sokolovi* sp. nov. into populations with and without introgressed mtDNA of S. *albipes* and *S. zimai* sp. nov. into its lineages 1 and 2 (see Table S1 and Bryja et al. 2018 for more details).

Results and Discussion

Gene pools revealed by nuclear phylogenomics

A phylogenomic species tree in ASTRAL (based on 388 nuclear markers and using *Myomyscus* sp. as outgroup) revealed that *Stenocephalemys* is composed of six highly supported (PP = 1) subclades (Fig. 2A) that correspond to six putative species previously defined by Bryja et al. (2018). Despite a large amount of genetic data, their relationships are only partly resolved, which might indicate rapid radiation. In agreement with Bryja et al. (2018), who used only six nuclear markers, *S. albipes* is sister to *S. zimai* sp. nov. (PP = 1). The most credible topology of the rest of the tree is, however, different. The four remaining species form a significantly supported (PP = 1) monophyletic group, but their relationships remain obscure. It seems that *S. griseicauda* is sister to *S. sokolovi* sp. nov., and *S. albocaudatus* is sister to *S. ruppi*, but only with PP = 0.95 and PP = 0.62, respectively (Fig. 2A).

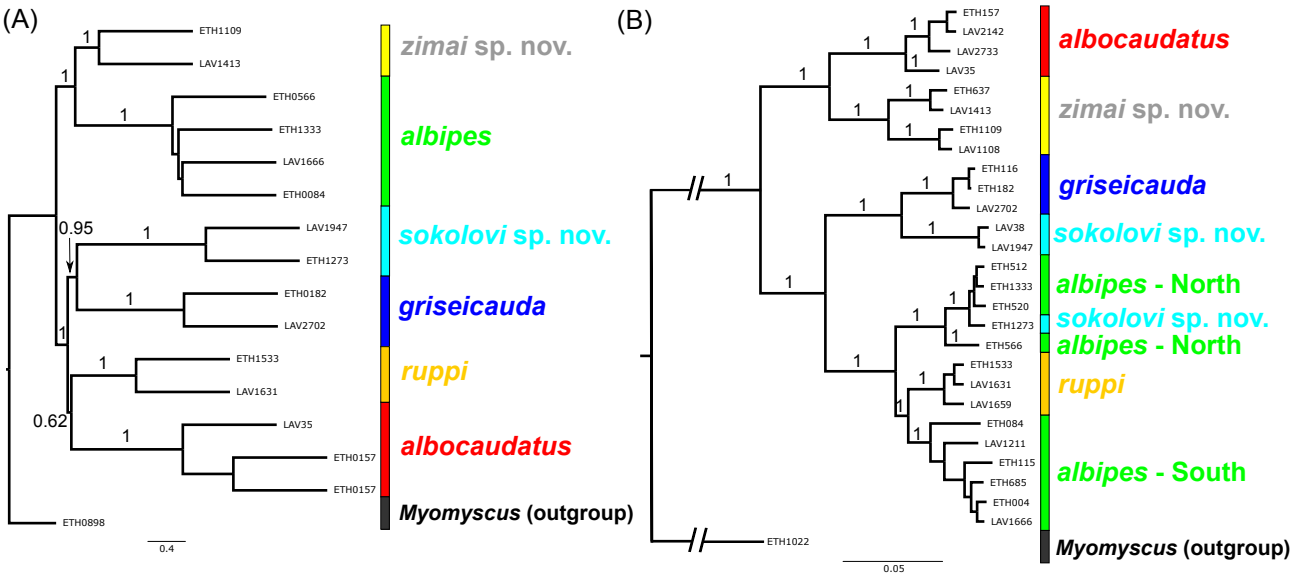

Fig. 2. Phylogenomic analysis of the genus *Stenocephalemys*. Six candidate species, including two new species, have different colours and were suggested on the basis of phylogenetic analysis of nuclear markers in Bryja et al. (2018). (A) Species tree based on 388 nuclear loci from ASTRAL (note that individual ETH0157, *S. albocaudatus*, was analysed twice to check for consistency of genotyping); (B) Bayesian tree from complete mitogenomes.

Phylogeny of complete mtDNA

Phylogenetic analysis of 28 complete mitogenomes (including *Myomyscus* sp. as an outgroup) revealed a well-resolved tree (Fig. 2B). The phylogeny previously inferred was based on one mitochondrial marker, cyt*b* (Bryja et al. 2018), and is completely consistent with that reconstructed from complete mitogenomes. The topology is different from the nuclear species tree, which suggests past mitochondrial introgression events. There are three monophyletic mitochondrial clades (PP = 1) that roughly correspond to different preferred elevations: (i) *S. albocaudatus* + *S. zimai* sp. nov. live in the highest altitudes in Afroalpine habitats of south-eastern and north-western plateaux; (ii) *S. griseicauda* and *S. sokolovi* sp. nov. prefer ericaceous habitats in mid-elevations; (iii) *S. albipes* and *S. ruppi* are primarily forest species occupying the lowest elevations. The only exception is the population of *S. sokolovi* sp. nov. from high elevation of the Borena Saynt NP (represented by the individual ETH1273 in our genomic datasets), whose mtDNA was completely (and probably recently) replaced by mtDNA of *S. albipes* from lower elevation of the same mountains (compare Figs. 2A and B; see more details in Bryja et al. 2018).

Morphometric variability

Table 1 and Fig. 3 show how the external body measurements and skull size varied within and between the species. The Afroalpine specialist *S. albocaudatus* is the largest species with average head and body length (HB) = 161.3 mm, followed by another high elevation species, *S. zimai* sp. nov. (HB = 151.4 mm). The smallest species is the forest-dwelling *S. albipes* (HB = 128.6 mm), followed by another forest species, *S. ruppi* (HB = 138.1 mm). The two species preferring ericaceous shrubs, *S. griseicauda* and *S. sokolovi* sp. nov., are of intermediate size. A similar pattern is observed in the skull size, where *S. albocaudatus* and *S. albipes* are on the opposite extremes of size variability and average values of the remaining species are intermediate. The average tail length (TL) separated the two forest-dwellers (*S. albipes*, TL = 161.2 mm and *S. ruppi*, TL = 155.8 mm) from the other species. The contrast was even more pronounced in the average relative tail length, which was as large as 125.7% in *S. albipes* and 113.4% in *S. ruppi*, but varied between 83.5% and 98.7% in the other species. Hind foot was considerably larger in *S. albocaudatus* (31.9 mm compared to 26.4-28.0 mm in the other species) and so was ear length (26.5 mm compared to 22.4-24.2 mm). There was a substantial intraspecific variation in some measurements, most notably in the lengths of head and body (*S. albocaudatus*) and ear (*S. zimai* sp. nov.)

