

Influence of Evolutionary Allometry on Rates of Morphological Evolution and Disparity in strictly Subterranean Moles (Talpinae, Talpidae, Lipotyphla, Mammalia)

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Abstract The adaptation to a particular function could directly influence the morphological evolution of an anatomical structure as well as its rates. The humeral morphology of moles (subfamily Talpinae) is highly modified in response to intense burrowing and fully fossorial lifestyle. However, little is known of the evolutionary pathways that marked its diversification in the two highly fossorial moles tribes Talpini and Scalopini. We used two-dimensional landmark-based geometric morphometrics and comparative methods to understand which factors influenced the rates and patterns of the morphological evolution of the humerus in 53 extant and extinct species of the Talpini (22 extant plus 12 extinct) and Scalopini (six extant plus 13 extinct) tribes, for a total of 623 humeri. We first built a synthetic phylogeny of extinct and extant taxa of the subfamily Talpinae based on all the available information from known phylogenies, molecular data, and age ranges of fossil records. We tested for evolutionary allometry by means of multivariate regression of shape on

size variables. Evolutionary allometric trajectories exhibited convergence of humeral shape between the two tribes, even when controlling for phylogeny, though a significant differences in the evolutionary rates was found between the two tribes. Talpini, unlike Scalopini, seem to have reached a robust fossorial morphology early during their evolution, and their shape disparity did not change, if it did not decrease, through time. Furthermore, the basal *Geotrypus* spp. clearly set apart from the other highly fossorial moles, exhibiting a significant acceleration of evolutionary shifts toward higher degree of fossorial adaptation. Our observations support the hypothesis that the evolution of allometry may reflect a biological demand (in this case functional) that constrains the rates of evolution of anatomical structures.

Keywords Talpinae · Humerus · Evolutionary allometry · Geometric morphometrics · Comparative methods

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Introduction

The heterogeneous dynamics of morphological evolution have been recently emphasized by different authors (Hopkins and Smith 2015; Slater 2015), who showed that different evolutionary processes could influence evolutionary rates and morphological disparity through time. Furthermore, these same authors highlighted that, although the peak of morphological diversification might occur at any time along a clade history, the possibility of detecting these changes strongly depends on the temporal and taxonomic scale of observation (Hopkins and Smith 2015; Slater 2015).

Assessing how rates of morphological evolution change through time is challenging. In recent years, many different methods have been proposed (O'Meara et al. 2006; Eastman et al. 2011; Beaulieu et al. 2012; Thomas and Freckleton 2012; Ingram and Mahler 2013; among others) to detect the rate bursts within a clade's phylogenetic history. Some of these methods can handle multivariate data by fitting multiple Ornstein Uhlenbeck (OU) optima on a tree (Thomas and Freckleton 2012; Ingram and Mahler 2013). The search for the mechanisms underlying the evolution of a particular anatomical structure in a clade could be directly influenced by that structure's adaptive function.

The subfamily Talpinae includes the most specialized species for burrowing within Talpidae (Yates and Moore 1990), representing a paradigmatic group for the study of phenotypic evolutionary rates under highly selective constraints (i.e., an underground lifestyle; Nevo 1979). The subfamily includes two tribes: the Talpini and the Scalopini (Hutterer 2005). The genus *Geotrypus* (which includes nine species), basal to Talpini, exhibits several primitive morphological characters linked to the highly fossorial status and, in the context of their tribe, represents a distinctive morphological and phylogenetic group (Schwermann and Thompson 2015). The fossil record of Talpinae is well documented, with several extinct species described in the literature (Ziegler 1999; Gunnell et al. 2008), and it is largely accepted that the North American (Scalopini) and Eurasian (Talpini) highly fossorial moles are sister clades (Shinohara et al. 2003; Cabria et al. 2006; Sánchez-Villagra et al. 2006; Crumpton and Thompson 2013; Schwermann and Thompson 2015). However, there are still some uncertainties about the moles' origin (Hutchison 1974; Sánchez-Villagra et al. 2006; Schwermann and Thompson 2015). Provided with evidence that the oldest representative of this clade comes from the early Oligocene of Germany, it was hypothesized that the subfamily originated in Eurasia (Ziegler 2012). During the Neogene, the Talpinae spread across the Palearctic (Ziegler 1999; Gunnell et al. 2008), although it is not clear how many invasions occurred through North America, across which route (s), or if there was any back-migration to Eurasia (Sánchez-Villagra et al. 2006).

Piras et al. (2012) combined geometric morphometrics and phylogenetic comparative methods to study the humeral biomechanical performance and morphology in 30 genera of both extant and extinct talpids. According to their results, the rate of the phenotypic evolution of the highly fossorial species' humeri underwent a drastic reduction when compared with the rate of other talpid clades not adapted to an underground lifestyle. Furthermore, Piras et al. (2012, 2015) showed that highly fossorial moles reached a functional optimum early in their evolution and then did not experience further significant changes. Their results appear to be congruent with an early burst mode of evolution and niche filling (Freckleton and Harvey 2006; Slater et al. 2010). However, as reported by Slater (2015), early rapid morphological evolution is rare (Harmon et al. 2010). In this framework, we present a new study on the rates of phenotypic evolution of humeri in talpids, focusing solely on highly fossorial moles to understand if the evolution of fossoriality followed different evolutionary pathways within and/or between the two tribes, Scalopini and Talpini. The humerus of moles is particularly interesting because it is the bone of the forearm that experienced the most remarkable transformations (Dobson 1883; Freeman 1886; Edwards 1937; Campbell 1939; Reed 1951; Yalden 1966; Gambaryan et al. 2003; Sánchez-Villagra et al. 2004). This bone is widened and flattened in response to intense burrowing. It has an elliptically shaped (ventrally directed) head, a heavily-expanded proximal end, an enlarged teres tubercle, a deep brachialis fossa, a large, hemicylindrical clavicular facet, and an enlarged medial epicondyle bearing a deep fossa for the attachment of the flexor digitorum profundus tendon-muscle (Hutchison 1968; Gambaryan et al. 2003). The complexity of humeral modifications makes this bone a potentially rich source of evolutionary informative characters (Sánchez-Villagra et al. 2004). Moreover, this bone experienced transformations that are of taxonomic and systematic relevance, and is further considered to be a good proxy for a mole's body size (Skoczen 1993; van den Hoek Ostende, 1997; Ziegler 2003, 2006; Klietmann et al. 2014; Sansalone et al. 2015, 2016). The humeral morphology of Talpinae is highly modified and adapted for complex tunnel digging in both Talpini and Scalopini, but the morphological differences exhibited in these two tribes are primarily related to the teres tubercle, the bicipital tunnel, and the pectoral ridge (Sánchez-Villagra et al. 2006). Despite the abundant literature about the functional morphology of the talpid forelimb (Gambaryan et al. 2003; Scott and Richardson 2005; Piras et al. 2012, 2015), there are no contributions aimed at directly testing the phylogenetic trajectories of the humeral morphology exclusively within highly fossorial moles.

