Running head: CRANIAL MORPHOLOGICAL DIVERSITY IN TENRECS

Morphological diversity of tenrec (Afrosoricida, Tenrecidae) crania is greater than their closest relatives, the golden moles (Afrosoricida, Chrysochloridae)

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Abstract

Introduction

Morphological diversity has long attracted the attention of biologists. There are many famous examples of morphological diversity including beak morphologies in Darwin's finches, body and limb morphologies in Caribbean *Anolis* lizards and pharyngeal jaw diversity in cichlid fish (Gavrilets & Losos, 2009). Apart from a few examples (REFS), it is common to study morphological diversity from a qualitative rather than quantitative perspective (REFS). However, it is important to quantify morphological diversity because it has implications for studies of adaptive radiations (Losos, 2010), convergent evolution (REF) and our understanding of biodiversity (Roy & Foote, 1997).

Tenrecs are an example of a morphologically diverse 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) which is 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). However, morphological diversity in tenrecs has not been quantified.

Morphological diversity is difficult to quantify. Studies are inevitably constrained to measure the diversity of specific traits rather than overall morphologies (Roy & Foote, 1997). Different trait axes (such as cranial compared to limb morphologies) may yield different patterns of morphological diversity (REF) Furthermore, linear measurements of morphological traits can restrict our understanding of overall morphological variation (REF). However, geometric morphometric approaches (Rohlf & Marcus, 1993; Adams et al., 2013) provide more detailed insights into morphological variation.

Here we present the first quantitative investigation of morphological diversity in tenrecs. We use geometric morphometrics to compare cranial morphological diversity in tenrecs to their sister taxa, the golden moles (Afrosoricida, Chrysochloridae). Tenrecs inhabit a wider variety of ecological niches (Soarimalala & Goodman, 2011) than golden moles (Bronner, 1995) so we expected tenrecs to be more morphologically diverse than their closest relatives. However, we find no significant difference in the diversity of cranial morphologies between the two groups. It is only when we restricted our data to include a subsample of the morphologically similar *Microgale* tenrec Genus that we found tenrecs to be more morphologically diverse than golden moles. Our results demonstrate the importance of using quantitative methods to assess otherwise subjective estimates of morphological diversity. We show that the apparently high morphological diversity in tenrecs is not necessarily reflected in all morphological traits.

Materials and Methods

Morphological data collection

One of us (SF) photographed cranial specimens 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) 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 identify our specimens.

We used a combination of both landmarks (type 2 and type 3, (Zelditch et al., 2012)) and semilandmarks to characterise the shapes of our specimens. Figure 1 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 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 (Zelditch et al., 2012) that defined which points in our tps files should be treated as semilandmarks. We conducted all subsequent analyses in R version 3.0.2 (R Core Team, 2014) within the geomorph package (Adams et al., 2013). We used the gpagen function 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

Results

Morphological disparity in tenrecs and golden moles

Figure 2 depicts the morphospace plots derived from our principal components analyses of average Procrustes-superimposed shape coordinates for each species in our skull and mandible data respectively. We used the principal components axes which accounted for 95% of the cumulative variation (number of axes: n = 7 (dorsal), n = 8 (ventral), n = 8 (lateral) and n = 12 (mandibles)) to calculate the disparity of each Family.

Tenrecs and golden moles clearly have very different cranial and mandible morphologies: in each analysis, the families occupy significantly different areas of morphospace (npMANOVA, table 3). In our analyses of the three different views of the skulls, there is an overall trend for tenrecs to have higher disparity than golden moles. However, none of these differences are statistically significant (table 1).

There is a less clear pattern from our analysis of disparity in mandibles. Two of our four metrics indicate that golden moles have significantly higher disparity in the shape of their mandibles than tenrecs (table 1) although one metric (sum of ranges) gives the opposite result.

The three curves at the back of the mandibles (figure **??**) place a particular emphasis on shape variation in the posterior of the bone; the ramus, coronoid, condylar and angular processes. Therefore, higher disparity in golden mole mandibles compared to tenrecs could be driven by greater morphological variation in these structures. To test this idea, we repeated our morphometric analyses of the mandibles with a reduced dataset of points; just the seven landmark points and one single curve at the base of the jaw between landmarks 1 and 7 (figure **??**). When we compared disparity with this reduced data set we found that golden moles no longer had significantly higher disparity than tenrecs (table 1).

Morphological disparity in non-*Microgale* tenrecs and golden moles

We repeated our disparity comparisons with a subset of the tenrec specimens to remove the large and phenotypically similar *Microgale* tenrec Genus. In this case we found that tenrecs have significantly higher disparity than golden moles when the skulls are analysed in lateral view (table 2). However, none of the other comparisons in any of the analyses were significant.