Fig. 4A shows ordination of skull shapes in the space of the first two between-group principal components (bg-PC). The main contrast is between *S. albipes* and *S. albocaudatus*, which occupy the opposite ends of bgPC1. Four other species, *S. griseicauda*, *S. ruppi*, *S. sokolovi* sp. nov. and *S. zimai* sp. nov., are intermediate along bgPC1, but they are located together along bgPC2. Skull shapes of

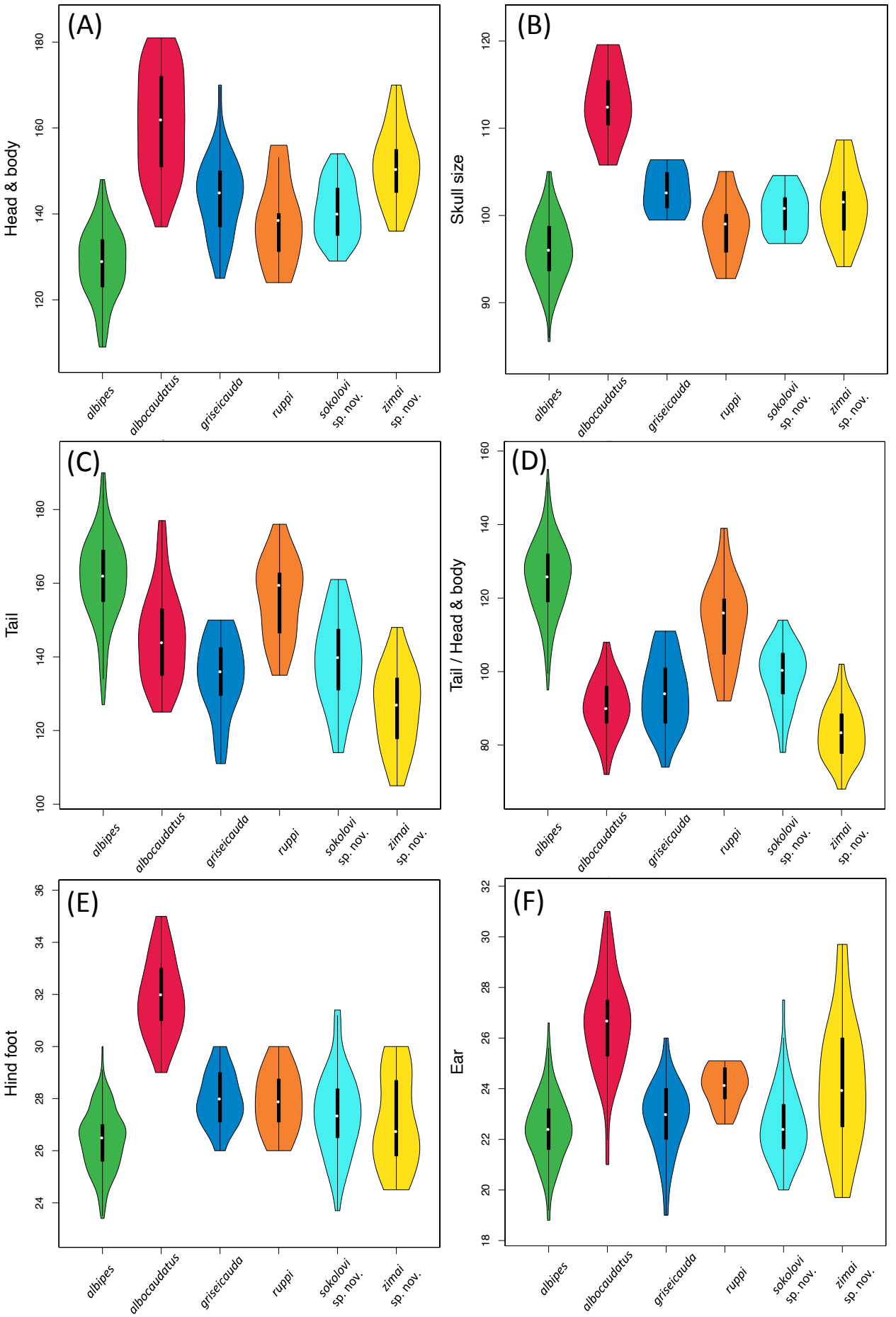

Fig. 3. Violin plots showing variations in external body measurements (A, C-F) and skull size (B) within and between six species of *Stenocephalemys*.

Table 1. Morphometric variability of six species of *Stenocephalemys*. Mean and standard deviation (SD) is shown. For the list of analysed specimens see Table S1 in Supplementary online material.

	Head + body (mm)			Tail (mm)			Hind foot (mm)			Ear (mm)			Relative tail length (%)			Skull centroid size (mm)		
	mean	SD	range	mean	SD	range	mean	SD	range	mean	SD	range	mean	SD	range	mean	SD	range
S. albipes	128.6	8.2	109-148	161.2	11.7	127-190	26.4	1.2	23.4-30.0	22.4	1.3	18.8-26.6	125.7	10.2	95-155	96.0	3.7	85.5-105.0
S. albocaudatus	161.3	12.1	137-181	145.6	12.5	125-177	31.9	1.5	29.0-35.0	26.5	1.9	21.0-31.0	90.5	7.7	72-108	112.9	4.2	105.8-119.6
S. griseicauda	144.0	9.2	125-170	135.1	10.5	111-150	28.0	1.0	26.0-30.0	22.8	1.4	19.0-26.0	94.3	9.5	74-111	102.9	2.6	99.5-106.4
S. ruppi	138.1	9.6	124-156	155.8	11.0	135-176	27.9	1.2	26.0-30.0	24.1	0.8	22.6-25.1	113.4	11.5	92-139	98.6	3.5	92.8-105.0
S. sokolovi sp. nov.	140.8	6.6	129-154	139.0	12.0	114-161	27.4	1.6	23.7-31.4	22.6	1.4	20.0-27.5	98.7	8.2	78-114	100.4	2.5	96.8-104.6
S. zimai sp. nov.	151.4	8.6	136-170	126.2	11.0	105-148	27.1	1.8	24.5-30.0	24.2	2.5	19.7-29.7	83.5	7.5	68-102	101.3	3.9	94.1-108.7

the type specimens are shown as filled circles in Fig. 4A, the ordination of the *S. albipes* holotype (based on the reduced dataset) is shown in Fig. 4B.

The cluster analysis showed a number of morphotypes, which are generally overlapping between the traits (Table 2). Moreover, their delimitation was often ambiguous with two solutions having similar support. Head and body length measurements differed mainly between the high-altitude (*S. albocaudatus* + *S. zimai* sp. nov. or just *S. albocaudatus*) and the lower-altitude morphotypes (all other species). A similar result was found with the skull sizes. Tail and relative tail lengths differed between the forest (*S. albipes* + *S. ruppi*) and non-forest morphotypes (all other species). Hind foot measurements separated *S. albocaudatus* (best model) or *S. albipes* and *S. albocaudatus* (alternative model) into their own morphotypes. Two morphotypes were found with ear length: the short-eared *S. albipes*, *S. griseicauda* and *S. sokolovi* sp. nov. differed from the long-eared *S. albocaudatus*, *S. zimai* sp. nov. and *S. ruppi*.

The most complex clustering was found in skull shape (Table 2). The best solution ($\tau = 0.9716$) had four morphotypes: one each for *S. albipes*, *S. ruppi* and *S. albocaudatus* and one ("*griseicauda*-like") for the three remaining species, *S. griseicauda*, *S. sokolovi* sp. nov. and *S. zimai* sp. nov. (Fig. 4C shows bgPCA ordination of this four-morphotype solution). An alternative solution ($\tau = 0.9645$) had an additional group, separating *S. sokolovi* sp. nov. from the other two *griseicauda*-like species (Table 2). While the first component makes a contrast between low-altitude and high-altitude species, the second separates *S. ruppi* by separating it from the altitudinal sequence. The associated wireframe plots (Fig. 5) show the average shape differences between the four skull morphotypes: *S. albocaudatus* differs from *S. albipes* conspicuously by having an overall more concave skull, longer rostrum, wider zygomatic arches, narrower interorbital constriction, relatively narrower braincase and smaller parietal as well as slightly more procumbent incisors and larger molars. The *griseicauda*-like morphotype was intermediate between the two. *Stenocephalemys ruppi* generally resembles *S. albipes*, but it has slightly narrower interorbital constriction and a longer rostrum. This is not associated, however, with differences in morphology of zygomatic arches and braincase.

