Morphological diversity of tenrec (Afrosoricida, Tenrecidae) skulls compared to their closest relatives, the golden moles (Afrosoricida, Chrysochloridae)

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Abstract

Morphologically diverse groups have long attracted the interest of biologists. Many studies now recognise the importance of quantifying patterns of morphological diversity to gain new insights into evolutionary patterns. Tenrecs (Afrosoricida, Tenrecidae) are a family of small mammals which is often cited as an example of an exceptionally morphologically diverse group. However, this assumption has not been tested. Here we use geometric morphometric analyses of skull shape to test whether tenrecs are more morphologically diverse than their closest relatives, the golden moles (Afrosoricida, Chrysochloridae). Contrary to our expectations, we find that tenrec skulls are only more morphologically diverse than golden moles when measured in lateral view. Furthermore, the similarities among the species-rich *Microgale* tenrec Genus appear to mask higher morphological diversity in the rest of the Family. Our results reveal new insights into the morphological diversity of tenrecs and highlight the importance of using quantitative methods to test qualitative assumptions about patterns of morphological diversity.

Introduction

Morphological diversity has long attracted the attention of biologists. There are many famous examples of exceptional morphological diversity including in the beaks of Darwin's finches, the body and limbs of Caribbean *Anolis* lizards, and the pharyngeal jaws of cichlid fish (Gavrilets & Losos, 2009). Morphological diversity is important because it has implications for studies of adaptive radiations - where close relatives exhibit a range of divergent morphologies - (Losos, 2010), convergent evolution - where distant relatives exhibit similar morphologies - (e.g. Muschick et al., 2012; Harmon et al., 2005) and our understanding of biodiversity (Roy & Foote, 1997). However, apart from a few examples (e.g. Goswami et al., 2011; Ruta et al., 2013; Brusatte et al., 2008), it is still common to study morphological diversity from a qualitative rather than quantitative perspective.

Morphological diversity is rarely studied from a quantitative perspective because it is difficult to quantify. Studies are inevitably constrained to measure the diversity of specific traits rather than overall morphologies (Roy & Foote, 1997). Different traits (such as cranial compared to limb morphologies) may yield different patterns of morphological diversity (Foth et al., 2012). Furthermore, linear measurements of morphological traits can restrict our understanding of overall morphological variation (Rohlf & Marcus, 1993) . Some of these problems can be solved by using geometric morphometric approaches (Rohlf & Marcus, 1993; Adams et al., 2013) that provide more detailed insights into morphological variation. Yet few studies have used these techniques to specifically address questions about morphological diversity

in mammals.

Tenrecs (Afrosoricida: Tenrecidae) are a morphologically diverse mammalian group (Soarimalala & Goodman, 2011; Olson & Goodman, 2003). The Family contains 34 species, 31 of which are endemic to Madagascar (Olson, 2013). Body sizes of tenrecs span three orders of magnitude (2.5 to $>$ 2,000g); a greater range than all other Families, and most Orders, of living mammals (Olson & Goodman, 2003). Within this vast size range there are tenrecs which convergently resemble shrews (*Microgale* tenrecs), moles (*Oryzorictes* tenrecs) and hedgehogs (*Echinops* and *Setifer* tenrecs) (Eisenberg & Gould, 1969) even though they are not closely related to these species (Stanhope et al., 1998). Despite these interesting features, the morphological diversity of tenrecs has never been properly quantified.

Here we present the first quantitative investigation of morphological diversity in tenrecs. We use geometric morphometric approaches to compare cranial morphological diversity in tenrecs to their sister taxa, the golden moles (Afrosoricida, Chrysochloridae). Tenrecs inhabit a wider variety of ecological niches than golden moles (Soarimalala & Goodman, 2011; Bronner, 1995) so we expect tenrecs to be more morphologically diverse. However, we only find a significant difference in the morphological diversity of skulls in lateral view, not dorsal or ventral. In contrast, when we restricted our data to include a subsample of the morphologically similar *Microgale* tenrecs, we found that tenrecs were more morphologically diverse than golden moles in all three analyses. Our results highlight the importance of using quantitative methods to test assumptions about patterns of morphological diversity.

Materials and Methods

Our methods for measuring cranial morphological diversity involved several steps of data collection, processing and analysis. For clarity, figure 1 summarises all of these steps which are described in detail below.

