**Skull morphological evolution in Malagasy endemic Nesomyinae rodents**

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**Abstract**

Madagascar is a large island to the south-east of Africa and in many ways con-tinental in size and ecological complexity. Here we aim to define how skull morpholo-gy of an endemic and monophyletic clade of rodents sub-family Nesomyinae, that show considerable morphological variation, have evolved and how their disparity is characterized in context of the geographical and ecological complexity of the island. We performed a two-dimensional geometric morphometric analysis on 371 dorsal and 400 ventral skull images of 19 species (comprising all nine extant nesomyine genera) and tested the influence of three ecological parameters (climate, locomotor habitat, and nychthemeral cycle) in a phylogenetic context on size and conformations. The results indicate that skull conformations appear to significantly reflect phylogeny, whereas skull size does not carry a significant phylogenetic signal. Skull shape is significantly influenced by climate, while skull size is not impacted by any of the ecolog-ical factors tested, which is converse to expectations in an insular context. In conclusion, Nesomyinae must have evolved under unusual types of local constraints preventing this radiation from

demonstrating strong ecological release.

**Description of the protocole**

Sampling

We used a data set of Nesomyinae skull photographs taken with a macro-photographic CANON EOS including 371 dorsal and 400 ventral images. The images were collected in a standardized way to prevent any bias due to the effect of parallax: in dorsal view the frontal part of the skull was horizontally oriented (parallel to the photographic plane), and in ventral view molar rows were oriented as to be parallel to the photographic plane. Juveniles (defined as having portions of the skull being unossified) and older individuals (with heavily worn teeth) are not included in our sample. To minimize any potential bias due to sexual dimorphism we have included for each species as many specimens as possible and of both sexes; although, we add that this subfamily is not known to show sexual dimorphism. Several species are known by only one or few individuals, such as *Brachytarsomys villosa*. The specimens come from different Institutions and are housed in the Field Museum of Natural History (FMNH), Chicago; The Natural History Museum (formerly British Museum of Natural History [BMNH]), London; the Mention Zoologie et Biodiversité Animale (formerly Département de Biologie Animale), Université d’Antananarivo (UADBA), Antananarivo, Madagascar; the Museum für Naturkunde (ZMB), Berlin; and the Muséum national d’Histoire naturelle (MNHN), Paris.

 Morphometric analyses

To capture skull shape variation, we used a 2-dimensional landmark-based approach. In dorsal view, 27 anatomical landmarks were chosen, as well as 42 in ventral view (**Figure 1**). Landmarks were selected to correspond as closely as possible to anatomical homologies. They have been digitized using the software tpsDig2 .

Ventral and dorsal data sets were analyzed separately. First, we performed a Generalized Procrustes Analysis (GPA). This procedure was realized using the *gpagen* function of the *geomorph* library under the free software R (R Core Team 2016).

We only examined the symmetric component of shape. Asymmetric component was explored using MorphoJ and explained 4.7% of shape variability in the ventral cranium and 5.8% in the dorsal cranium. These aspects were removed using *bilat.symmetry* from the *geomorph* library. Further statistical testing was performed under the free software R.

In order to reduce data dimensionality, principal component analyses (PCA) were performed on conformations. This step was carried out using the *gm.prcomp* function of the *geomorph* library [24]. To reduce the number of variables, we retained 95% of shape variation, the latest principal components being usually considered as negligible because they explain little of the global shape variation. Further analyses have been carried out on principal components instead of Procrustes coordinates.

Sexual dimorphism in ventral and dorsal view were tested when sufficient information was available (12 species: *Eliurus carletoni*, *Eliurus majori*, *Eliurus grandidieri*, *Eliurus tanala*, *Eliurus minor*, *Eliurus myoxinus*, *Eliurus webbi*, *Gymnuromys roberti*, *Brachyuromys betsileoensis*, *Nesomys rufus*, *Monticolomys koopmani* and *Macrotarsomys bastardi*). On shape we performed a Procrustes ANOVA with the function *procD*.*lm* from the package *geomorph* using the formula: shape ~ sex + species + sex:species. For size we used the *lm* function from the *stats* package using the formula: size ~ sex + species + sex : species. In both cases interactions between sex and species shape/size were examined to assess the presence of sexual dimorphism.