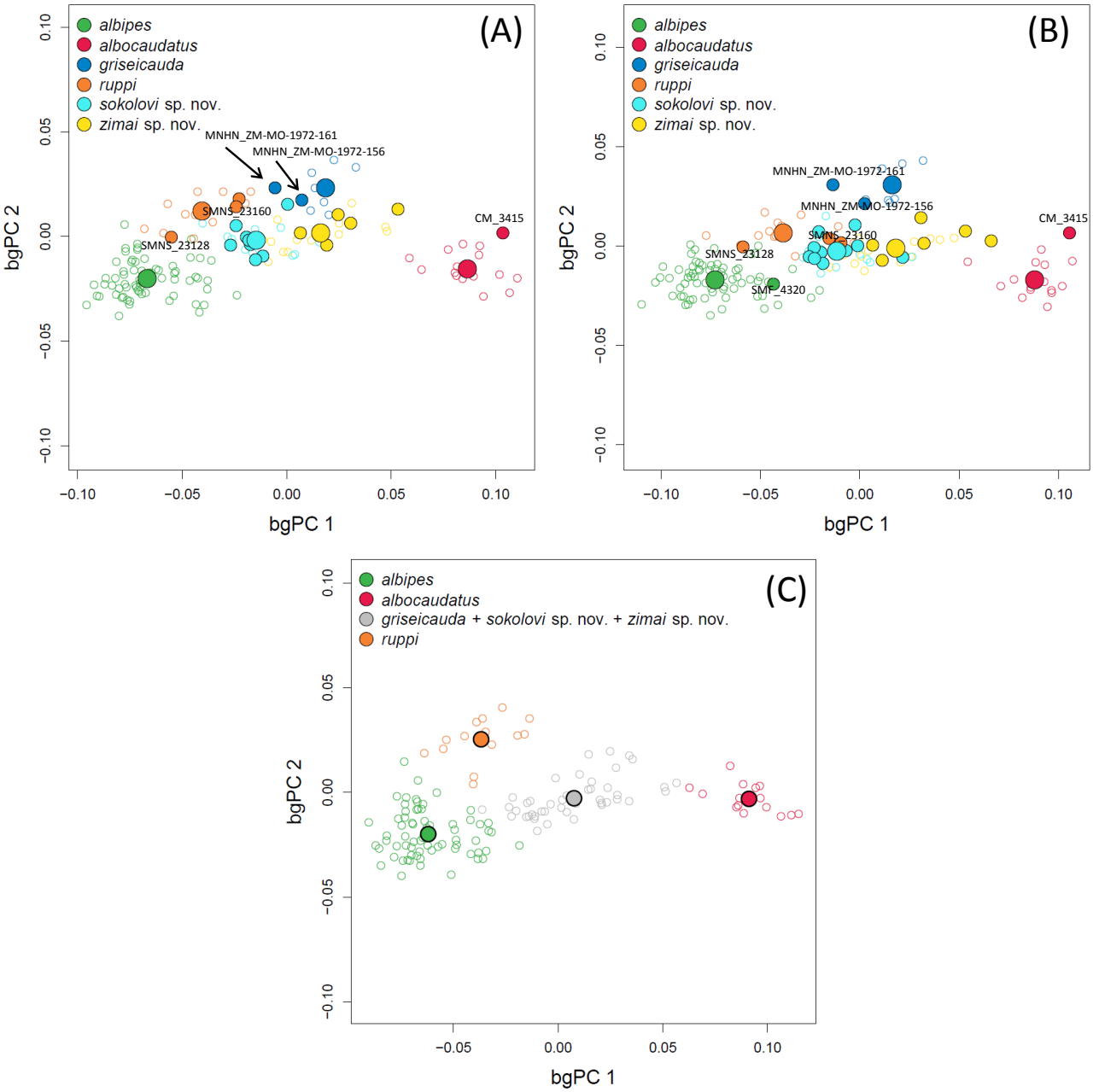

Fig. 4. (A) Ordination of skull shapes of six *Stenocephalemys* species (empty circles, whose colours correspond to particular species) in the shape space of the first two between-group principal components (bgPC). The means of groups (either species or morphotypes) are shown by large filled circles. Skull shapes of type specimens (small full circles) with all landmarks were projected into this space to show they fit their respective species. Type specimens without genetic information are labelled by museum codes. (B) Ordination with reduced set of landmarks, allowing the placement of the severely damaged skull of *S. albipes* holotype (SMF_4320). (C) bgPCA ordination of the four morphotypes, i.e. the best solution identified by the clustering of the skull shape: one for each *S. albipes*, *S. ruppi* and *S. albocaudatus* and one ("*griseicauda*-like") for three remaining species, *S. griseicauda*, *S. sokolovi* sp. nov. and *S. zimai* sp. nov.

The comparison at a finer phylogenetic scale (Figs. S2 and S3 in Supplementary online materials) showed internal lineages of *S. albipes*, *S. albocaudatus* and *S. zimai* sp. nov. generally similar to each other. In *S. sokolovi* it can be seen that populations with recently introgressed mtDNA of *S. albipes* are slightly more similar to that species in the length of head and body, skull size and hind foot length. The shift was opposite in tail length, however, and generally the two lineages of *S. sokolovi* sp. nov. were more similar to each other than to other species, which is especially obvious in skull shape (Fig. S3).

Taxonomic summary of the genus and the description of two new species

The combined genomic and morphometric analyses suggest the presence of six species in the genus *Stenocephalemys*, including two, which are formally described below. The dorsal and ventral views on the skin of all six species is shown at

Table 2. Classification of species to morphotypes. In each column (solution of cluster analysis) morphotypes are arbitrarily numbered and species with the same number belong to the same morphotype. If there were multiple solutions of comparable quality for the same trait, more (two) columns are shown and they are ordered according to difference in AICc or τ.

	Head + body		Tail		Hind foot		Ear	Rel. tail length	Skull centroid size		Skull shape	
ΔAICc/Δτ	0.00	0.22	0.00	0.73	0.00	0.96	0.00	0.00	0.00	0.74	0.0000	0.0071
S. albipes	1	1	1	1	1	1	1	1	1	1	1	1
S. albocaudatus	2	2	2	2	2	2	2	2	2	2	2	2
S. griseicauda	1	1	2	2	1	3	1	2	1	2	3	3
S. ruppi	1	1	1	2	1	3	2	1	1	1	4	4
S. sokolovi sp. nov.	1	1	2	2	1	3	1	2	1	1	3	5
S. zimai sp. nov.	2	1	2	2	1	3	2	2	1	1	3	3

Fig. 6, the morphology of skulls at Fig. 7, and their geographic distribution is summarized at Fig. 1.

Stenocephalemys zimai Lavrenchenko & Bryja, sp. nov.
Stenocephalemys griseicauda, part. (Yalden et al. 1976)
Stenocephalemys griseicauda (Müller 1977)
Stenocephalemys sp. A (Lavrenchenko & Verheyen 2006, Bryja et al. 2018, 2019a, Meheretu et al. 2019, Craig et al. 2020, Kostin et al. 2020, Lövy et al. 2020)

Holotype: **ZMMU S-178783**; adult female; skull and dry skin; collected by L.A. Lavrenchenko (14 May 2005); collecting number 1413.

