

1 **Running head:** CRANIAL MORPHOLOGICAL DIVERSITY IN
2 TENRECS

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10 diversity, tenrecs

11 Introduction

12 Analysing patterns of morphological diversity has important implications
13 for our understanding of ecological and evolutionary traits. For example,
14 from a functional ecology perspective, morphological characteristics of
15 limbs inform us about locomotory style (e.g. Bou et al., 1987) and the
16 trophic niches associated with particular dental morphologies affect
17 speciation and diversification rates through time (Price et al., 2012).
18 Morphological diversity is also an important aspect of evolutionary
19 patterns such as adaptive radiations and convergent evolution. High
20 morphological diversity is a unifying (Losos and Mahler, 2010; Olson and
21 Arroyo-Santos, 2009), although not defining (Glor, 2010; Olson and
22 Arroyo-Santos, 2009), characteristic of adaptive radiations. Furthermore,
23 analysing morphological convergences in groups such as freshwater
24 cichlid fish (Muschick et al., 2012) and anole lizards (Mahler et al., 2013)
25 gives interesting insights into the relative repeatability of evolution (Losos,
26 2011).

27 Although studies of morphological diversity have clear implications
28 for our understanding of ecological and evolutionary patterns, apart from
29 a few examples (e.g. Ruta et al., 2013; Goswami et al., 2011; Brusatte et al.,
30 2008), it is still common to study morphological diversity from a
31 qualitative rather than quantitative perspective. However, we need to
32 quantify the morphological similarities and differences among species to
33 gain a better understanding of their ecological interactions and
34 evolutionary history. Unfortunately, morphological diversity is difficult to
35 quantify. Studies are inevitably constrained to measure the diversity of
36 specific traits rather than overall morphologies (Roy and Foote, 1997). In

37 addition, our perception of morphological diversity is influenced by the
38 trait being used. One study of pterosaurs demonstrated that comparing
39 the diversity of different morphological traits using varying methods
40 produced similar results (Foth et al., 2012). However, it remains unclear
41 whether this finding can be applied to all vertebrate groups: in some
42 species, comparing the relative diversity of cranial and limb morphologies
43 may yield different results (Foth et al., 2012). Furthermore, linear
44 measurements of morphological traits can restrict our understanding of
45 overall morphological variation. A distance matrix of measurements
46 between specific points is unlikely to give a completely accurate
47 representation of a three dimensional structure (Rohlf and Marcus, 1993).

48 These are important limitations to consider but geometric
49 morphometric approaches help to overcome some of the issues associated
50 with traditional morphological studies (Adams et al., 2004).

51 Morphometric studies based on caliper measurements of particular
52 features can only describe a limited set of distances, ratios and angles
53 which often fail to capture the overall shape of a specific structure (Slice,
54 2007). Geometric morphometrics circumvents these issues by using a
55 system of Cartesian landmark coordinates to define anatomical points.
56 This method captures more of the true, overall anatomical shape of
57 particular structures (Mitteroecker and Gunz, 2009). These more detailed
58 approaches are useful tools for studying patterns of morphological
59 diversity.

60 Here we apply geometric morphometric techniques to quantify
61 morphological diversity in a Family of small mammals, the tenrecs.
62 Tenrecs (Afrosoricida, Tenrecidae) are a morphologically diverse group
63 that is commonly cited as an example of both convergent evolution and an