Morphological data collection

One of us (SF) photographed crania of tenrecs and golden moles at the Natural History Museum London (BMNH), the Smithsonian Institute Natural History Museum (SI), the American Museum of Natural History (AMNH), Harvard's Museum of Comparative Zoology (MCZ) and the Field Museum of Natural History, Chicago (FMNH). We photographed the specimens with a Canon EOS 650D camera fitted with an EF 100mm f/2.8 Macro USM lens using a standardised procedure to minimise potential error (see supplementary material for details).

We collected pictures of the skulls in dorsal, ventral and lateral views (right side of the skull). A full list of museum accession numbers and details on how to access the images can be found in the supplementary material.

In total we collected pictures from 182 skulls in dorsal view (148 tenrecs and 34 golden moles), 173 skulls in ventral view (141 tenrecs and 32 golden moles) and 171 skulls in lateral view (140 tenrecs and 31 golden moles) representing 31 species of tenrec (out of the total 34 in the family (Olson, 2013)) and 12 species of golden moles (out of a total of 21 in the family (Asher et al., 2010)). We used the taxonomy of Wilson and Reeder (2005) supplemented with more recent sources (Olson, 2013) to define our

species.

We used a combination of landmarks (type 2 and type 3, (Zelditch et al., 2012)) and semilandmarks to characterise the shapes of our specimens. Figure 2 shows our landmarks (points) and semilandmarks (outline curves) for the skulls in each of the three views. Corresponding definitions of each of the landmarks can be found in the supplementary material.

We used the TPS software series (Rohlf, 2009) to process and landmark the pictures (Fig. 1). We digitised all landmarks and semilandmarks in tpsDIG, version 2.17 (Rohlf, 2013). We re-sampled the outlines to the minimum number of evenly spaced semilandmark points required to represent each outline accurately (MacLeod, 2013, details in supplementary material). We used TPSUtil (Rohlf, 2012) to create "sliders" files that defined which points in our TPS files should be treated as semilandmarks (Zelditch et al., 2012). We conducted all subsequent analyses in R version 3.0.2 (R Core Team, 2014, Fig. 1).

We used the `gpagen` function in the `geomorph` package (Adams et al., 2013) to run a general Procrustes alignment (Rohlf & Marcus, 1993) of the landmark coordinates while sliding the semilandmarks by minimising Procrustes distance (Bookstein, 1997). We used these Procrustes-aligned coordinates of all species to calculate average shape values for each species (n = 43) which we then used for a principal components analysis (PCA) with the `plotTangentSpace` function (Adams et al., 2013).

Calculating morphological diversity

We calculated morphological diversity using the results of our principal components analyses. We selected the principal components (PC) axes which accounted for 95% of the cumulative variation for each of our three skull analyses. These axes represent the dimensions of our full cranial morphospace (Polly et al., 2013).

We used the scores from the PC axes to compare cranial morphologies in two ways (Fig. 1). First, we used non parametric MANOVAs (Anderson, 2001) to test whether tenrecs and golden moles occupied significantly different positions within our full cranial morphospaces (e.g Serb et al., 2011; Ruta et al., 2013). We then compared morphological diversity within tenrecs to the diversity within golden moles, defining morphological diversity as the mean Euclidean distance between each species and its Family centroid. If tenrecs are more morphologically diverse than golden moles, then they should be more dispersed within our full cranial morphospaces.

Our groups have unequal sample sizes (31 tenrec species compared to 12 golden mole species). Morphological diversity is usually decoupled from taxonomic diversity (e.g. Ruta et al., 2013; Hopkins, 2013) so larger groups are not necessarily more morphologically diverse. However, comparing morphological diversity in tenrecs to the diversity of a smaller Family could still bias our results. To account for this, we used pairwise permutation tests as follows.

We assigned each species to either "tenrecs" or "golden moles" at random and then calculated the difference in morphological diversity for the new groupings as described above. We repeated this procedure 1000

times to generate a null distribution of the expected differences in morphological diversity between a group with 31 members ("tenrecs") compared to one with 12 members ("golden moles"). If there is no difference between the morphological diversity of tenrecs and golden moles, then the group identity ("tenrec" or "golden mole") of each species is arbitrary,

Finally, we compared our observed measures of the differences in morphological diversity between the two Families to our null distributions to determine whether there were significant differences after taking sample size into account.