Discussion

Our analyses are the first quantitative investigation of morphological disparity in tenrecs. We show that tenrecs' cranial morphologies are no more diverse than their closest relatives and therefore phenotypic variety in tenrecs is perhaps not as exceptional as it first appears.

When we compared the diversity of skull shapes in the two Families, we found a trend towards higher disparity in tenrecs compared to golden moles but none of these differences were significant (table 1). Even when we removed the phenotypically similar *Microgale* Genus, tenrecs were still no more diverse than golden moles in most of the analyses of their skull shapes (table 2).

In contrast to these results for the skulls, two of our disparity metrics indicate that golden moles have more disparate mandible shapes than tenrecs (table 1). We recognised that our landmarks and curves for the mandibles focus particular attention on the ascending ramus (condyloid, condylar and angular processes, figure **??**). Therefore we deleted the three semilandmark curves around these structures and repeated our disparity calculations. In this case we found no significant differences in disparity between the two Families (table 1). Therefore, our results seem to indicate that golden moles have greater morphological variation in the posterior structures of their mandibles compared to tenrecs.

Given that these posterior structures act as muscle attachment and articulation sites for connections with the upper jaw, one might expect that golden moles with highly disparate posterior mandible morphologies should also show high variability in the corresponding mandible articulation areas of the skull. However, we could not locate reliable, homologous points accurately on those areas of the skull pictures in lateral view. Instead, our landmarks and semilandmark curves for the skulls in lateral view focus attention on morphological variation in the dentition and the overall shape of the top and back of the skulls (figure **??**). This may explain why golden mole skulls in lateral view do not show the same pattern of higher disparity compared to tenrecs that we see in our analyses of the mandibles. However, further investigation is required to identify possible reasons why golden moles appear to show such variation in the posterior structures of their mandibles.

We used variation in skull and mandible shapes as proxy measures for overall morphological diversity within the two Families. Many other studies also use skulls to study phenotypic 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 phenotypic (dis)similarities (e.g. Ruta et al., 2013; Goswami et al., 2011; Wroe & Milne, 2007).

However, studies of morphological disparity are inevitably constrained to measure diversity within specific traits rather than overall phenotypes (Roy & Foote, 1997). Disparity calculations based on skull shape can yield similar results compared to analyses of whole-skeleton discrete characters and limb proportion data sets (Foth et al., 2012). Yet it is still possible that comparing disparity in tenrecs and golden moles using non-cranial morphological measures could produce different results. For example, tenrecs inhabit a wide variety of ecological niches and habitats including terrestrial, arboreal, semi-aquatic and semi-fossorial environments (Soarimalala & Goodman, 2011). In contrast, although golden moles occupy a wide altitudinal, climatic and vegetational spectrum of habitats (Bronner, 1995), they are are all fossorial species which, superficially at least, appear to be less functionally diverse than tenrecs. Therefore, comparing the disparity of limb morphologies within the two Families could indicate that tenrecs are more morphologically diverse than golden moles and therefore support the claim that tenrecs are an exceptionally diverse group.

Our analyses are the first measures of morphological diversity within tenrecs, a group which is commonly cited as an example of an adaptive radiation (Olson, 2013). Evidence of exceptional morphological diversity is one criterion for designating a clade as an adaptive radiation (Losos & Mahler, 2010). However, we found that tenrecs are no more morphologically diverse than their their closest relatives and therefore, within our tests, do not appear to show the exceptional diversity which characterises an adaptively radiated group.

The evolution of cranial shape (both upper skull and mandible), particularly dental morphology, has obvious correlations with dietary specialisations and occupation of specific ecological niches (e.g. Wroe & Milne, 2007). Considering the wide ecological diversity of the tenrec Family; semi-fossorial, arboreal, terrestrial and semi-aquatic (Soarimalala & Goodman, 2011), we think that it is reasonable to expect that this variety should be reflected in skull morphology. However, we have not included any measures of the 'adaptiveness' of cranial shape in our analyses and therefore our analyses should not be considered to be an explicit test of whether or not tenrecs are an adaptive radiation (Losos & Mahler, 2010). Instead we have made the first step towards understanding the apparent phenotypic diversity within tenrecs within a quantitative framework. Future work should focus on explicit measures of the 'adaptiveness' and functional importance of tenrec cranial and post-cranial morphologies to understand the significance of morphological diversity within the Family (e.g. Mahler et al., 2010). However, we also recognise that strict, statistically based categorisations of clades as being adaptive radiations or not are not always biologically meaningful or helpful when it comes to trying to understand patterns of phenotypic diversity (Olson & Arroyo-Santos, 2009).