64 adaptive radiation (Soarimalala and Goodman, 2011; Eisenberg and
65 Gould, 1969). The Family is comprised of 34 species, 31 of which are
66 endemic to Madagascar (Olson, 2013). Body masses of tenrecs span three
67 orders of magnitude (2.5 to $\geq 2,000$ g); a greater range than all other
68 Families, and most Orders, of living mammals (Olson and Goodman,
69 2003). Within this vast size range there are tenrecs which convergently
70 resemble shrews (*Microgale* tenrecs), moles (*Oryzorictes* tenrecs) and
71 hedgehogs (*Echinops* and *Setifer* tenrecs, Eisenberg and Gould, 1969). Their
72 similarities include examples of morphological, behavioural and
73 ecological convergence (Soarimalala and Goodman, 2011). Tenrecs are one
74 of only four endemic mammalian clades in Madagascar and the small
75 mammal species they resemble are absent from the island (Garbutt, 1999).
76 Therefore, it appears that tenrecs represent an adaptive radiation of
77 species which filled otherwise vacant ecological niches (Soarimalala and
78 Goodman, 2011). The similarities among tenrecs and other small
79 mammals are even more remarkable when you consider their
80 phylogenetic history. Tenrecs were originally classified within the general
81 "Insectivora" clade and only molecular studies revealed their true
82 phylogenetic affinities within the Afrotherian mammals (Stanhope et al.,
83 1998). Therefore, despite initial appearances, tenrecs are more closely
84 related to elephants, manatees and armadillos than they are to shrews,
85 moles or hedgehogs.

86 Although tenrecs are often cited as an example of both an adaptive
87 radiation and exceptional convergent evolution, these claims have not
88 been investigated quantitatively. There are qualitative similarities among
89 the hind limb morphologies of tenrecs and several other unrelated species
90 with similar locomotory styles (Salton and Sargis, 2009) but the degree of

91 morphological similarity has not been established. Morphological
92 diversity is an important feature of adaptive radiations (Losos and
93 Mahler, 2010) and it also informs our understanding of convergent
94 phenotypes (Muschick et al., 2012). Therefore, it is important to quantify
95 patterns of morphological diversity in tenrecs to gain an insight into their
96 evolution. My thesis is the first study to address this issue.

97 We present the first quantitative study of patterns of morphological
98 diversity in tenrecs. We use geometric morphometric techniques (Rohlf
99 and Marcus, 1993) to compare cranial morphological diversity in tenrecs
100 to that of their closest relatives, the golden moles (Afrosoricida,
101 Chrysochloridae). We expect tenrecs to be more morphologically diverse
102 than golden moles because tenrecs occupy a wider variety of ecological
103 niches. The tenrec Family includes terrestrial, semi-fossorial, semi-aquatic
104 and semi-arboreal species (Soarimalala and Goodman, 2011). In contrast,
105 all golden moles occupy very similar, fossorial ecological niches (Bronner,
106 1995). Greater ecological variety is often (though not always) correlated
107 with higher morphological diversity (Losos and Mahler, 2010).

108 **Materials and Methods**

109 The methods we used involved several steps of data collection, geometric
110 morphometrics analyses and comparisons of morphological diversity. For
111 clarity, Figure 1 summarises all of these steps and we describe them in
112 detail below.

Data collection

One of us (SF) used the collections of five museums: the Natural History Museum, London (BMNH), the Smithsonian Institute Natural History Museum, Washington D.C. (SI), the American Museum of Natural History, New York (AMNH), the Museum of Comparative Zoology, Cambridge M.A. (MCZ) and the Field Museum of Natural History, Chicago (FMNH). We recorded species names as they were written on museum specimen labels and then corrected them to match the taxonomy in Wilson and Reeder's Mammal Species of the World (2005). For recently identified species, which are not included in Wilson and Reeder (2005), we used the taxonomy recorded on the specimen labels. Wilson and Reeder (2005) record 30 species of tenrec but more recent studies indicate that there are now 34 species (Olson, 2013). The additional species belong to the shrew tenrec (*Microgale*) Genus and represent either recognition of cryptic species boundaries (Olson et al., 2004) or discovery of new species (Goodman et al., 2006; Olson and Arroyo-Santos, 2009). Only one of these four recent additions, *M. jobihelyi*, was present in the museum collections and therefore we could not include the three other newly recognised species in the analyses. We photographed all of the tenrec and golden mole skulls available in the collections. This included 31 of the 34 species in the tenrec Family and 12 of the 21 species of golden moles (Wilson and Reeder, 2005).

We took pictures of the skulls using photographic copy stands consisting of a camera attachment with an adjustable height bar, a flat stage on which to place the specimen and an adjustable light source. To take possible light variability into account, on each day we took a

139 photograph of a white sheet of paper and used the custom white balance
140 function on the camera to set the image as the baseline "white"
141 measurement for those particular light conditions.