In the present paper we provide, for the first time, a comprehensive set of analyses directly aimed at investigating, by means of two-dimensional geometric morphometrics, if the strong selective pressure exerted by the underground

environment differently shaped the rates of morphological evolution in Talpini and Scalopini. In addition to the strong adaptive constraints linked to a subterranean lifestyle, other factors such as evolutionary allometry and phylogenetic history likely intervened as potential sources of phenotypic disparity through time. Accordingly, we first built the most complete synthetic phylogeny for the Talpinae clade including both extant and extinct species. By using this synthetic phylogeny we were able to investigate the humeral rates of morphological evolution in a phylogenetic context. Then, applying comparative methods, we investigated the humeral evolutionary allometric trajectories looking for the occurrence of convergence or parallelism between the two tribes. Finally, we tracked the course of size and shape disparity to determine whether phenotypic variability increased through time.

Materials and Methods

Phylogenetic Tree

Building a synthetic phylogeny of Talpinae at the species level was challenging. There is a lack of phylogenetic hypotheses accounting for a robust sampling of fossil species. Further, several extinct species are represented primarily by isolated teeth and fragmented mandibles. However, the talpid fossil record also accounts for a high number of humeri. Unfortunately, the use of characters coded only on one anatomical structure from a morphologically highly self-similar group does not provide a high degree of resolution. Therefore, we decided to develop our phylogeny based on all the contributions to Talpinae phylogeny available in the literature. We initially assembled a tree that included only the extant species using the information provided by the most recent molecular phylogenies (Shinohara et al. 2003, 2014; Cabria et al. 2006; Colangelo et al. 2010; Crumpton and Thompson 2013; He et al. 2014; Bannikova et al. 2015a, b). Following Renaud et al. (2005) and Piras et al. (2012, 2013, 2015), we assigned each extinct taxon to a clade based on three criteria: 1) the taxonomic validity of the taxon; 2) its stratigraphic range; and 3) the most agreed phylogenetic position of genera and species (see Supplementary Table III for the references used). For the time calibration we considered: 1) the first and last occurrence ages of extinct species in the fossil record; 2) the ages of the first occurrence of extant species; and 3) the molecular clock estimate (when available).

Several studies, using both molecular and morphological characters, recognized Scalopini and Talpini to be two monophyletic sister groups (Shinohara et al. 2003; Cabria et al. 2006; Sánchez-Villagra et al. 2006; Crumpton and Thompson 2013; Schwermann and Thompson 2015; Hooker 2016). According to several different contributions, we considered *Geotrypus* basal to Talpinae (Ziegler 1990,

2012; van den Hoek Ostende 2001; Sánchez-Villagra et al. 2004; Piras et al. 2012; Schwermann and Martin 2012; Schwermann and Thompson 2015). However, recently, Hooker (2016) suggested to exclude *Geotrypus* from the Talpini and to consider it as sister to Talpini + Scalopini clade. For the phylogenetic relationships within the extant members of the genus *Talpa*, we followed Colangelo et al. (2010) and Bannikova et al. (2015a). According to Ziegler (1990, 1999) and van den Hoek Ostende and Fejfar (2006), *T. tenuidentata* and *T. minuta* are considered basal to the genus *Talpa*. According to Ziegler (1999, 2006) and Dahlmann (2001), *T. vallesensis* and *T. gilothi* are considered basal to the modern (Pliocene to Holocene) *Talpa* species. The Plio-Pleistocene species *T. minor*, *T. fossilis*, *T. tyrrhenica*, and *T. episcopolis* are placed in a polytomy due to the absolute lack of phylogenetic hypotheses; however, Dahlmann (2001) and Abbazzi et al. (2004) hypothesized a closer relationship between *T. tyrrhenica* and *T. minor* due to their small size. The East Asia Talpini lack a well documented fossil record and for the extant members' relationships we followed Shinohara et al. (2014), which provided the most complete molecular phylogeny. The origin of Scalopini is still highly debated (Sánchez-Villagra et al. 2006; Bannikova et al. 2015b). For this reason, we decided to regard the European and Asian-North American Scalopini as two distinct lineages. Following Ziegler (2003), we considered *Leptosaptor* as sister taxon of *Proscapanus* + *Hugueneya* lineage (Ziegler 1999). According to Hooker (2016) and Gunnell et al. (2008), the clade *Domninoidea* + *Wilsonius* is considered basal to the other North American Scalopini. We considered *Parascalops breweri* to be outside the *Scapanus* + *Scalopus* clade (Crumpton and Thompson 2013; Bannikova et al. 2015a, b). According to Crumpton and Thompson (2013), *Scalopus* is basal to *Scapanus*. Following Voorhies (1977), *Scalopus mcgrewi* is sister to *Scalopus aquaticus*.

According to Hutchison (1987), *Scapanus schultzi* is basal to the three extant *Scapanus* species. The phylogenetic position of the extant *Scapanus* species has been based on Crumpton and Thompson (2013), where *S. latimanus* is outside *S. townsendii* and *S. orarius*. The phylogenetic position of *Scapanulus* is highly debated, and only recently Bannikova et al. (2015b) were able to define a clearer scenario. In fact, they recognized closer relationships between *Scapanulus oweni* and *Parascalops breweri*. However, we placed *S. oweni* within the Asian Scalopini forms. This is justified by the close morphological similarities with *Yunosaptor scalprum* (Storch and Qiu 1991) and by the presence of the Thai lower Miocene *S. lampounensis* (Mein and Ginsburg 1997), which reveals a longstanding evolutionary history in Asia for the genus *Scapanulus*. According to Storch and Qiu (1983, 1991), *Yanshuella* belongs to a different lineage anyway related to *Yunosaptor* + *Scapanulus* clade.

