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 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* tenrec Genus, we found that tenrecs were more morphologically diverse than golden moles in all three analyses.

Our results demonstrate that the apparently high morphological diversity in tenrecs is not necessarily reflected in all morphological traits. Therefore the choice of morphological traits is a critical consideration when it comes to quantitative investigations 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 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 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 to process and landmark the pictures (figure 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 (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, Figure 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 axes which accounted for 95% of the cumulative variation for each of our three skull analyses. These axes represent the dimensions of our morphospace (REF). We used the scores from the PC axes to compare cranial morphologies in

two ways (figure 1).

First, we used non parametric MANOVAs (Anderson, 2001) to test whether tenrecs and golden moles occupied significantly different positions within our cranial morphospaces (e.g Serb et al., 2011; Ruta et al., 2013). Secondly, we compared morphological diversity within tenrecs to the diversity within golden moles. If tenrecs are more morphologically diverse, then they should be more spread-out within our cranial morphospaces. We calculated the morphological diversity of each Family as the mean Euclidean distance between every species and the centroid for that Family. We used a t test to assess whether there was any significant difference in the morphological diversity of tenrecs and golden moles.

Our groups have unequal sample sizes (31 tenrec species compared to 12 golden mole species). Therefore, we could find higher morphological diversity in tenrecs simply because it is the larger group (REF). We used pairwise permutation tests to account for this potential bias in sample size. Our null hypothesis was that there is no difference in morphological diversity between tenrecs and golden moles. If this were true, then the group identity of each species would be arbitrary: if you randomly assign the species as being either a tenrec or golden moles and then re-calculate morphological diversity there would still be no difference between the two groups.

We assigned Family identities at random to each species and calculated the differences in morphological diversity (mean Euclidean distances to the Family's centroid) for the new groupings. We repeated these permutations 1000 times to generate a null distribution of the

expected differences in morphological diversity between a group that has 31 members (tenrecs) compared to one which has 12 members (golden moles). Finally, we compared our observed (true) measures of the differences in morphological diversity to these permuted distributions to test whether there were significant differences in morphological diversity of the two Families after taking sample size differences into account.

The majority of tenrec species (19 out of 31 in our dataset) are members of the *Microgale* (shrew-like) Genus which is notable for its relatively low morphological diversity (Soarimalala & Goodman, 2011; Jenkins, 2003). Therefore, the strong similarities among these species may mask signals of higher morphological diversity among other tenrecs. To test this idea, we created a subset of our tenrec data which included just five of the *Microgale* species. Each species represents one of the five sub-divisions of *Microgale* outlined by Soarimalala and Goodman (2011). We compared the morphological diversity of this subset of tenrecs (n=19: 5 *Microgale* with 12 other tenrec species) to the morphological diversity within the 12 species of golden moles. We repeated the same morphological diversity comparisons and permutation tests to account for differences in sample size on this reduced data set (figure 1).

Results

Figure 3 depicts the morphospace plot derived from our principal components analysis of average Procrustes-superimposed shape coordinates for skulls in dorsal view. Similar plots for our analyses of skulls in ventral and lateral 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 cranial morphologies.

Secondly, we compared the morphological diversity within each Family. Based on our measures of mean Euclidean distances to the Family's centroid, tenrec crania are more morphologically diverse than golden mole crania 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 5 *Microgale* species) to the 12 golden mole species, we found that tenrecs had significantly higher cranial morphological diversity than golden moles in all analyses (table 1).

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

Discussion

Our analyses are the first quantitative investigation of morphological diversity in tenrecs. Tenrecs are often cited as an example of a 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. However, tenrecs were only more morphologically diverse than golden moles in just one (lateral view) of our three skull analyses (table1). The morphologically similar *Microgale* Genus seems to mask the high morphological diversity in the rest of the tenrec Family: reducing our data to include a sub-sample of this Genus revealed the remaining tenrecs to be significantly more morphologically diverse than golden moles (table 1). Our 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 (figure 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. In contrast, lateral aspects of the skull could not be included in the dorsal and ventral analyses which instead focused on the palate and overall skull outline morphologies. In particular, our landmarks in ventral view focus on morphological variation in the palate (figure 2). Given that most tenrecs have broad, non-specialised diets (Olson, 2013) so it makes sense that their ventral skull morphologies should be no more diverse than the insectivorous golden moles.