Type locality: The vicinity of the Chennek campsite of the Semien Mountains National Park (13°15′ N 38°13′ E, 3800 m a.s.l.), Ethiopia.

Paratypes: **ZMMU S-178785** (adult male; skull and dry skin; collector's number 1419); **ZMMU S-178789** (adult female; skull and dry skin; collector's number 1428); two specimens from the type locality collected by L.A. Lavrenchenko on 13 and 14 May 2005. **ZMMU S-172748** (adult female; skull and dry skin; collector's number 1107); collected by L.A. Lavrenchenko (5 December 2001) in the Guna Mountain (11°43′ N; 38°15′ E; 3800 m a.s.l.). **ZMMU S-202822** (adult male; skull; collector's number 3232); **ZMMU S-202846** (adult male; skull and dry skin; collector's number 3258); **ZMMU S-202855 (**adult male; skull and dry skin; collector's number 3267); **ZMMU S-202856** (adult male; skull; collector's number 3268); all four specimens collected by L.A. Lavrenchenko in the Choke Mountain (10°42′17.4″ N; 37°50′40.5″ E; 3961 m a.s.l.) between 24 and 30 March 2018. **NMP-96963** (adult male; skull and dry skin; collector's number ETH1110); **NMP-96964** (adult female; skull and dry skin; collector's number ETH1198); two specimens collected on Abohoy Gara Mountain (12°04′19.2″ N; 39°22′08.04″ E; 3783 m a.s.l.; and 12°05′02.76″ N; 39°21′42.12″ E; 3533 m a.s.l., respectively) by Y. Meheretu, V. Mazoch and T. Aghová between 10 and 13 November 2014. **NMP-96965** (adult female; skull and dry skin; collector's number ETH0630); **NMP-96966** (adult female; skull and dry skin; collector's number ETH0636); two specimens collected on Choke Mountain (10°38′15″ N; 37°50′06″ E; 3780 m a.s.l.) by V. Mazoch, A. Ribas and K. Welegerima (12 November 2012).

Etymology: Patronymic. We selected the specific epithet to recognize Professor Jan Zima (14 August, 1952 - 26 March, 2019). Jan Zima was an outstanding Czech zoologist, a specialist on the diversity of small mammals, one of the founders of karyological research of Palaearctic mammals, and an enthusiastic supporter of the application of molecular genetic methods in zoological and evolutionary research. He served as director of the Institute of Vertebrate Biology of the Czech Academy of Sciences (CAS), as member of the Academic Council of CAS and was long-term Editor-in-Chief of Folia Zoologica (= now Journal of Vertebrate Biology). His surname "Zima" means "cold" in the Czech language, which also acknowledges that the species lives at the highest elevations in Ethiopia where researchers typically encounter cold weather.

Diagnosis: A typical medium-sized representative of the *Stenocephalemys* genus, similar in skull size and shape to *S. griseicauda* but on average it is larger (151.4 *vs.* 144.0 mm), with longer ears (24.2 *vs.* 22.8

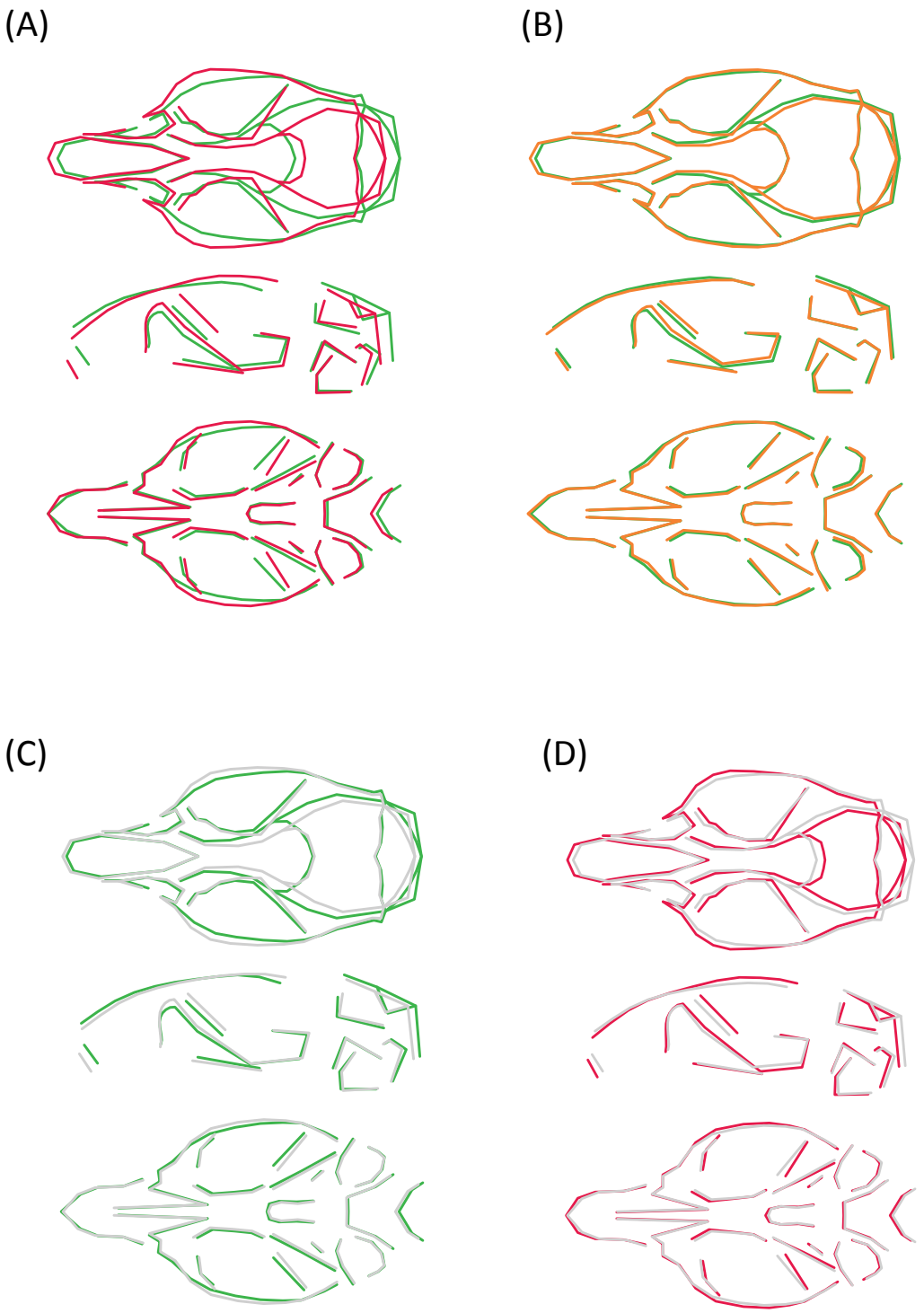

Fig. 5. Wireframe plots showing differences between mean skull shapes of four morphotypes, identified by the best solution of the clustering analysis. Wireframe colours match those in the scatter plot at Fig. 4C, i.e. green for *S. albipes*, orange for *S. ruppi*, red for *S. albocaudatus* and grey for the "*griseicauda*-like" group clustering remaining species, *S. griseicauda*, *S. sokolovi* sp. nov. and *S. zimai* sp. nov.

mm; Table 1). Karyotype is distinctive (2n = 50, NFa = 52 or 56; Bulatova & Lavrenchenko 2005). It can also be easily diagnosed by DNA sequences, at both mitochondrial (as sister to *S. albocaudatus*) and nuclear (as sister to *S. albipes*) markers (Fig. 2). It is the only species of the genus living in Afroalpine habitats in its distribution range.