Materials

In the present paper, we carefully reviewed the entire literature to identify all species for which at least one well-preserved humerus was available. We examined 623 left humeri belonging to 53 species, including 19 Scalopini (six extant and 13 extinct) and 34 Talpini (22 extant and 12 extinct). Based on the ossification status (absence of epiphyseal plates) of the humeral epiphysis, all individuals analyzed were adults or sub-adults (Vaughan et al. 2015). Details on sampling efforts are summarized in the supplementary Tables I and II.

Geometric Morphometrics

The humeri were photographed in caudal view at a fixed distance of 50 cm with a Nikon D100 camera with a Micro-Nikkor 105-mm lens. We digitized 22 landmarks and 14 semi-landmarks (Fig. 1) using the tpsDig2 software (Rohlf 2006). Semi-landmarks were used to capture the morphology of complex outlines where homologous anatomical points were missing. Semi-landmarks assume that curves or contours are homologous among specimens (Adams et al. 2004; Perez et al. 2006). Because the error in orientation and landmark placement can be significant, each specimen was photographed and digitized three times and then averaged. Successively, a generalized Procrustes analysis (GPA) (Bookstein 1986; Rohlf and Slice 1990; Goodall 1991), implemented in the procSym() function from the R-package “Morpho” (Schlager 2014), was used to rotate, translate, and scale landmark configurations to unit centroid size (CS = the square root of the sum of squared distances of the landmarks

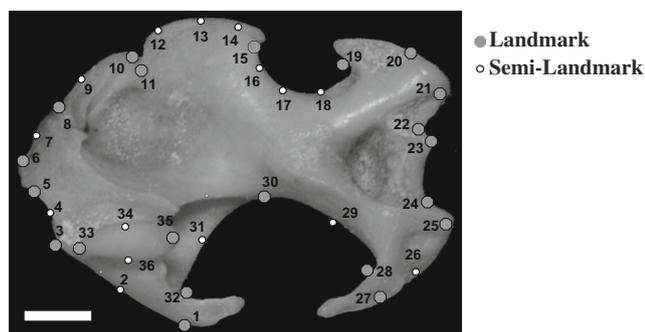


Fig. 1 Landmarks (large grey circles) and semi-landmarks (small white circles) digitized on the humerus in caudal norm: 1) lateral end of greater tuberosity; 2) articular facet of clavícula; 3) proximal edge of the articular facet of clavícula; 4) bicipital notch; 5) proximal end of lesser tuberosity; 6) medial edge of minor tuberosity; 7) lateral edge of lesser tuberosity; 8) bicipital ridge; 9) middle point of bicipital tunnel; 10) lateral end of scalopine ridge; 11) proximal end of teres tubercle; 12–14) surface of teres tubercle; 15) distal end of teres tubercle; 16–18) minor sulcus; 19) posterior margin of lateral epicondyle; 21–22) lateral epicondyle; 22–24) trochlear area; 25–27) medial epicondyle; 28) posterior margin of medial epicondyle; 29–32) greater sulcus; 33–36) humeral head. Scale bar is 1 mm

from their centroid) (Bookstein 1991). To visualize the multivariate ordination of the aligned specimens, we performed a between-group PCA (bgPCA), using the function groupPCA() included in the R-package “Morpho,” considering the species as groups. The bgPCA provides a projection of the data onto the principal components of the group means, resulting in an ordination of the shape variables between the group means. The new axes are orthogonal and can be computed even when the per-group data matrices are not of full rank. This method provides a good performance when the number of observations in each group is smaller than the number of variables (Boulesteix 2005; Mitteroecker and Bookstein 2011). The significance of the observed shape changes between Talpini and Scalopini was evaluated by performing a Procrustes ANOVA (Goodall 1991) on aligned Procrustes coordinates using the function procD.lm() included in the R package “geomorph” (Adams and Otarola-Castillo 2013).

Measurement Error

The measurement error associated with the digitization of landmarks was measured on three replicas of 120 specimens. The mean Procrustes distances between all the combinations of pairs of specimens were computed for each replicated dataset using the TPSsmall software (Rohlf 2014). We then calculated the mean Procrustes distances for each triplet of the same subjects occurring in the three replicas. We then computed the averages of all the mean values of the minimum and maximum values of each triplet. The amount of digitization error, with respect to the total variation in the shape, can be expressed as a percentage. We calculated the ratio of the mean value for total digitization and the mean of the total dataset.

Evolutionary Allometry and Comparative Methods

The relationship between size (independent variable) and shape (dependent variable) in the two tribes (Talpini and Scalopini) was tested by performing a multivariate regression of shape on size (averaged by species). Thus, the allometric trajectories were interpreted in terms of the evolutionary allometry not influenced by intraspecific ontogenetic processes. To test for differences in slope among tribes we ran a permutational multivariate analysis of covariance, using Procrustes coordinates as dependent variables, centroid size (CS) as an independent variable and tribe (Talpini–Scalopini) as a factor (Zelditch et al. 2004, 2012). This analysis was performed using the function procD.lm() in the package “geomorph” (Adams and Otarola-Castillo 2013). If the slopes did not significantly differ among tribes, it is possible to control for the allometric effect and compute the size-corrected shape variables (Viscosi and Cardini 2011, 2012; Zelditch et al. 2012). In the case of significant differences in slopes between the two tribes, we performed the “ontogenetic convergence test” as

described in Piras et al. (2010, 2011) to assess whether the allometric trajectories were parallel, convergent, or divergent. Further, we evaluated the rates of shape change per unit size for both Talpini and Scalopini using the predictions of separate multivariate regressions. In the case of a linear regression of degree 1, the rate of phenotypic change is calculated as Procrustes distance in a pair (even chosen randomly) of predictions divided by the difference in sizes at which these predictions were calculated.