We have presented the first quantitative study which tests the common claim that tenrecs are an exceptionally diverse group (Olson, 2013; Soarimalala & Goodman, 2011; Eisenberg & Gould, 1969). Focusing on cranial diversity is only one aspect of morphological variation and further analyses are required to test whether other morphological traits yield similar patterns. However, our results provide a clear indication that phenotypic variety within tenrecs is perhaps not as exceptional as it first seems.

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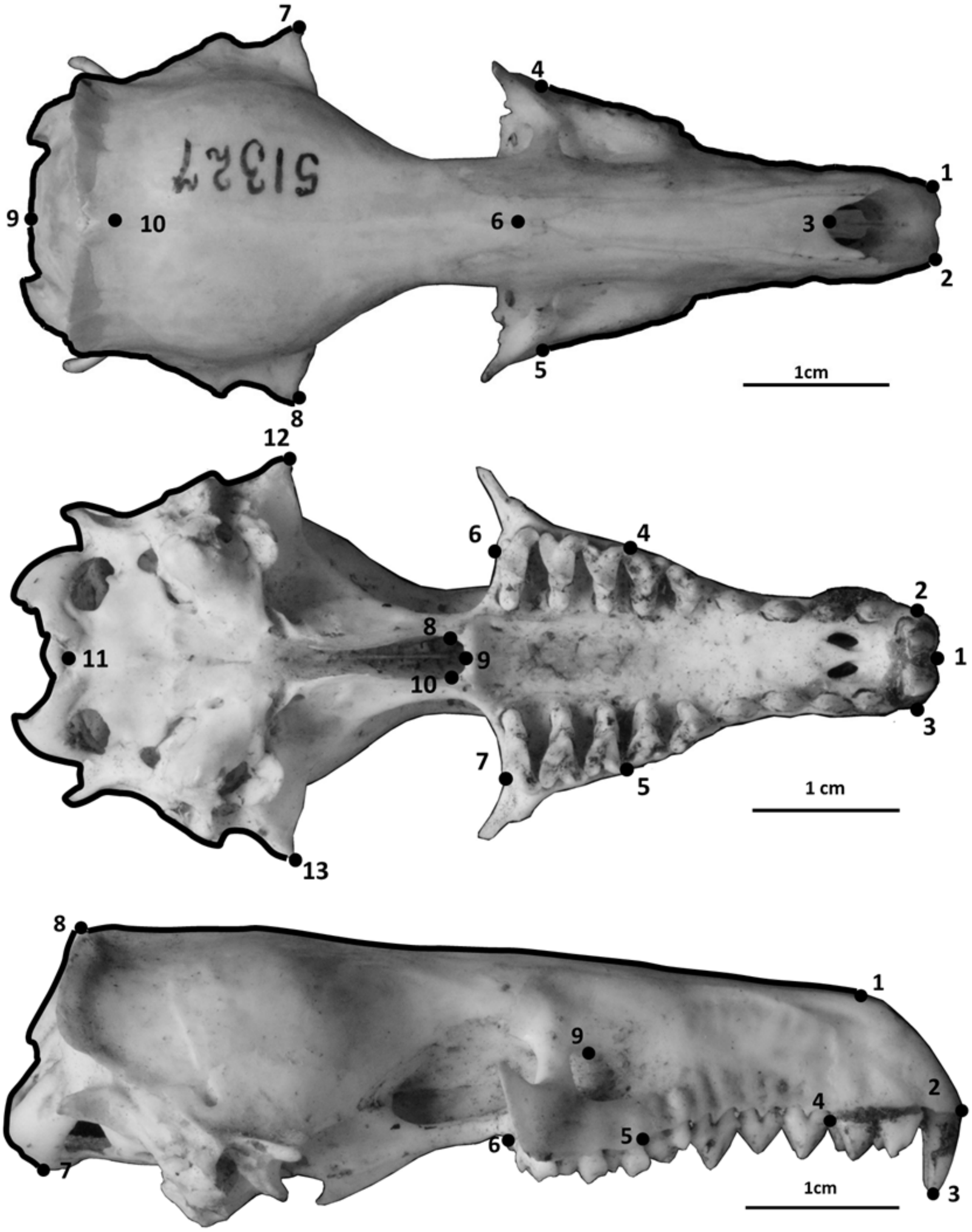

Figure 1: Landmarks (numbered points) and curves (black lines) used to capture the morphological shape of skulls in dorsal, ventral and lateral views respectively. Curves were re-sampled to the same number of evenly-spaced points. See Supplementary Material for descriptions of the curves and landmarks. The specimens belong to two different *Potamogale velox* (Tenrecidae) skulls: accession number AMNH 51327 (dorsal) and BMNH 1934.6.16.2 (ventral and lateral)