Description: S. zimai sp. nov. is a medium-sized representative of the *Stenocephalemys* genus. The dorsal pelage is variable, ranging from brownish-grey to reddish-grey. The bristles are black, the guard hairs are grey at the base and pale or yellowish in the distal half. Ventral pelage is variable, ranging from whitish-grey to yellowish-

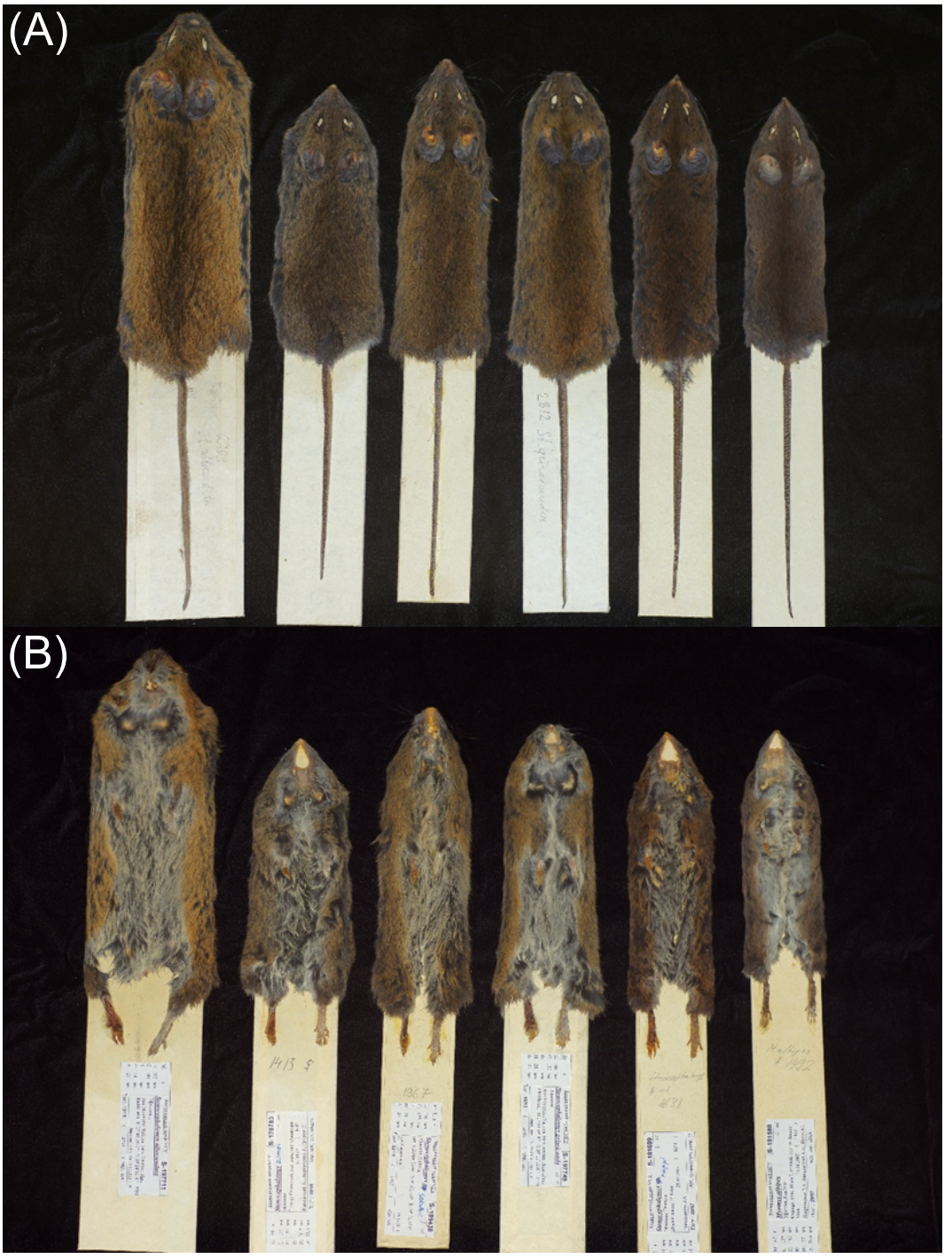

Fig. 6. Dorsal (A) and ventral (B) view of *Stenocephalemys* skins. From left to right: *S. albocaudatus* ZMMU S-197711, *S. zimai* sp. nov. ZMMU S-178783 (holotype), *S. sokolovi* sp. nov. ZMMU S-189438 (holotype), *S. griseicauda* ZMMU S-197749, *S. ruppi* ZMMU S-181699, *S. albipes* ZMMU S-181588.

greyish, the individual hairs grey at the base and tipped with white or pale-yellowish. The transition between dorsal and ventral colouration is conspicuous. Dorsal surfaces of the forefeet and hindfeet are whitish. The relatively large ears are blackish (including on the inner surface). The tail is relatively the shortest of all *Stenocephalemys* species (83.5% of HB; Table 1 and Fig. 3D) and appears moderately or distinctly bicoloured, the dorsal caudal hairs are grey or blackish in contrast to the white ventral hairs. The skull is similar in size and shape to *S. griseicauda* and *S. sokolovi* sp. nov. but with a more gracile occipital region. Two distinct karyomorphs with the same diploid number (2n = 50) were identified for *S. zimai* sp. nov. The karyotype of the specimens from the Semien Mts. (NFa = 52) differs from that of the specimens from Guna Mountain (NFa = 56) in the proportion of

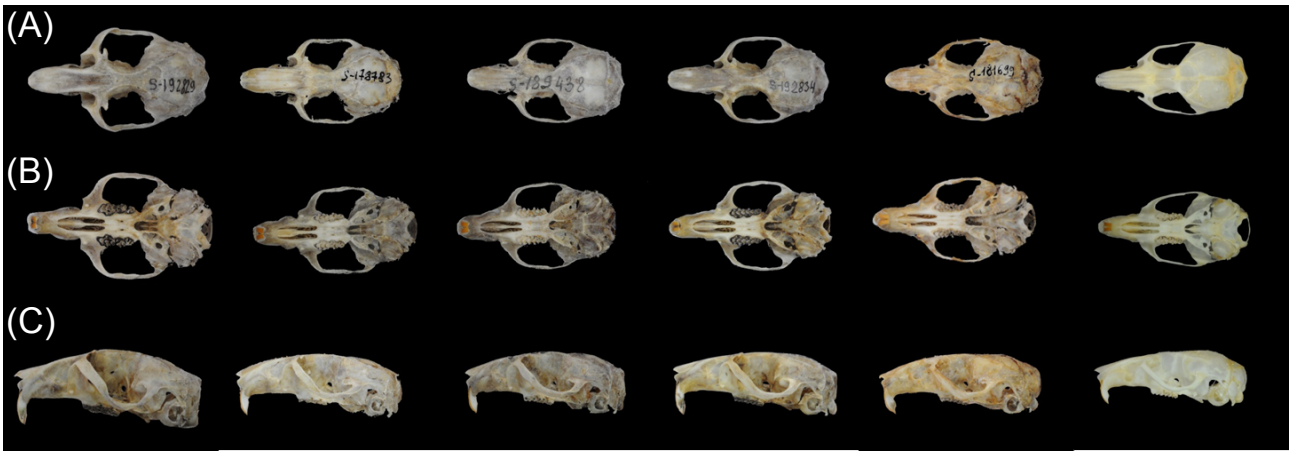

Fig. 7. Dorsal (A), ventral (B) and lateral (C) view of *Stenocephalemys* skulls. From left to right: *S. albocaudatus* ZMMU S-192829, *S. zimai* sp. nov. ZMMU S-178783 (holotype), *S. sokolovi* sp. nov. ZMMU S-189438 (holotype), *S. griseicauda* ZMMU S-192834, *S. ruppi* ZMMU S-181699, *S. albipes* IVB ETH0202.