The majority of tenrecs are members of the morphologically similar *Microgale* genus. 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 overall higher morphological diversity in tenrecs compared to golden moles (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 studies have 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.

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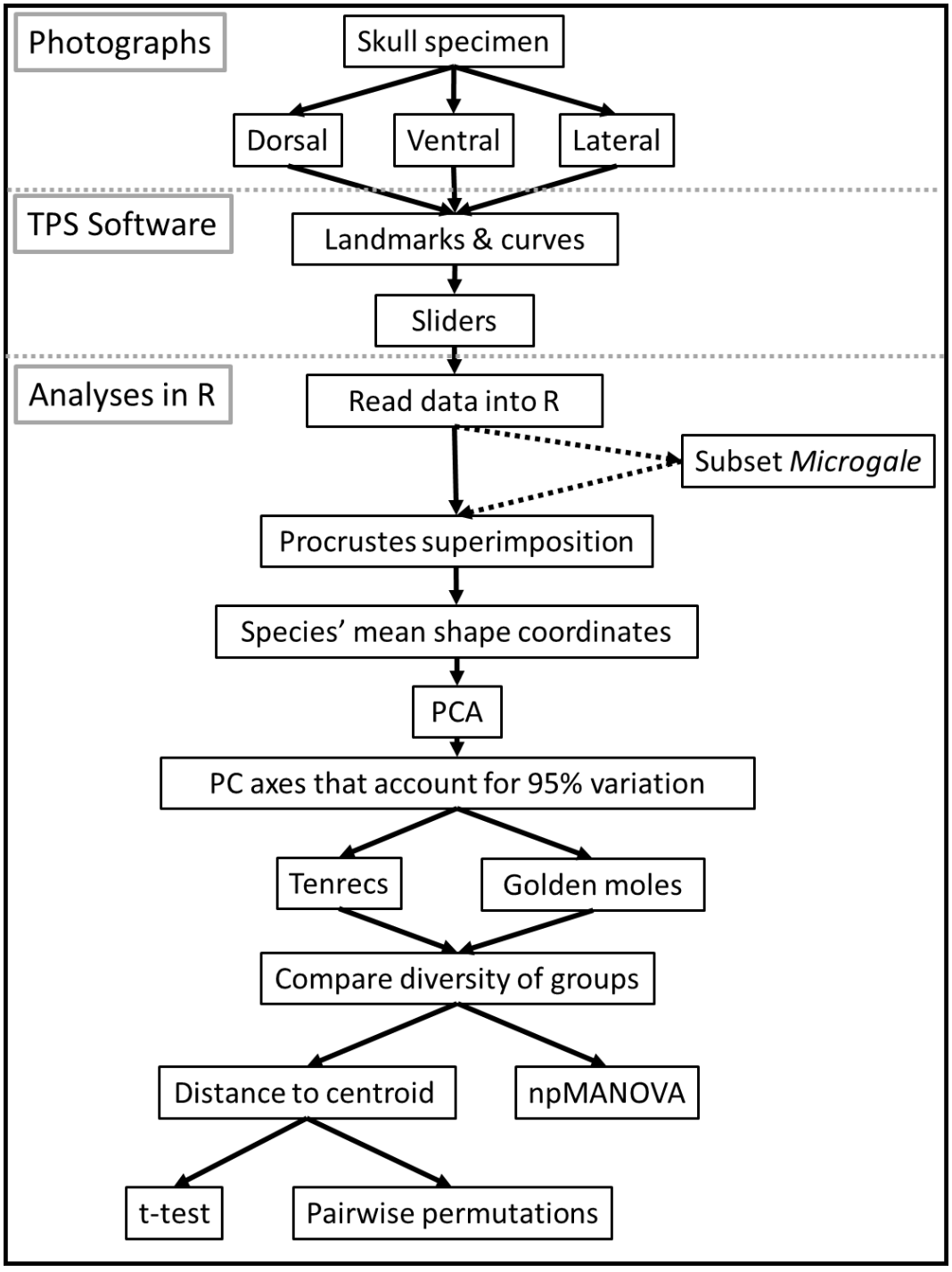