For the sake of visualization, we performed a canonical correlation analysis (CCA; Oksanen et al. 2013), which determines a Y-axis that represents the amount of Y (shape variables) that is best explained by the independent variable X (CS). Further, we provided an interactive three-dimensional visualization of the allometric trajectories (identified by model predictions) in Supplementary Fig. II, accessible online. Closely related species tend to be more similar to each other than to more distantly related taxa (Felsenstein 1985). Therefore, the species means cannot be treated as independent units of information (Garland and Ives 2000). We used a phylogenetic generalized least squares (PGLS) linear model (Harvey and Pagel 1991; Rohlf 2001; Revell 2012; Zelditch et al. 2012; Adams and Collyer 2015) that accounts for the non-independence among observations due to the phylogenetic history. To analyze the relationships between size and shape, we tested the differences between the evolutionary allometric trajectories of the two tribes in a phylogenetic context, performing a phylogenetic MANCOVA (Garland et al. 1993) using Procrustes coordinates as dependent variables, centroid size (CS) as an independent variable, and tribe (Talpini/Scalopini) as a factor. The test was performed using the function `procD.pgls()` in the package “geomorph” (Adams and Otarola-Castillo 2013). Because *Geotrypus* spp. possess a peculiar and plesiomorphic humeral morphology, we evaluated the evolutionary allometry twice: including *Geotrypus* spp. the first time; and the second time excluding them.

Evolutionary Rates

Starting from the time-calibrated phylogenetic tree, we evaluated the evolutionary rates in the two different clades (Talpini and Scalopini) for both size and shape variables. Rate differences between tribes were tested using the function `compare.evol.rates()` of the R package “geomorph” (Adams and Otarola-Castillo 2013). Moreover, we looked for shifts in the rates in the phylogeny using the trait MEDUSA approach (Thomas and Freckleton 2012). This method allows one to detect where accelerations or slowdowns in the rate of phenotypic evolution occur within the phylogeny. This was achieved by using the `transformPhylo.ML2()` function of the R package “motmot” (Thomas and Freckleton 2012).

The function first fits a constant-rate Brownian model to the data, and then works iteratively through the phylogeny

fitting a two-rate model at each node in turn. Each two-rate model is compared to the constant rate model and the best two-rate model is retained. Keeping the location of this rate shift intact, it then repeats the procedure for a three-rate model and so on. Because we included two clades (tribes), we searched for two major shifts. Again, we evaluated the evolutionary rates with and without *Geotrypus* spp. For the sake of visualization, we provided a dynamic three-dimensional plot of time, the first PC score and size (see Supplementary Fig. III).

Shape and Size Disparity and Multivariate Heteroskedasticity through Time

We calculated the mean clade disparity through time for both the shape variables and the CS. We compared both the morphological and size disparity across our tree with that expected under a pure Brownian process by simulating the traits evolution 10,000 times across our tree. The mean subclade disparity values for the observed and simulated data were plotted against the node age and the calculated morphological disparity index (MDI). The MDI quantifies the overall difference in the relative disparity of a clade compared with the expectation under the null Brownian motion model (McPeck 1995; Harmon et al. 2003; Slater et al. 2010; Pearman et al. 2014). Negative MDI values indicate a lower clade disparity than expected under Brownian motion and are a common property of adaptively radiating clades. Positive MDI values indicate a higher subclade disparity than expected under Brownian motion that is typical of a character evolution independent from time (Slater and Pennell 2014). We calculated the mean subclade disparity through time using the function `dtm()` from the package “geiger” (Harmon et al. 2014). Further, we tested whether morphological disparity increased or not through time in the two tribes. For this purpose, we built a MANCOVA model using the ancestral shape state reconstruction (first 10 PC scores) at nodes, and appended OTU’s (Operational Taxonomic Unit) observed values (as dependent variables), the corresponding time (as an independent variable), and tribe affiliation as a factor. We computed the sum of the squared residuals of the model for each tribe, and we performed a linear regression between them and time. This is a strategy adopted to investigate the course of heteroscedasticity through time (Piras et al. 2011). Heteroscedasticities around predictions indicate the variability of each group according to the group-specific phenotype-time model.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Phylogenetic Tree

Figure 2 shows the time calibrated phylogenetic tree produced for all extant and extinct taxa, which is the most complete tree ever produced for the subfamily Talpinae. The bold portions of branches represent the observed range of occurrence for

each species included in our sample. Taxa for which the phylogenetic relationships were not resolved were placed on polytomic nodes in Fig. 2.

Measurement Error

The measurement error analysis revealed that 2.4% of the total variation was due to digitization error. Because the