The majority of tenrec species (19 out of 31 in our dataset) belong to the *Microgale* (shrew-like) Genus that has relatively low morphological diversity (Soarimalala & Goodman, 2011; Jenkins, 2003). This may mask signals of higher morphological diversity among other tenrecs. To test this, we created a subset of our tenrec data that included just five of the *Microgale* species, each representing one of the five sub-divisions of *Microgale* outlined by Soarimalala and Goodman (2011), i.e. small, small-medium, medium, large and long-tailed species. We compared the morphological diversity of this subset of tenrecs (n=19: five *Microgale* and 12 non *Microgale* species) to that of golden moles using the methods described above (Fig. 1).

Results

Figure 3 depicts the morphospace plot derived from our principal components analysis of average Procrustes-superimposed shape

coordinates for skulls in lateral view. Similar plots for our analyses of skulls in dorsal and ventral views can be found in the supplementary material. To compare morphological diversity in the two families, we used the principal components axes which accounted for 95% of the cumulative variation in each of our skull analyses: dorsal (n=6 axes), ventral (n=7 axes) and lateral (n=7 axes).

First, we compared the position of each Family within the morphospace plots. Tenrecs and golden moles occupy significantly different positions in the dorsal (npMANOVA, $F_{1,42} = 68.13$, $R^2 = 0.62$, $p=0.001$), ventral (npMANOVA, $F_{1,42} = 103.33$, $R^2 = 0.72$, $p=0.001$) and lateral (npMANOVA, $F_{1,42} = 76.7$, $R^2=0.652$, $p=0.001$) skull morphospaces, indicating that the Families have very different, non-overlapping cranial morphologies.

Secondly, we compared the morphological diversity within each Family. Based on our measures of mean Euclidean distance to the Family's centroid, tenrec skulls are more morphologically diverse than golden mole skulls when they are measured in lateral view but not in dorsal or ventral view (table 1). In contrast, when we compared morphological diversity within the sub-sample of 19 tenrecs (including just five *Microgale* species) to the 12 golden mole species, we found that tenrecs had significantly higher morphological diversity than golden moles in all analyses (table 1).

Our pairwise permutation tests for each analysis confirmed that differences in morphological diversity were not artefacts of differences in sample size (see supplementary material).

Discussion

Tenrecs (Tenrecidae) are often cited as an example of a mammalian group with high morphological diversity (Olson, 2013; Soarimalala & Goodman, 2011; Eisenberg & Gould, 1969) and we expected them to be more morphologically diverse than their closest relatives the golden moles (Chrysochloridae). However, tenrecs were only more morphologically diverse than golden moles in one of our three skull analyses (lateral view; table1). Furthermore, the morphologically similar *Microgale* tenrecs seem to mask high morphological diversity in the rest of the tenrec Family; reducing our data to include a sub-sample of this *Microgale* species revealed that the remaining tenrecs were significantly more morphologically diverse than golden moles across all three skull analyses (table 1). These results highlight the importance of using quantitative methods to test qualitative assumptions about patterns of morphological diversity.

In our full analyses, tenrecs only had higher morphological diversity than golden moles when the skulls were measured in lateral view. This is most likely due to our choice of landmarks. The two outline curves in lateral view (Fig. 2) emphasise morphological variation in the back and top of the skulls, indicating that tenrecs are more morphologically diverse than golden moles in their three dimensional height. These lateral aspects of the skull morphology could not be included in the dorsal and ventral analyses. In contrast, our landmarks in the dorsal, and particularly ventral, views focus on morphological variation in the overall outline shape of the skull and palate (Fig. 2). The result that tenrecs are no more diverse than golden moles in these areas makes intuitive sense: most

tenrecs have broad, non-specialised diets (Olson, 2013) so there is no obvious functional reason why they should have significantly diverse palate morphologies. Therefore, comparing the morphologies in three separate views allowed us to identify the more morphologically variable skull regions.

Measures of morphological variation are sensitive to the sampling used. If a particular morphotype is over-represented then the similarities among those species will reduce the overall morphological variation within the group (Foote, 1991). This appears to be the case for our data: it is only when we included a sub-sample of *Microgale* tenrecs that we found higher morphological diversity in tenrecs compared to golden moles across all three skull analyses (table 1). These results indicate that the overall morphological diversity within tenrecs is not as large as is often assumed (e.g. Eisenberg & Gould, 1969; Olson, 2013) because the majority of the Family are members of a single, morphologically similar Genus.