Figure 1: Summary of the main steps in our data collection, processing and analysis protocol. Note that skulls were photographed in three views and then the following analyses were repeated separately for each view. The dashed arrows refer to the analyses we repeated while including only a subset of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

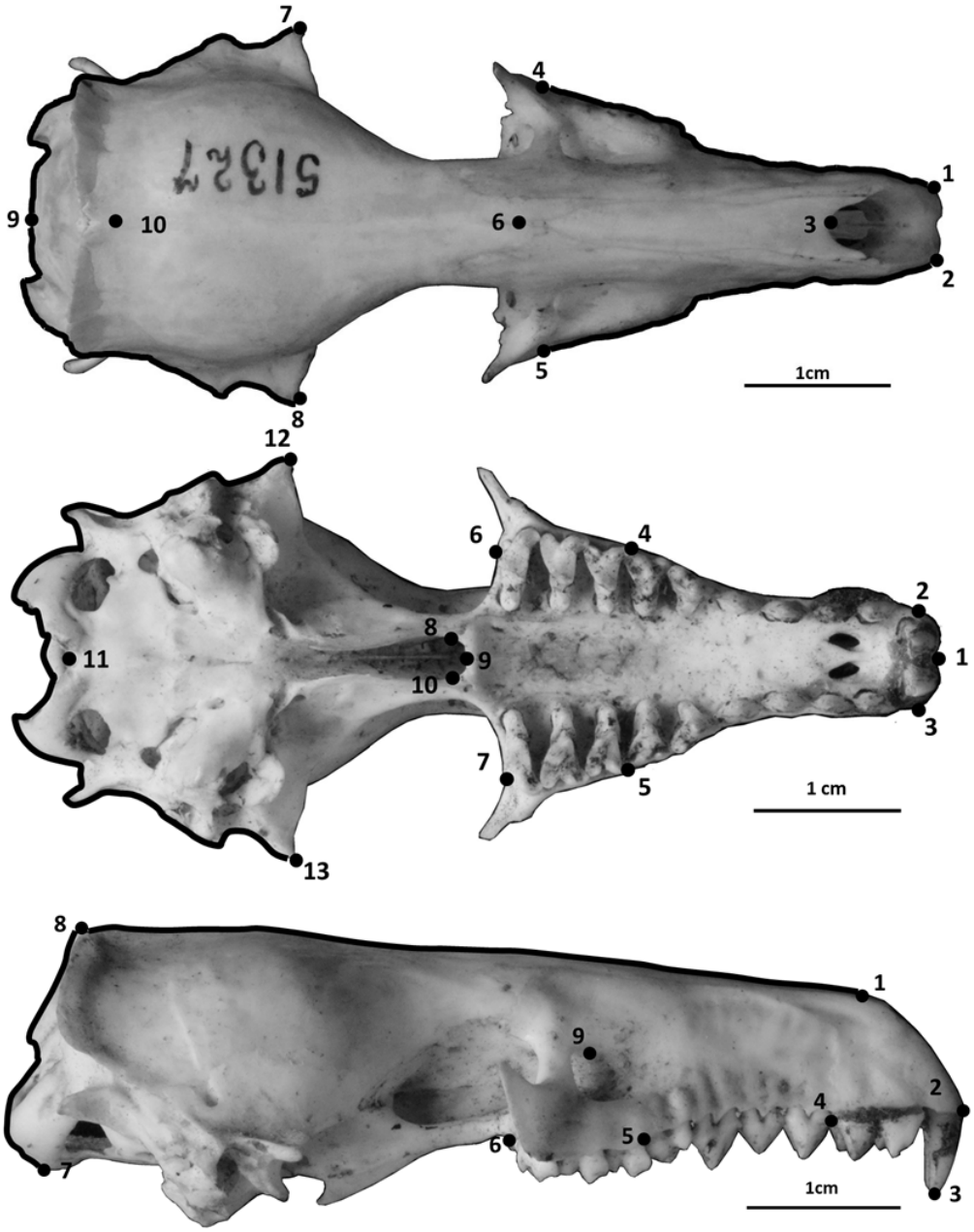

Figure 2: Landmarks (numbered points) and curves (black lines) used to capture the morphological shape of skulls in dorsal, ventral and lateral views respectively. Curves were re-sampled to the same number of evenly-spaced points. See Supplementary Material for descriptions of the curves and landmarks.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