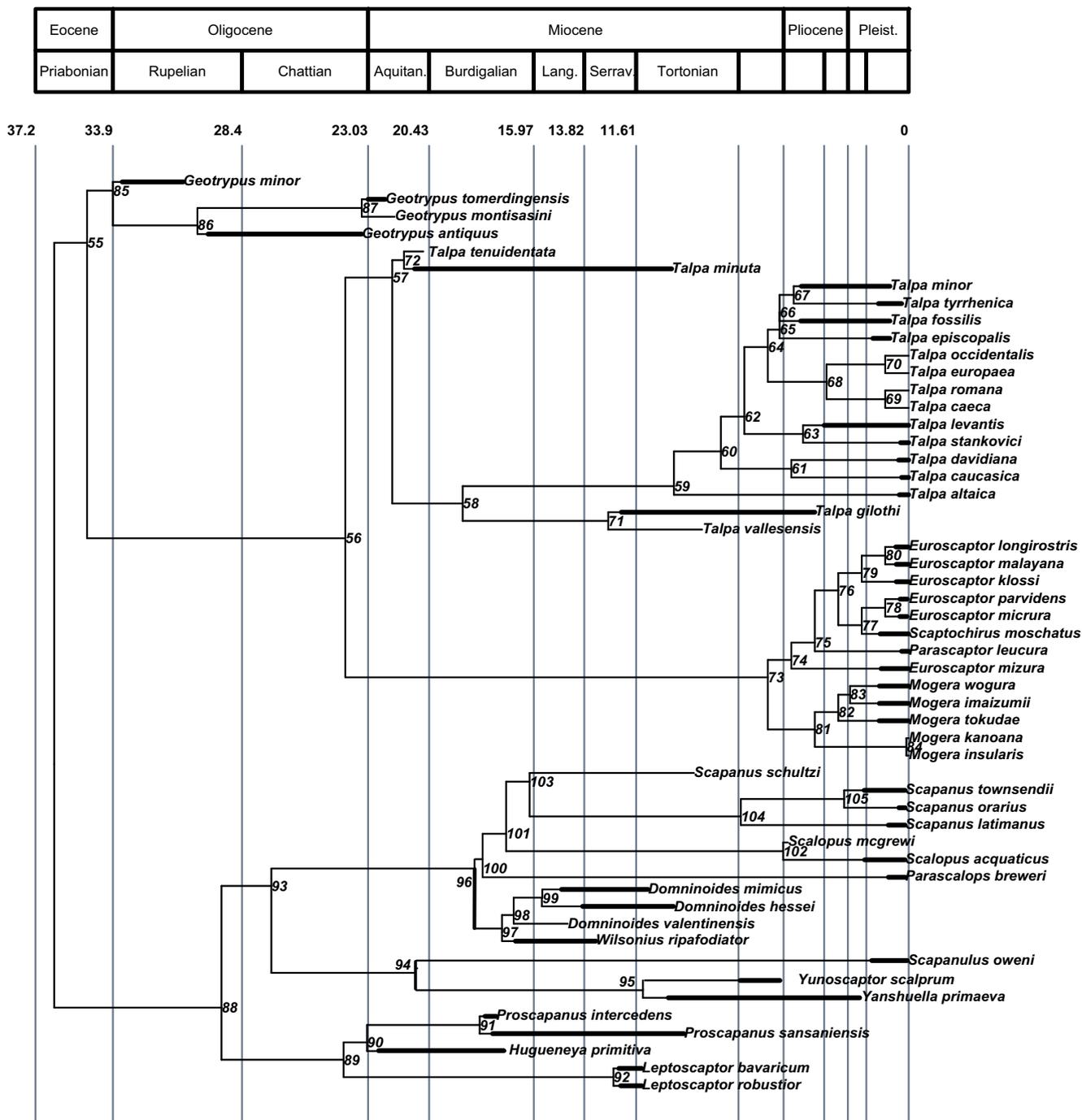


Fig. 2 Time-calibrated phylogeny of extant and extinct Talpinae. Bold branches represent the species' stratigraphic range of occurrence. Numbers of internal nodes are reported. See Supplementary Table III for further details

measurement error was smaller than 5%, it was judged to not significantly affect further analyses.

Shape Analysis

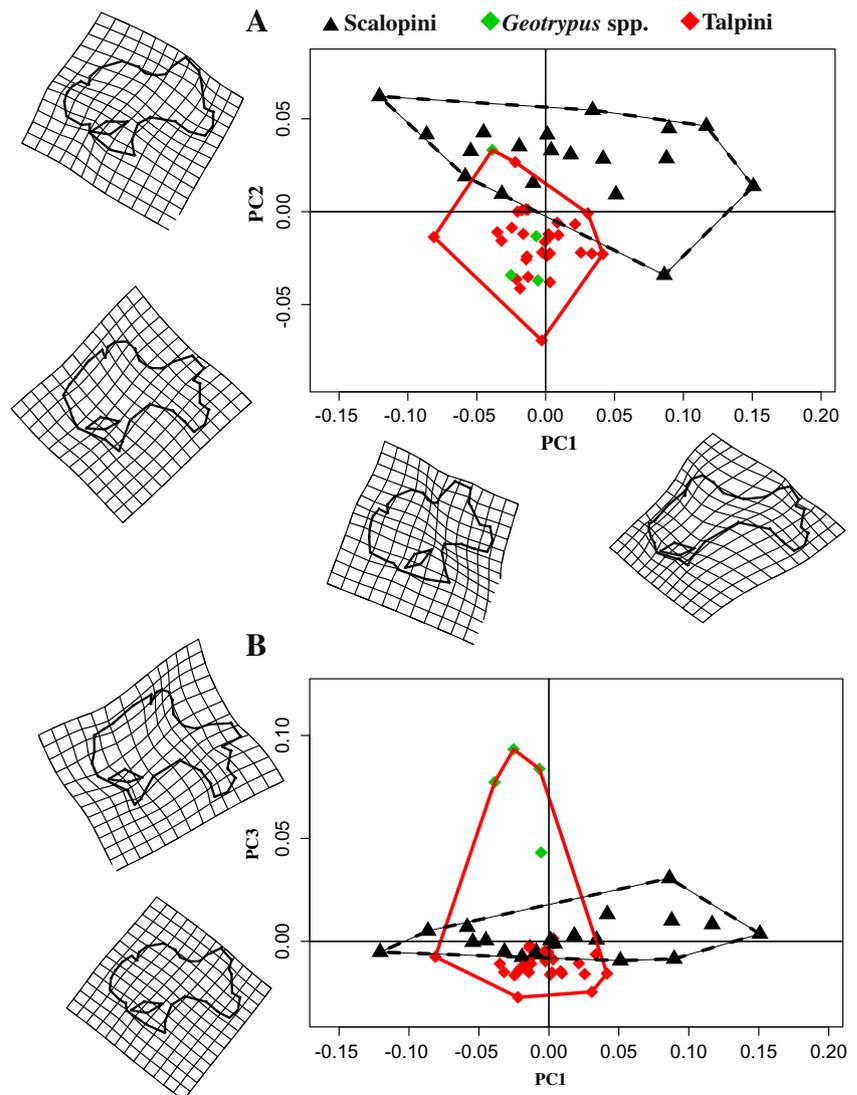
The bgPCA on the Procrustes coordinates showed that Talpini and Scalopini are well separated in the three-dimensional morphospace (Supplementary Fig. 1). The Procrustes ANOVA performed on the shape variables returned a highly significant result (p -value = 0.001). Along PC1 (43.86% of the total variance), there is a clear distinction between the slender (positive values) and the robust (negative values) Scalopini, whereas Talpini occupy a narrower portion of the shape space and are partially superimposed with the Scalopini robust forms. The slender humerus of the Scalopini exhibits a reduced teres tubercle and a less expanded pectoral ridge (see Fig. 3a). Scalopini and Talpini are clearly separated along PC2

(22.6% of the total variance), the former having a longer teres tubercle and a shorter pectoral ridge (see Fig. 3a). Along PC3 (14.3% of the total variance), *Geotrypus* spp. are clearly set apart with a humeral morphology characterized by a small and pointed teres tubercle, a very large pectoral ridge and an expanded minor tuberosity (see Fig. 3b).