Of course our results are based on a single morphological axis; the diversity of skull shape. It is difficult to quantify overall morphological diversity because any study is inevitably constrained by its choice of specific traits (Roy & Foote, 1997). Many other studies have also used skulls to study morphological variation within species (Blagojević & Milošević-Zlatanović, 2011; Bornholdt et al., 2008), to delineate species boundaries within a clade (e.g. Panchetti et al., 2008) or for cross-taxonomic comparative studies of morphological (dis)similarities (e.g. Ruta et al., 2013; Goswami et al., 2011; Wroe & Milne, 2007). However, variation in skull shape is only one aspect of overall morphology. Quantifying variation in other morphological traits could yield different patterns. Therefore future work should extend our

approach beyond just skulls to gain a more complete understanding of the overall morphological diversity of tenrecs and golden moles.

We have presented the first quantitative investigation of morphological diversity in tenrecs. We found that tenrec skulls are more morphologically diverse than their closest relatives the golden moles, but only in some aspects of their morphology. Furthermore, our results indicate that the similarities among the species rich *Microgale* tenrecs mask signals of higher morphological diversity among the rest of the Family. Of course our results are restricted to just one axis of morphological variation and further analysis of other traits is required. However, our results represent a significant step towards a more quantitative understanding of patterns of morphological diversity in tenrecs.

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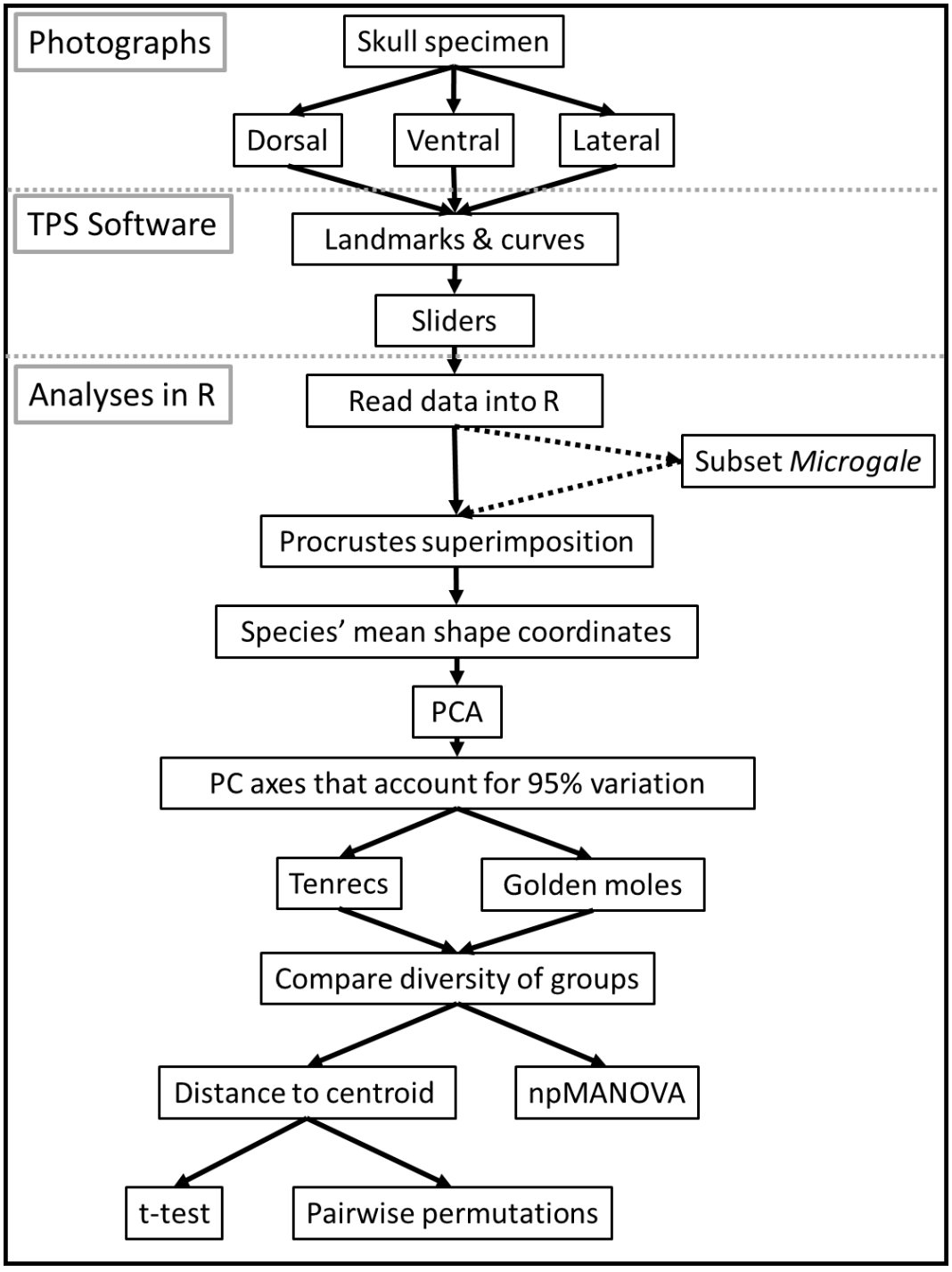