acrocentric and metacentric chromosomes and the presence of a large acrocentric pair (Bulatova & Lavrenchenko 2005, Bryja et al. 2018). This karyotypic variation is also reflected in the presence of two phylogenetic subclades at both mtDNA and nDNA markers with allopatric distribution (Bryja et al. 2018). The X-chromosome is large submetacentric, the Y-chromosome is medium-sized metacentric.

Comparisons: (see Tables 1, 2 and Fig. 3). *Stenocephalemys zimai* sp. nov. differs from *S. albipes* and *S. ruppi* in its relatively shorter tail, larger skull size, relatively long and broad nasal bones and broad zygomatic arches.
Stenocephalemys zimai sp. nov. differs from *S. sokolovi* sp. nov. in a larger skull size, relatively long and broad nasal bones and robust occipital region.
Stenocephalemys zimai sp. nov. differs from *S. griseicauda* in relatively larger ears and more gracile occipital region.
Stenocephalemys zimai sp. nov. differs from *S. albocaudatus* in significantly smaller body size and skull, blackish or greyish colouration of dorsal surface of the tail.
Stenocephalemys zimai sp. nov. differs from any known *Stenocephalemys* species in its diploid chromosome number of 2n = 50, nuclear and mitochondrial DNA markers, and distributional range (with the exception of *S. albipes* as both species can rarely meet even at high elevation, but only around human settlements; see Craig et al. 2020 and Kostin et al. 2020).

Distribution: The species has been found in four mountain regions of northern Ethiopia: the Semien Mountains, Guna Mountain, Choke Mountain and Abohoy Gara Mountain (Fig. 1C). Because the species is adapted to high-elevation Afro-alpine habitats, it is likely that its distribution is currently highly discontinuous.

Ecology: All known specimens of *S. zimai* sp. nov. were collected in Afro-alpine habitats between 3600 and 4000 m a.s.l. The holotype and two paratypes were captured in moorland and grassland habitats, sparsely covered with *Lobelia rhynchopetalum*, *Festuca macrophylla*, *Helichrysum citrispinum* and *Alchemilla* sp. The new species occurs together with at least the following rodent species: *Dendromus lovati* (de Winton, 1900), *Otomys typus* (Heuglin, 1877), *Otomys simiensis* P.J. Taylor et al., 2011, *Arvicanthis abyssinicus* (Rüppel, 1842), *Mus imberbis* (Rüppel, 1842), *Lophuromys simensis* Osgood, 1836, and *Tachyoryctes splendens* (Rüppel, 1835). Around human settlements (e.g. the camp sites in the Semien Mountains and on Choke Mountain), the species can co-occur with its congener *S. albipes*, which is usually found at lower elevations (Craig et al. 2020, Kostin et al. 2020). A recent study by Craig et al. (2020) suggested that *S. zimai* sp. nov. used to live at lower elevation in the recent past (below 3000 m a.s.l., ca. 90 years ago), but its distribution has currently shifted to higher elevations probably as a result of ongoing climate change and a shift in the vegetation line.

Stenocephalemys sokolovi Lavrenchenko & Bryja, sp. nov.
Stenocephalemys griseicauda, part. (Yalden et al. 1976)
Stenocephalemys "pseudogriseicauda" (Bryja et al. 2018, 2019a)

Holotype: **ZMMU S-189438**; adult male; skull and dry skin; collected by L.A. Lavrenchenko (30 March 2011); collecting number 1967.

Type locality: The vicinity of the Debre Sina (09°49′37″ N; 39°44′07″ E; 3233 m a.s.l.), Ethiopia.

Paratypes: **ZMMU S-189426** (adult male; skull and dry skin; collector's number 1937); **ZMMU S-189431** (adult male; skull and dry skin; collector's number 1955); **ZMMU S-189435** (adult male; skull and dry skin; collector's number 1961); all three specimens from the type locality collected by L.A. Lavrenchenko between 29 and 31 March 2011. **NMP-96967** (adult female; skull and dry skin; collector's number ETH1889); **NMP-96968** (adult male; skull and dry skin; collector's number ETH1895); two specimens collected in Ankober, close to Kundi village (09°39′24.552″ N; 39°44′48.12″ E; 3194 m a.s.l.) by J. Bryja, D. Mizerovská, Y. Meheretu and M. Uhrová (21 November 2018). **NMP-96969** (adult male; skull and dry skin; collector's number ETH1822); **NMP-96970** (adult male; skull and dry skin; collector's number ETH1874); two specimens collected in Guwasa (10°17′37.86″ N; 39°48′01.224″ E; 3320 m a.s.l.) by J. Bryja, D. Mizerovská, Y. Meheretu and M. Uhrová (18-19 November 2018). **NMP-96971** (adult male; skull and dry skin; collector's number ETH1703); **NMP-96972** (adult female; skull and dry skin; collector's number ETH1704); two specimens collected in Borena Saynt NP (10°52′39.576″; 38°48′57.312″ E; 3514 m a.s.l.) by J. Bryja, D. Mizerovská, Y. Meheretu and M. Uhrová (11 November 2018).

Etymology: Patronymic. The new species is named in honour of the late Academician Vladimir E. Sokolov (1 February, 1928 - 19 April, 1998), the famous Russian zoologist. Vladimir Sokolov was for many years the director of the A. N. Severtsov Institute of Evolutionary Animal Morphology and Ecology (now Institute of Ecology and Evolution) of the Russian Academy of Sciences. He initiated the Russian-Ethiopian collaborative zoological studies in Ethiopia and founded the Joint Ethio-Russian Biological Expedition (JERBE).

Diagnosis: A medium-sized representative of the *Stenocephalemys* genus, similar in skull size and shape to *S. ruppi*, but with a relatively shorter tail (98.7% *vs.* 113.4% on average), broad and angular zygomatic arches (Fig. 5), and distinctive karyotype (2n = 52, NFa = 52). Our previous report of the karyotype formula of the species (Bryja et al. 2018) was erroneous because of the poor quality of chromosome slides (for details see Bulatova et al. 2020). The species can be also diagnosed by DNA sequences, where it forms distinctive clades at both nuclear (Bryja et al. 2018, Fig. 2A) and mitochondrial markers. At the latter, it is a sister group of *S. griseicauda* (Fig. 2B). The only exception is the population in Borena Saynt NP, where *S. sokolovi* sp. nov. possess recently introgressed mtDNA of *S. albipes* (Fig. 1B). The distribution of *S. sokolovi* sp. nov. is separated from that of morphologically similar species either by the Blue Nile valley and its tributaries (*S. zimai* sp. nov.) or by the Great Rift Valley (*S. griseicauda*) (Fig. 1C).