Evolutionary Allometry and Comparative Methods

Per-clade multivariate regressions returned a significant result for both Talpini and Scalopini (p -value = 0.001, $r^2 = 0.11$; p -value < 0.001, $r^2 = 0.44$, respectively). The `procD.lm()` function revealed that the slopes are significantly different in the two tribes (interaction p -value = 0.001). The ontogenetic convergence test also returned a significant result (p -value = 0.017), revealing that the two trajectories were convergent (Supplementary Fig. II). In fact, the Euclidean distance

Fig. 3 **a** PC1/PC2 scatterplot of the bgPCA on humeral shape variables. Deformation grids refer to positive and negative extremes of the axes. **b** PC1/PC3 scatterplot of the bgPCA on humeral shape variables. Deformation grids refer to positive and negative extremes of the axes



between the predictions at a common small size (CS value =1.9) was greater than that between the predictions at a common large size (CS value =4.49). Further, Scalopini showed a higher rate of shape change per unit size (0.08) compared with Talpini (0.03; p -value =0.004). The same applies when excluding *Geotrypus* spp. (0.08 and 0.016, respectively; p -value =0.001). From small to large sizes the humerus shape exhibit significant changes from a slender to robust configuration (Fig. 4). The association between shape and size through PGLS was significant for Scalopini (p -value =0.006), but not for Talpini (p -value =0.11). The phylogenetic MANCOVA revealed a non-significant interaction (albeit at the limit of 0.05 significance) between size and tribe when using the entire sample (p -value =0.072), whereas it was significant when excluding *Geotrypus* spp. (p -value =0.045).

Evolutionary Rates

Morphological evolutionary rates did not differ significantly between the two tribes when we included all taxa (p -value =0.16). A positive shift was detected corresponding to *Geotrypus* spp., and a significant slowdown corresponded to the genus *Talpa* (Fig. 5b). When we excluded *Geotrypus* spp. from the analysis, we found a significant difference in the evolutionary rates between Talpini and Scalopini (p -value =0.01), with higher rates in the latter. Two positive shifts were found in correspondence of the robust *Dominoides* spp. and the East Asian *Euroscaptor* spp. (Fig. 5c). No significant differences were found in the evolutionary rates of size between the two tribes (p -value =0.26).

Fig. 4 Scatterplot of the CCA of shape on size. Deformation grids refer to the shape predicted at small or large size

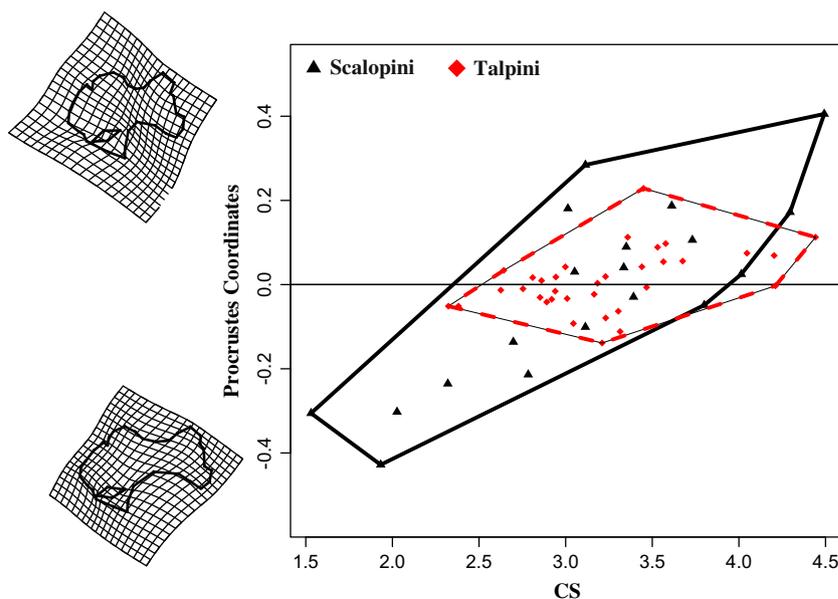
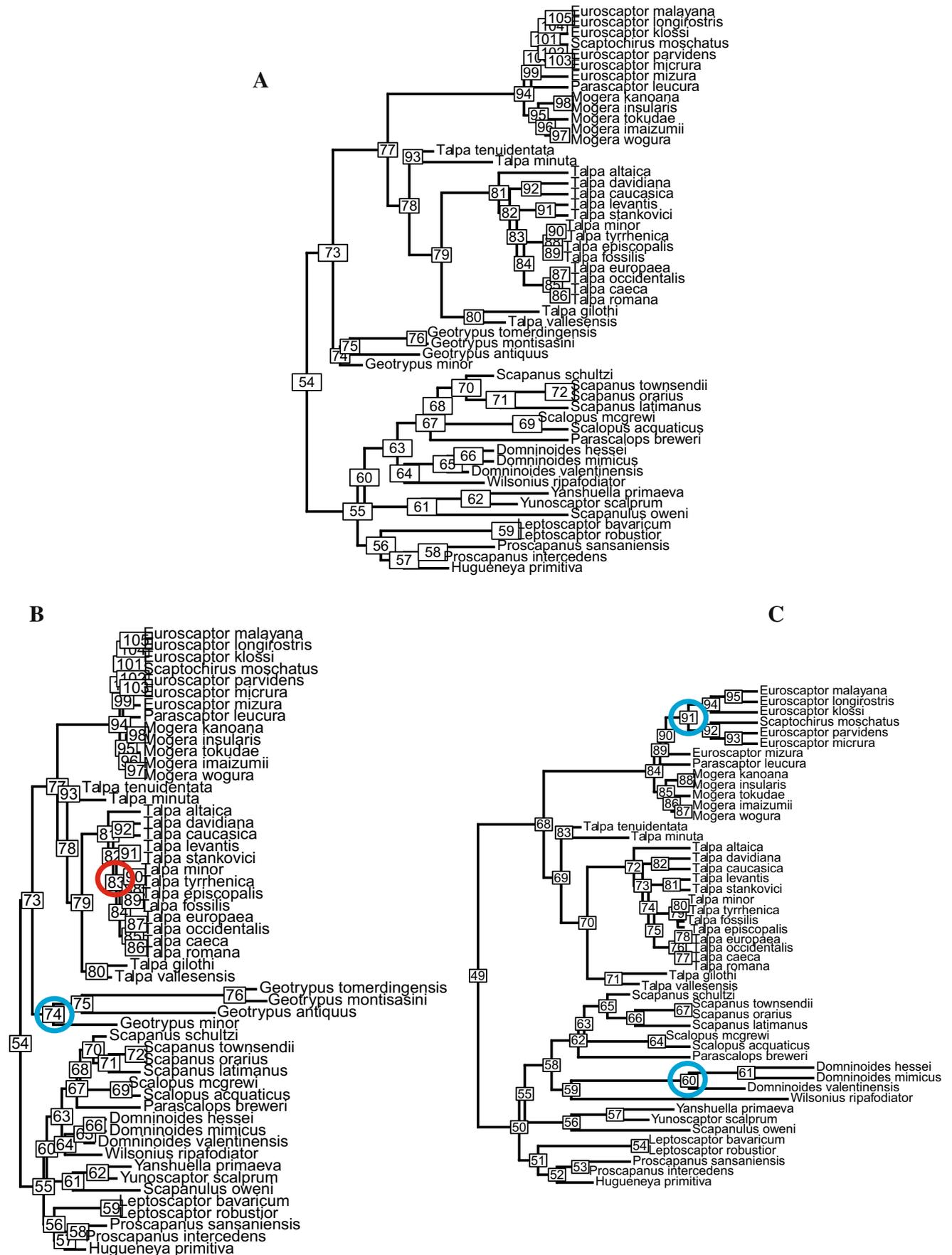