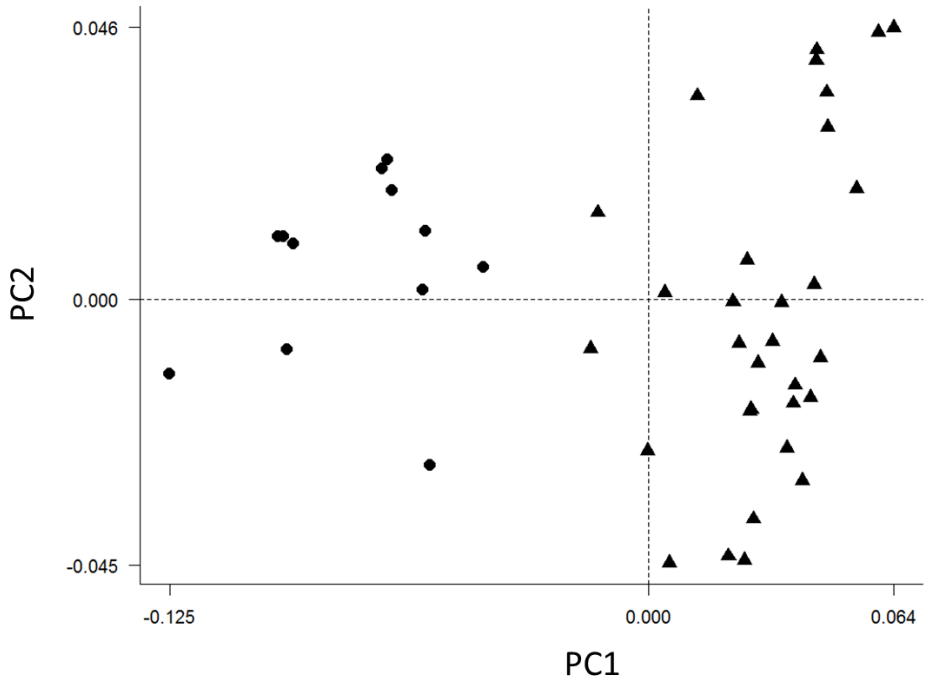

Figure 3: Principal components plot of the morphospace occupied by tenrecs (triangles, n = 31 species) and golden moles (circles, n = 12) for the skulls in dorsal view. Axes are PC1 and PC2 of the average scores from a PCA analysis of mean Procrustes shape coordinates for each species.

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Table 1:
Morphological diveristy in tenrecs and golden moles for each of the three analyses (skulls in dorsal, ventral and lateral view). We measured morphological diversity as the mean Euclidean distance between each species and the centroid for their Family. We compared the morphological diversity of 12 species of golden mole to a) all 31 species of tenrec (left) and b) 19 species of tenrec (right) which included just 5 species of *Microgale* tenrec. Significant differences (p values from t-test comparisons) are highlighted in bold.

Skulls analysis	Tenrecs (31) (mean± s.e)	Golden moles (mean± s.e)	t	p	Tenrecs (19) (mean± s.e)	Golden moles (mean± s.e)	t	p
Dorsal	0.036 (±0.0029)	0.029 (±0.0032)	-1.63	0.11	0.044 (±0.0025)	0.029 (±0.003)	-3.62	**0.001**
Ventral	0.048 (±0.0034)	0.044 (±0.0041)	-0.676	0.51	0.054 (±0.004)	0.042 (±0.004)	-2.23	**0.04**
Lateral	0.044 (±0.0041)	0.032 (±0.0037)	-2.16	**0.04**	0.054 (±0.005)	0.031 (±0.0037)	-3.47	**0.002**