142 We photographed the specimens with a Canon EOS 650D camera fitted
143 with a EF 100 mm f/2.8 Macro USM lens. We used a remote control
144 (Hähnel Combi TF) to take the photos to avoid shaking the camera and
145 distorting the images. We photographed the specimens on a black
146 material background with a light source in the top left-hand corner of the
147 photograph. We used small bean bags as necessary to hold the specimens
148 in position while being photographed to ensure that they lay in a flat
149 plane relative to the camera and did not tilt in any direction. We used the
150 grid-line function on the live-view display screen of the camera to position
151 the specimens in the centre of each image.

152 We photographed the skulls in three views: dorsal (top of the
153 cranium), ventral (underside of the skull with the palate roof facing
154 upwards) and lateral (right side of the skull). When the right sides of the
155 skulls were damaged or incomplete we photographed the left sides and
156 later reflected the images so that they could be compared to pictures of
157 the right sides (e.g. Barrow and Macleod, 2008).

158 We converted the raw files to binary (grey scale) images and re-saved
159 them as TIFF files

Geometric morphometric analyses

Landmark placement on images

We used a combination of landmark and semilandmark analysis approaches to assess the shape variability in skull. We used the TPS software suite (Rohlf, 2013) to digitise landmarks and curves on the photos. We set the scale on each image individually to standardise for the different camera heights that I used when photographing my specimens. We created separate data files for each of the three morphometric analyses (skulls in dorsal, ventral and lateral views). One of us (SF) digitised landmarks and semilandmark points on every image individually. Some specimens were too damaged to use in particular views so there were a different total number of images for each analysis: skulls dorsal (356), ventral (346) and lateral (336).

When using semilandmark approaches there is a potential problem of over - sampling: simpler structures will require fewer semilandmarks to accurately represent their shape (MacLeod, 2012). To ensure that we applied a uniform standard of shape representation to each outline segment (i.e. that simple structures would not be over-represented and more complex features would not be under-represented), we followed the method outlined by MacLeod (2012). For each data set we chose a random selection of photos of specimens which represented the breadth of the morphological data (i.e. specimens from each sub-group of species). We drew the appropriate curves on each specimen and over-sampled the number of points on the curves. We measured the length of the line and regarded that as the 100%, true length of that outline. We then re-sampled the curves with decreasing numbers of points and measured the length of

186 the outlines. We calculated the length of each re-sampled curve as a
187 percentage of the total length of the curve and then found the average
188 percentage length for that reduced number of semilandmark points across
189 all of the specimens in my test file. We continued this process until I
190 found the minimum number of points that gave a curve length which was
191 at least 95% accurate. We repeated these curve-sampling tests for each
192 analysis to determine the minimum number of semilandmark points
193 which would give accurate representations of morphological shape.

194 Figure (REF) depicts that landmarks and curves which we used for
195 each of the sets of photographs. For landmarks which are defined by
196 dental structures, we used published dental sources (Repenning, 1967;
197 Eisenberg and Gould, 1969; Nowak, 1983; MacPhee, 1987; Knox Jones and
198 Manning, 1992; Davis and Schmidly, 1997; Quérrouil et al., 2001; Nagorsen,
199 2002; Wilson and Reeder, 2005; Goodman et al., 2006; Karataş et al., 2007;
200 Hoffmann and Lunde, 2008; Asher and Lehmann, 2008; Muldoon et al.,
201 2009; Lin and Motokawa, 2010) where available to identify the number
202 and type of teeth in each species. Detailed descriptions of the landmarks
203 can be found in the supplementary material.