Description: S. sokolovi sp. nov. is a medium-sized representative of the *Stenocephalemys* genus. The dorsal pelage is brownish-greyish without a reddish shade (as in *S. albocaudatus* and partly *S. zimai* sp. nov.). The bristles are black and the guard hairs are greyish at the base and yellowish in the distal half. The ventral pelage is yellowish-greyish and the individual hairs grey at the base and tipped with pale-yellow. The transition between dorsal and ventral colouration is generally conspicuous. Dorsal surfaces of the forefeet and hindfeet are whitish. The ears are blackish (including inner surface). The tail is moderately short (mean = 98.7% of HB) and appears distinctly bicoloured, the dorsal caudal hairs are blackish in contrast to the white ventral hairs. The skull is similar in size and shape to *S. ruppi*, but with broader and angular zygomatic arches. The chromosomal set of *S. sokolovi* sp. nov. (2n = 52, NFa = 52) comprises 24 pairs of gradually decreasing acrocentric and one pair of small metacentric autosomes. The X-chromosome is large submetacentric, the Y-chromosome is medium-sized submetacentric (Bulatova et al. 2020).

Comparisons: Stenocephalemys sokolovi sp. nov. differs from *S. albipes* and *S. ruppi* in relatively shorter tail and broad zygomatic arches.
Stenocephalemys sokolovi sp. nov. differs from *S. griseicauda* and *S. zimai* sp. nov. in relatively short nasal bones and more gracile occipital region, distribution (Fig. 1C) and nuclear DNA markers (Fig. 2A).
Stenocephalemys sokolovi sp. nov. differs from *S. albocaudatus* in significantly smaller skull and body size, blackish colouration of dorsal surface of tail.
Stenocephalemys sokolovi sp. nov. differs from any known *Stenocephalemys* species in its diploid chromosome number of 2n = 52.

Distribution: The species has been found in four regions: the vicinities of Debre Sina, Ankober area, Guwassa area and Borena Saynt NP (Fig. 1C) in altitudes between 3200 and 3500 m a.s.l.

Ecology: The type series (holotype and three paratypes) of *S. sokolovi* sp. nov. was collected in a grassland habitat (3233 m a.s.l.) dominated by *Festuca macrophylla*. The following rodent species were trapped in the type locality and the geographically proximate Ankober area: *Dendromus lovati* (de Winton, 1900), *Dendromus* sp., *Otomys typus* (Heuglin, 1877), *Otomys* cf. *simiensis* P.J. Taylor et al., 2011, *Arvicanthis* cf. *blicki* Frick, 1914, *Lophuromys flavopunctatus* Thomas, 1888, and *Tachyoryctes splendens* (Rüppel, 1835). In Guwassa, the species lives in a mosaic of Afro-alpine habitats (*Helichrysum*, *Alchemilla*) with Erica shrubs, in sympatry/micro-allopatry with *S. albocaudatus*. Our preliminary data suggest that both species differ in their preferred microhabitats, with *S. sokolovi* sp. nov. living in more closed *Erica* growths. In Borena Saynt NP the species lives in the highest Afro-alpine habitats as well as in the *Erica* belt in lower elevation and is accompanied by *Dendromus* sp., *Otomys typus* (Heuglin, 1877), *Otomys simiensis* P.J. Taylor et al., 2011, *Arvicanthis abyssinicus* (Rüppel, 1842), and *Lophuromys simensis* Osgood, 1836. The population density can be high (e.g. captured individuals in ca. 40% of set traps in Ankober) and it can be an important agricultural pest (we observed damaged crops, close to *Erica* shrubs, in Ankober).

Stenocephalemys albipes (Rüppell, 1842)
Mus albipes Rüppell, 1842
Mus leucopus Fitzinger, 1867 – Eritrea, Massawa (error!)
Mus albipes var. *minor* Heuglin, 1877 – Eritrea, Modat valley, W of Massawa (error!)
Epimys rufidorsalis alettensis Frick, 1914 – Aletta, Ethiopia
Epimys rufidorsalis ankoberensis Frick, 1914 – Ankober, Ethiopia

Type locality: Massawa, Eritrea (error!).

Taxonomic notes: Yalden et al. (1976) stated that the original Rüppell´s specimens from Massawa area were re-examined by D. Kock and there is no doubt that they represent *S. albipes* (see also Fig. 4B). It is equally clear that all records of this species in the Massawa area (including the names *leucopus* Fitzinger and *minor* Heuglin) are based upon Rüppell´s collection and all these materials appear to have been incorrectly labelled. Massawa, Moncullo and the Modat valley (all currently in Eritrea) lie well below the altitudinal limits of this montane species. The type material was likely collected somewhere in the northern Ethiopian Highlands during Rüppell´s expedition in 1831-1834 (see Bekele & Yalden 2013). Musser & Carleton (2005) examined the holotype of *ankoberensis* and concluded that it is an example of *S. albipes.* Despite the name, the specimens lack white hindfeet (they have a dark mark on the dorsal surface in most cases). The external morphology is similar to hypothetical ancestral phenotype of Praomyini rodents, which is likely the reason why the species have been reported previously as *Praomys*, *Myomys* or *Myomyscus*, until the study based on cytogenetic and allozyme analyses was performed (Lavrenchenko et al. 1999; see also Musser & Carleton 2005).

Ecological note: This is one of the most widespread rodent species in the Ethiopian Highlands (Fig. 1A; Bryja et al. 2019a), living at altitudes between 800 and 3300 m a.s.l. It occurs in sympatry with *S. griseicauda* in the Arsi Mountains (Kostin et al. 2019), *S. ruppi* in the Chingawa Forest (our unpublished data) and *S. zimai* sp. nov. around the camp sites in the Semien Mountains and Choke Mountain (Craig et al. 2020, Kostin et al. 2020), but it can usually be readily recognized by its external morphology. The species is abundant, especially in the forests of southern Ethiopia, where it forms the predominant component of small mammal assemblages.

Stenocephalemys albocaudatus Frick, 1914
Stenocephalemys albocaudata Frick, 1914

Type locality: Chilalo Mountains, Inyala Camp ("Nyala Camp"?), Ethiopia.

Taxonomic notes: The name was changed to *Stenocephalemys albocaudatus* by Denys et al. (2017) for gender agreement.

Ecological note: This is the most specialized species of the genus, adapted to life in open Afro-alpine habitats. It has been considered as endemic to Bale and Arsi Mountains (Yalden et al. 1976, Bekele & Yalden 2013), but Bryja et al. (2018) recently discovered another population in the Guwassa area on the other side of the Great Rift Valley (Fig. 1B). The species lives there in sympatry with *S. sokolovi* sp. nov., but they probably differ in their preferred

microhabitats (see above). A similar situation was observed in the Arsi Mountains, where the mosaic of different habitats allows sympatric occurrence of *S. albocaudatus* and *S. griseicauda* (Kostin et al. 2019). On the other hand, in the Bale Mountains these two species occupy different parts of the elevational gradient (Yalden 1988, Lavrenchenko et al. 1997).

Stenocephalemys griseicauda F. Petter, 1972

Type locality: Bale Mountains, Dinsho, Ethiopia.