Figure 1: Summary of the main steps in our data collection, processing and analysis protocol. Note that skulls were photographed in three views and the analyses were repeated separately for each view. The dashed arrows refer to the analyses we repeated including only a subset of *Microgale* tenrecs.

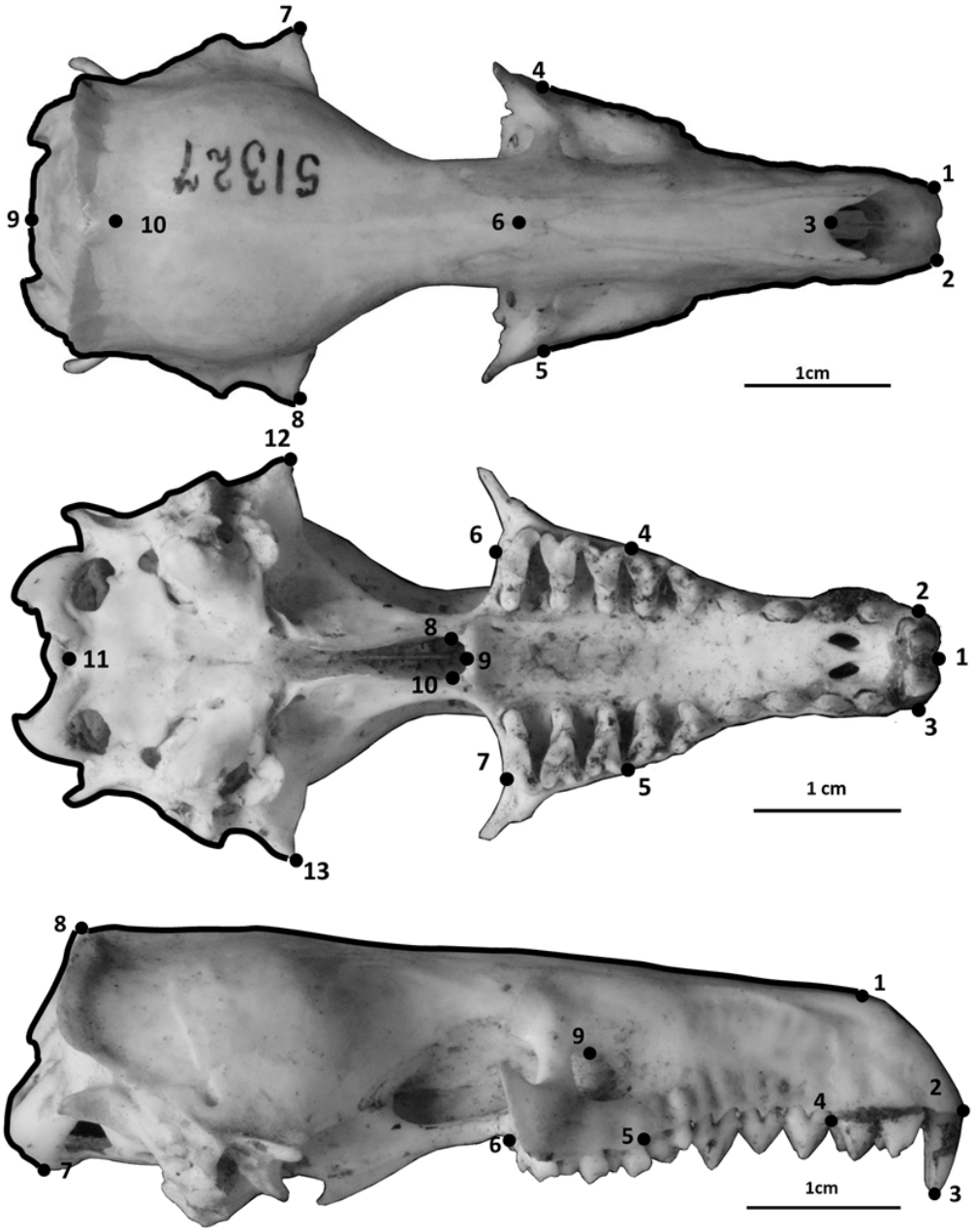

Figure 2: Landmarks (numbered points) and curves (black lines) used to capture the shape of skulls in dorsal, ventral and lateral views respectively. Curves were re-sampled to the same number of evenly-spaced points. See supplementary material for descriptions of the curves and landmarks. The specimens belong to two different *Potamogale velox* (Tenrecidae) skulls: accession number AMNH 51327 (dorsal) and BMNH 1934.6.16.2 (ventral and lateral)

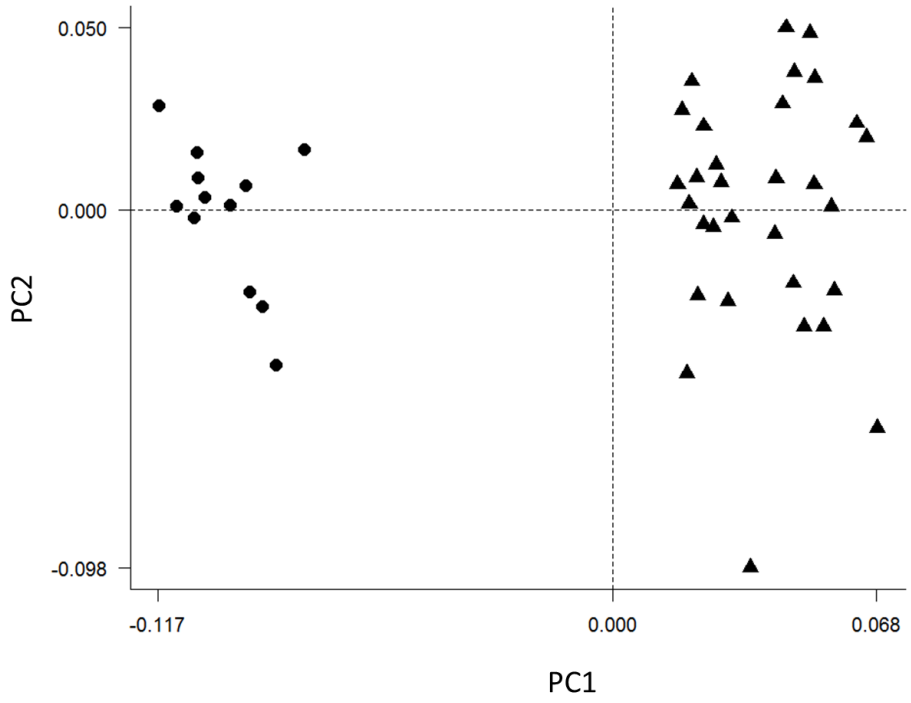

Figure 3: Principal components plot of the morphospace occupied by tenrecs (triangles, n = 31 species) and golden moles (circles, n = 12) for the skulls in lateral view. Each point represents the average skull shape of an individual species. Axes are PC1 and PC2 of the average scores from a PCA analysis of mean Procrustes shape coordinates for each species.

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Table 1:
Morphological diversity in tenrecs and golden moles for each of the three analyses (skulls in dorsal, ventral and lateral view). Results are shown for all 31 species of tenrec (left) and 19 species of tenrec (right) including just five *Microgale* species. Significant differences ($p < 0.05$) are highlighted in bold.

Tenrec species	Analysis	Tenrecs (mean± s.e)	Golden moles (mean± s.e)	t	p
31	Skulls dorsal	0.036 ± 0.0029	0.029 ± 0.0032	-1.63	0.11
	Skulls ventral	0.048 ±0.0034	0.044 ± 0.0041	-0.676	0.51
	Skulls lateral	0.044 ± 0.0041	0.032 ± 0.0037	-2.16	**0.04**
	Mandibles	0.049 ± 0.0044	0.067 ± 0.0054	2.62	**0.014**
17	Skulls dorsal	0.044 ± 0.0025	0.029 ±0.003	-3.62	**0.001**
	Skulls ventral	0.054 ± 0.004	0.042 ± 0.004	-2.23	**0.04**
	Skulls lateral	0.054 ± 0.005	0.031 ± 0.0037	-3.47	**0.002**
	Mandibles	0.055 ± 0.0049	0.062 ± 0.005	1.003	0.325