Fig. 5 Phylogenetic trees. (a) Untransformed tree. (b) Transformed tree with branch lengths proportional to morphological evolutionary rates; red circles indicate acceleration; blue circles indicate slowdown. (c) Transformed tree excluding *Geotrypus* spp.; red circles indicate acceleration

Shape and Size Disparity, and Multivariate Heteroscedasticity through Time

Although the morphological disparity of Talpini and Scalopini through time was higher than expected under the null model of Brownian motion (MDI = 0.22), the difference was not significant (p -value =0.94; Fig. 6a). The $dt()$ function returned similar results for size (MDI = 0.56; p -value =0.74). The results from MANCOVA on time (independent variable) and shape (dependent variables) (OTUs observed values + ancestral state reconstructions) revealed that in Talpini the humerus shape is significantly correlated with time (with or without *Geotrypus* spp.), whereas this does not hold for Scalopini. When exploring the multivariate heteroscedasticities through time (with or without *Geotrypus* spp.), the β coefficient (slope) was negative and significant for Talpini (p -value =0.021; $R^2 = 0.10$; not significant when excluding *Geotrypus* spp.; p -value =0.7, $\beta = -5.31 \times 10^{-5}$; with or without *Geotrypus* spp.) and was positive but not significant for Scalopini (p -value =0.01; $R^2 = 0.13$, $\beta = 2.48 \times 10^{-4}$). The multivariate heteroscedasticities computed for Talpini and Scalopini are depicted in Fig. 6b.



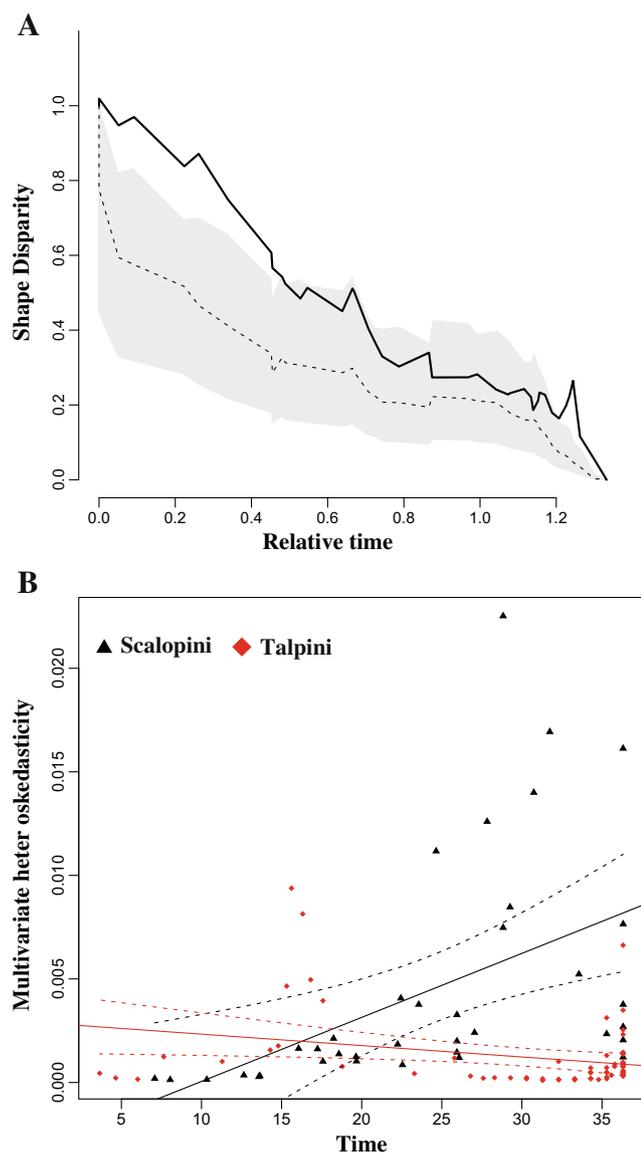


Fig. 6 **a** Plot of the $dtt()$ function performed on the humerus shape variables. The solid line represents the empirical data, and the dotted line represents the simulated data under Brownian motion. The grey shaded area indicates the 95% disparity through time for the simulated data. **b** Plot of the multivariate heteroscedasticity trough time

Discussion

Our results highlight that the evolution of the humerus of highly fossorial moles demonstrates a strong interaction between size and shape. When considering the phylogenetic effect, this interaction was significant when we excluded the plesiomorphic *Geotrypus* spp., revealing the presence of different evolutionary allometries in the other members of the Talpini and Scalopini tribes.