Taxonomic notes: All previous records reported under this species name from north-western part of the Ethiopian Highlands belong either to *S. zimai* sp. nov. or *S. sokolovi* sp. nov. (Fig. 1C)

Ecological notes: The species is known only from the Afromontane *Erica* belt in the Arsi and Bale Mountains, where it can live in sympatry or elevational parapatry with *S. albocaudatus* (in higher elevation) and *S. albipes* (in lower elevation) (Yalden 1988, Lavrenchenko et al. 1997, Kostin et al. 2019).

Stenocephalemys ruppi Van der Straeten & Dieterlen, 1983
Praomys ruppi Van der Straeten & Dieterlen, 1983

Type locality: Bonke, north of Bulta (Gamo Gofa region), 2800-3200 m a.s.l., Ethiopia.

Taxonomic notes: A short sequence of mitochondrial cyt*b* marker was obtained from the paratype of *S. ruppi* (SMNS23151) and it clearly clustered with the clade ap_2 from the Chingawa (= Inegawa) Forest in south-western Ethiopia (Bryja et al. 2018). Two genetically and morphologically distinct species co-occur in this forest; one is *S. albipes* and we assigned the name *S. ruppi* to the second, based on the mtDNA sequence of the paratype (see Bryja et al. 2018 for more details). This assignment was confirmed by morphometric analysis in the current study (see the position of the holotype and paratypes of *S. ruppi* in Fig. 4). The morphology of *S. ruppi* is intermediate between *S. albipes* and other mid-sized taxa, which is the reason why it was described as *Praomys*, and subsequently reported as *Myomys* or *Myomyscus* (see Musser & Carleton 2005 for more details).

Ecological notes: The Chingawa Forest is currently the only known locality of this species. The type locality, Bonke, was visited by L.A. Lavrenchenko in 2011. The local people still remember the expedition of Hans Rupp in 1974 (who collected the type series), but the habitat has been significantly altered since that time and the forest has disappeared. Therefore, it is highly probable that the species is extinct in this area. In the Chingawa Forest, *S. ruppi* and *S. albipes* were found in the same trapping line. With some experience, they can be recognized by the colouration pattern (pelage, tail, hind foot) and other external traits, e.g. shorter relative tail length and longer body length. We have not found any evidence of possible hybridization (Bryja et al. 2018), but more extensive genetic study is required. This forest is much more humid compared to other forests in south-western Ethiopia, which can be the reason why we have not found *S. ruppi* in any other forest, despite intensive trapping over the last decade (only *S. albipes*, often abundant, was found in other forests, e.g. Sheko, Godare, Charada).

Ethiopian rats as a model for evolutionary research

Ethiopian rats of the genus *Stenocephalemys* usually form the most abundant members of small mammal assemblages in the Ethiopian Highlands (see e.g. recent studies of Kostin et al. 2019, 2020, Craig et al. 2020). They are also important agricultural pests (Meheretu & Leirs 2019, Welegerima et al. 2020) and reservoirs of potentially harmful hantaviruses (Meheretu et al. 2012). Here we showed that they are also highly variable at their nuclear and mitochondrial DNA (and the topologies of their phylogenetic trees are not congruent), as well as in external and cranial morphology. Different species often occupy different parts of environmental elevational gradients, from closed canopy montane forest to completely open Afro-alpine grasslands.

These properties potentially make the Ethiopian rats a valuable model taxon for evolutionary research. We see at least two major fields where future studies could contribute to our understanding of fundamental evolutionary questions. First, the different species occupy different elevational zones along a gradient and this system is replicated (with different species) on both sides of the Great Rift Valley and even on different banks of large rivers. Therefore, they can be used for the assessment of the role of elevation (tree cover, partial concentration of oxygen, etc.) on the evolution of morphological (skull properties, relative tail length, body size, etc.) and physiological traits (metabolic rates, haemoglobin,

etc.; an example is a study of haematocrit levels affected by elevation by Lövy et al. 2020 in this issue). Even without performing a formal analysis, the topology of the species tree suggests that the ancestral phenotype (especially morphology of skull) is maintained in the group of three to four morphologically relatively similar species, shown in the middle part of bgPC1 (Fig. 4C). It appears that strong selection in specific environments subsequently drove the evolution of a "forest" phenotype (*S. albipes* and partly *S. ruppi*) and "Afro-alpine" phenotype (*S. albocaudatus*), a hypothesis that warrants further testing. What exactly the terms "forest" and "Afro-alpine" phenotype mean in a functional sense can thus be assessed e.g. by detailed morphometric analyses in 3D.

Second, mtDNA phylogeny is incongruent with the nuclear species tree (Fig. 2) suggesting past, and in one case of very recent, interspecific introgression. Because species living at similar altitudes have similar mtDNA (Fig. 2B), one can hypothesize that past introgressions were adaptive, at least to some extent. The approaches of modelling the structure of proteins encoded by mtDNA can bring a more detailed understanding of the function of mitochondrial proteins in conditions with different ambient temperature and relative oxygen pressure (see an example in Kostin & Lavrenchenko 2018). Further, the comparison (e.g. using ecophysiological approaches) of populations of *S. sokolovi* sp. nov. with introgressed (in Borena Saynt NP) and their "own" (at other localities) mtDNA can illuminate the adaptive value of mtDNA introgression, and might also be instrumental in testing the prediction of the recently defined mito-nuclear compatibility species concept (Hill 2017).

Conclusion

In this study we employed a combination of genomic and morphometric approaches to review the taxonomy and evolutionary diversity of the endemic Ethiopian rats (genus *Stenocephalemys*). We delimited species boundaries and formally described two new species. Using the most comprehensive available material, we characterized the distribution of all six species of the genus. We showed that if two species occur in sympatry or elevational parapatry, they are well distinguished by a combination of morphological and genetic traits as well as (micro-)habitat preferences (with the exception of *S. albipes* and *S. ruppi*, where both species seem to occupy exactly the same habitat). Mitogenomic and nuclear species trees showed different topologies; the most parsimonious explanation being so-called "reticulate evolution", i.e. the repeated hybridization of not yet completely reproductively isolated taxa, followed by the mitochondrial introgression. The variability of mito-nuclear combinations, together with other properties (high abundance, amenability to captivity, etc.), makes the genus *Stenocephalemys* a prospective model for evolutionary studies in general, and for testing the predictions of the mito-nuclear compatibility species concept in particular.

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Supplementary online material

Table S1. List of specimens used for genomic and morphological analyses (https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-2-2020-Mizerovska-et-al.-Table-S1.xlsx).

Fig. S1. Set of landmarks used for the analysis of skull geometric morphometry.
Fig. S2. Violin plots of external body measurements and skull centroid size within and between 10 phylogenetic lineages of *Stenocephalemys*.
Fig. S3. Ordination of skull shapes of ten phylogenetic lineages of *Stenocephalemys* in the shape space of the first two between-group principal components.

(https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-2-2020-Mizerovska-et-al.-Figs.-S1-S2-S3.pptx)

Data S1. Alignments of 388 nuclear loci obtained by the anchored phylogenomic approach in the Nexus format (https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-2-2020-Mizerovska-et-al.-SupplementaryMaterial_DataS1-1.txt).

Data S2. Phylogenetic trees from MrBayes (388 gene trees) used as input for ASTRAL analysis in the Nexus format (https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-2-2020-Mizerovska-et-al.-SupplementaryMaterial_DataS2.txt).

NOMENCLATURAL ACTS REGISTRATION ✷

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