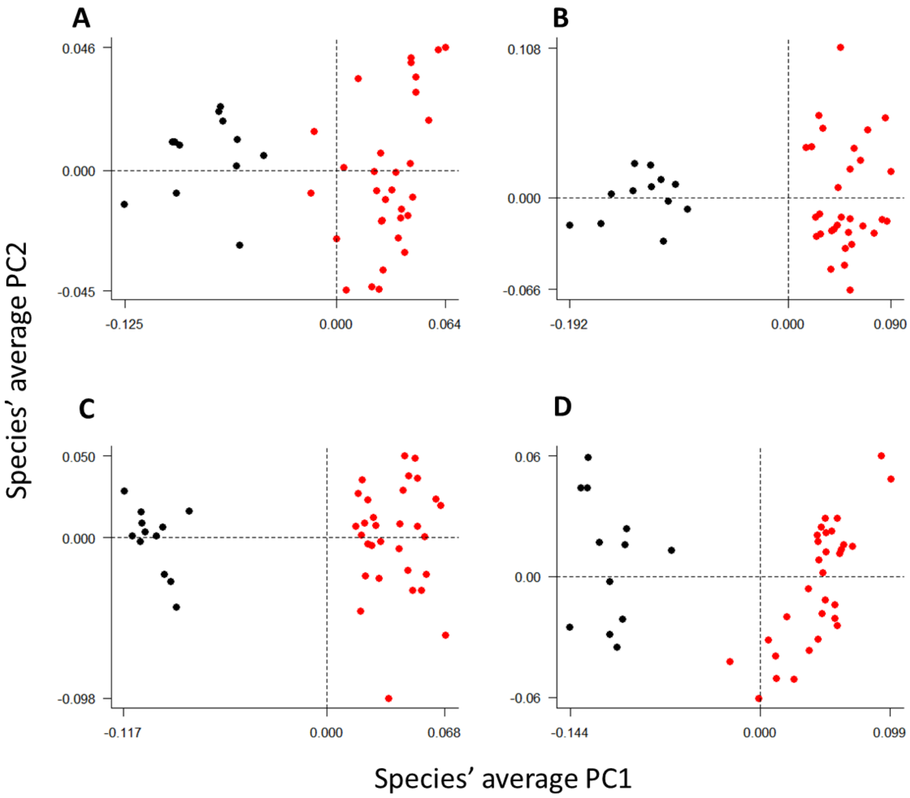

Figure 2: Principal components plots of the morphospaces occupied by tenrecs (red, n = 31 species) and golden moles (black, n = 12) for the skulls: dorsal (A), ventral (B), lateral (C) and mandibles (D) analyses. Axes are PC1 and PC2 of the average scores from a PCA analysis of mean Procrustes shape coordinates for each species.

List of Tables

Table 1: Disparity comparisons between tenrecs (T) and golden moles (G) for each of our data sets(rows) and four disparity metrics (columns). 'Mandibles:one curve' refers to our shape analysis of mandibles excluding the three curves around the posterior structures of the jaw (figure **??**). Significant differences are highlighted in bold with the corresponding p value in brackets. Disparity metrics are: sum of variance, product of variance, sum of ranges and product of ranges

Disparity metric	**SumVar**	**ProdVar**	**SumRange**	**ProdRange**
Skulls dorsal	T>G	T>G	T>G	T>G
Skulls lateral	T>G	T>G	T>G	T>G
Skulls ventral	T>G	G>T	T>G	T>G
Mandibles	G>T	**G>T* (0.008)**	**T>G* (0.025)**	**G>T* (0.009)**
Mandibles:one curve	G>T	G>T	T>G	T>G

Table 2: Disparity comparisons between non-*Microgale* tenrecs (T) and golden moles (G) for each of our data sets(rows) and four disparity metrics (columns). Significant differences are highlighted in bold with the corresponding p value in brackets. Disparity metrics are; sum of variance, product of variance, sum of ranges and product of ranges.

Disparity metric	**SumVar**	**ProdVar**	**SumRange**	**ProdRange**
Skulls dorsal	T>G	T>G	T>G	T>G
Skulls lateral	**T>G* (0.014)**	T>G	**T>G* (0.001)**	**T>G*(0.003)**
Skulls ventral	T>G	T>G	T>G	T>G
Mandibles	T>G	G>T	T>G	G>T

Table 3: npMANOVA comparisons of morphospace occupation for tenrecs and golden moles in each of the four analyses (three views of skulls and mandibles). In each case the two families occupy significantly different areas of morphospace.

Analysis	**F**	$\mathbf{R^2}$	**p value**
Skulls dorsal	66.02	0.62	0.001
Skulls ventral	100.74	0.71	0.001
Skulls lateral	75.07	0.65	0.001
Mandibles	59.34	0.59	0.001