204 Results and Discussion

205 Conclusions

206 Acknowledgements

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325 **List of Figures**

326 1 Flowchart diagram of data collection and analysis 17

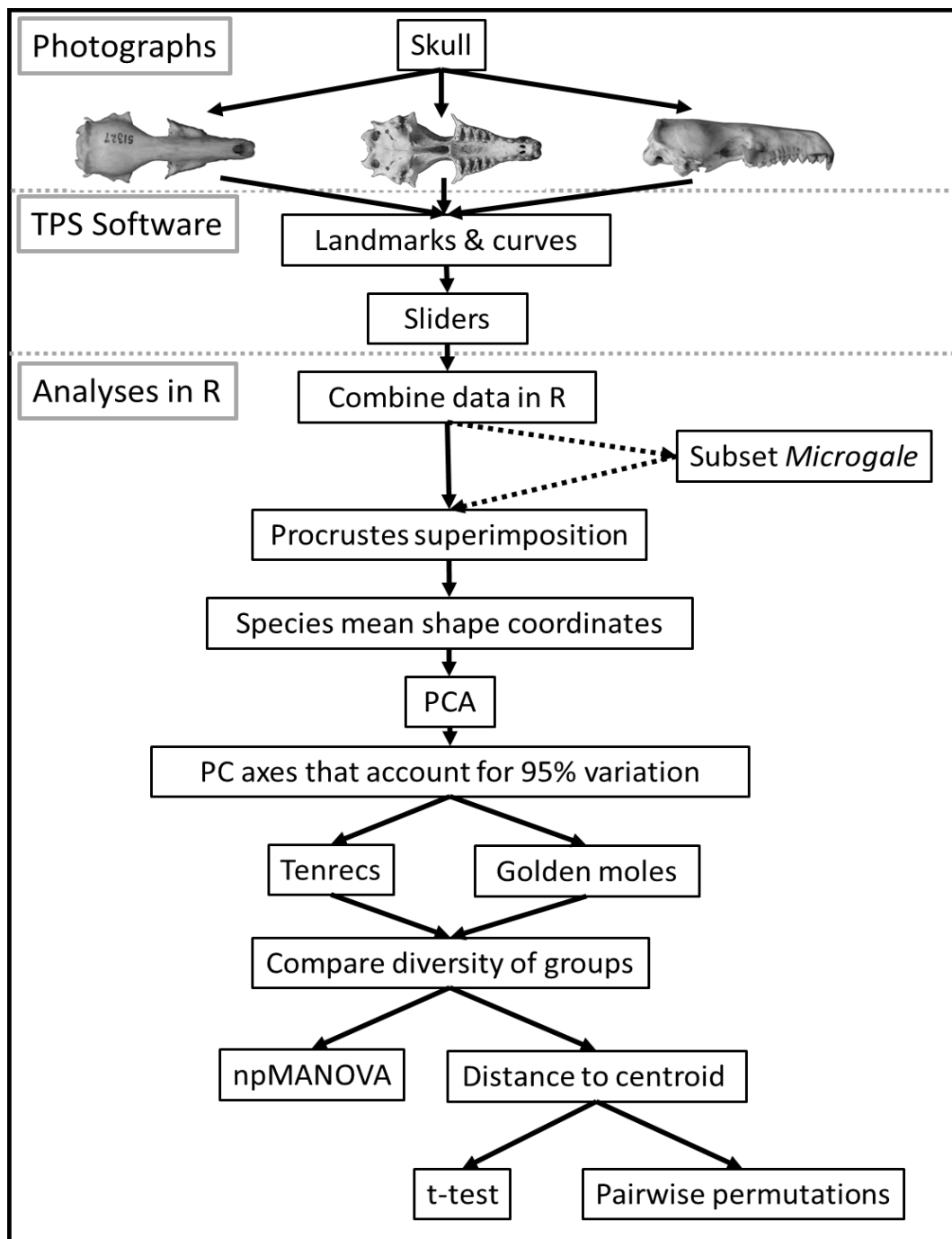


Figure 1: Summary of the main steps in our data collection, processing and analysis protocol. Note that the analyses were repeated separately for each set of photographs: skulls in dorsal, ventral and lateral views. The dashed arrows refer to the stage at which we selected a subsample of the tenrecs (including just five species of the *Microgale* Genus) so that we could compare the morphological diversity of this reduced subsample of tenrec species to the diversity of golden moles.