Allometry at the interspecific level was investigated with *procD*.*lm* from *geomorph* using the formula: shape ~ size + species + size:species. The log centroid size was used as an estimator of size. Interactions between species and size were examined to assess homogeneity of allometric slopes between species. This aspect was explored on all species with the exception of two with insufficient samples (*Eliurus antsingy* and *Brachytarsomys villosa*).

All subsequent analyses have been performed on species means, that include all the specimens of a given taxa. For each data set comparative analyses were carried out on 1) conformations, which correspond to the principal components computed on the symmetric component of Procrustes coordinates, and 2) centroid size, which is also obtained from the Procrustes superimposition method and is defined as the square root of the sum of square distance of each landmark from the centroid of the object.

Phylogenetic signal

As a basis for phylogenetic analyses we used the phylogeny of muroid rodents of Steppan et al. 2017 (Plos One), which is based on 900 muroid species. The tree was pruned to keep only species of interest using the function *keep.tip* of the library *ape*.

To quantify phylogenetic signal on size we used the K-statistic method for univariate traits. To quantify it on conformations, we used the same method extended to multivariate data by Adams (2014: Syst.Biol.). This approach compares observed traits variations to their expected variations under Brownian motion. If K-value = 1 the considered trait evolved according to Brownian motion. If tested groups resemble each other more than expected, i.e. strong phylogenetic signal, K-value >> 1. On the contrary, K-value close to 0 indicates no phylogenetic signal. This signal has been computed with *physignal* from *geomorph*.

To visualize to what extent conformations reflect phylogeny, we performed PCA on mean shape per species and projected phylogeny on it. This step was performed using *phylomorphospace* from the *phytools* library [32]. Method for ancestral states reconstruction, morphometric branch lengths estimation and phylomorphospace reconstruction are described in Sidlauskas (2008 in Evolution). Visualization of shape variation along axes were obtained using *plotRefToTarget* from *geomorph* and are deformations in comparison to the global mean shape.

Influence of ecological factors

We tested the three best informed and relevant ecological parameters whose influence on mammalian skull morphology has been well documented: climate, locomotor habitat, and nychthemeral cycle. Based on recognized ecological characteristics of Nesomyinae, we assigned categories to characterize the three parameters: locomotor habitat (“terrestrial”, “arboreal”, and “semi-arboreal”), nychthemeral cycle (“nocturnal”, “twilight”, and “arrhythmic”) and climate (“tropical wet” and “hot and dry”) . Specimens have been assigned to climatic areas based on their collection locality.

 To quantify the influence of ecological factors on size, we performed ANOVA (F test), analyses of variance, which aims to determine whether qualitative factors (ecological factors) have significant effects on one quantitative variable (size). F is the ratio between inter- and intra-group variability. Thus, the more the average sizes of two groups are different, the higher the F statistic. Regarding conformations we used MANOVA analyses (Wilks test). MANOVA, multivariate analysis of variances, is the extension of the ANOVA to multivariate data. It computes the λ of Wilks, which measures the part of intra-class inertia in total inertia. λ is comprised between 0 and 1, a value close to 0 indicating a good discrimination between the groups. When morphological descriptors were found to carry significant phylogenetic signal we used phylogenetics MANOVA (MANOVAphy), which takes phylogeny into account for p-value estimation. We used *manova.gls* from *MvMORPH*  to test for the generalized least square linear model using the penalized likelihood method.Four evolutionary models were tested and compared with the Generalized Information Criterion (GIC): Brownian Motion (BM), Ornstein-Uhlenbeck (OU), Early Burst (EB) and Pagel’s lambda transformation (L), all using *GIC* from *MvMORPH*. When no significant phylogenetic signal was found in morphological descriptors, the influence of ecological factors was determined using the function *aov* of the *Stats* library. For each case tested, ecological factors and their interaction with the log centroid size were employed using the formula: shape ~ size + ecology + size:ecology.When tests were significant, shape variation related to factors were investigated and we computed mean shape per category of each factor using *mshape*  from *geomorph*.