The convergence of allometric trajectories between the tribes suggests that evolutionary allometry channelled the humeral morphology in highly fossorial moles, particularly at larger sizes. Our results also showed that the evolution of the humerus

in Scalopini was more closely linked to the variation in size than it was in Talpini. This evidence was stressed by the higher rate of shape change per unit size that is associated with a higher morphological evolutionary rate when *Geotrypus* spp. were excluded from the phylogeny, thus suggesting that evolutionary allometry plays a key role in modulating the tempo and mode of the phenotypic evolution of moles. In fact, the Scalopini tribe includes small-sized species that exhibit a slender humeral morphology with plesiomorphic characters such as a reduced tuberosity, a reduced pectoral crest and a teres tubercle (Sánchez-Villagra et al. 2004, 2006; Piras et al. 2012). The Talpini tribe occupies a smaller portion of the morphological space compared to Scalopini, sharing with the latter the more robust phenotypes but not the slender forms. Allometry can promote also morphological diversification (Marroig and Cheverud 2005). Allometry played a key role in the ecomorphological diversification within different mammalian clades such as primates, carnivorans, artiodactyls, and rodents (Renaud et al. 2006; Meloro and Raia 2010, 2015; Raia et al. 2010;).

Specialization via adaptive evolution can be attained through the allometric change of shape and proportions (Schmidt-Kittler 2002, 2006; Meloro et al. 2015; among others). Based on our findings, we hypothesize that Scalopini, constrained by the strong selective pressure exerted by the underground environment, achieved a more robust humeral morphology accompanied by an increase in size. When we accounted for size, the higher degree of phenotypic disparity experienced across the phylogeny by Scalopini compared to Talpini was even more evident (see Supplementary Fig. III). In fact, the multivariate heteroscedasticity analysis revealed that the variability of humeral morphology around predictions was positively correlated with time in Scalopini, suggesting that shape disparity increased through time in this clade.

Multivariate ordination evidenced the separation of the *Geotrypus* spp. from all other taxa, whereas the results of MANCOVA and the rates tests obtained by including or excluding *Geotrypus* spp. suggest that this clade deserves further specific considerations. The genus *Geotrypus* is considered basal to Talpini (Schwermann and Martin 2012; Schwermann and Thompson 2015). However, according to Schwermann and Martin (2012), many features distinguish *Geotrypus* spp. from the other true moles. The greater tuberosity ends terminally, as in Talpini and Scalopini, in an enlarged facies articularis, which merges anteriorly with the humeral shaft. When we looked for major evolutionary shifts in the phylogenetic tree, we found a significant acceleration in correspondence of *Geotrypus* spp., with the exception of *Geotrypus minor*, and a slowdown in correspondence of *Talpa* spp. *Geotrypus minor* exhibits a very odd humerus morphology (Ziegler 2012), with many plesiomorphic features. Among these, the most relevant is the open bicapital tunnel, which is closed in the other *Geotrypus* species as well as in all other true moles (Sánchez-Villagra et al. 2004, 2006).

The presence of a plesiomorphic open bicipital tunnel suggests reconsidering the generic allocation of this species. Furthermore, the shape analysis (in particular on PC3, see Fig. 3b and Supplementary Fig. 1) evidenced that *Geotrypus* spp. clearly sets apart from the other highly fossorial moles by virtue of having a highly developed pectoral ridge and a tiny and pointed teres tubercle. The development of a large teres tubercle played a key role in the evolution of fossoriality (Hutchison 1968; Sánchez-Villagra et al. 2006; Piras et al. 2012, 2015; Schwermann and Thompson 2015). The teres tubercle enables the insertion of the teres major and latissimus dorsi muscles, two of the most powerful muscles involved in digging (Gambaryan et al. 2003; Piras et al. 2015; Sansalone et al. 2015). Moreover, the presence of fossil taxa with an outlying morphological variability that has been lost through extinction could significantly affect the inference of morphological evolutionary rates and the results of phylogenetic comparative methods (Hopkins and Smith 2015).

Talpini, differently from Scalopini, seem to have reached robust fossorial morphology early during their evolution as evidenced by the slowdown recorded in the shape evolutionary rate when *Geotrypus* spp. were excluded from the analysis. According to Piras et al. (2012, 2015), the Talpini tribe likely reached a functional optimum at the beginning of their evolution and did not experience further significant structural changes. Highly fossorial talpids are known to have differential timing of expression of *SOX9*, which is an early marker of autopodial chondrification, compared to other non-specialized taxa (Bickelmann et al. 2012). It is possible that transcriptional heterochrony, related to ecological specialization, evolved primitively across the Talpini phylogeny (Bickelmann et al. 2012). Further, the Talpini tribe does not include species with slender humeri, even in the fossil record (Ziegler 1999; Piras et al. 2012, 2015). The multivariate heteroscedasticity analysis revealed that the Talpini humeral morphological variability around predictions was negatively correlated with time and not correlated when *Geotrypus* spp. were excluded from the phylogeny, suggesting that in the Eurasian moles shape disparity did not change, if it did not decrease, through time. Based on these considerations and the occurrence of both a robust phenotype (characterized by a large teres tubercle and pectoral ridge) and larger size, even in basal Talpini members, we hypothesize that the evolutionary allometry imposed a strong phenotypic constraint on both the size and shape of the humerus at the beginning of the tribes' evolution (Gorman and Stone 1990).

At the subfamily level, we accounted for a constant rate of phenotypical evolution for Scalopini (the disparity through the time analysis and node height test were not significant for both shape and size), whereas for Talpini, we found a *p*-value that was very close to the significance level, thus indicating Brownian motion. In this context, evidence was provided for the direct selective pressure exerted by the evolutionary allometry on strictly related taxa (Pélabon et al. 2014). The two tribes

exhibit the same adaptation to an underground lifestyle, although the evolutionary allometry influenced their rates of morphological evolution differently through time resulting in a neat convergence pattern. Our observations support the hypothesis that the evolution of allometry may reflect a biological demand (in this case functional) that constrains the rates of evolution of a particular anatomical structure (Klingenberg and Zimmermann 1992; Klingenberg 2005; Pélabon et al. 2014).

Conclusions

We revealed that even in a clade subjected to strong environmental constraints, such as that experienced by highly fossorial moles, significant differences could be found in the tempo and mode of phenotypic traits evolution. In fact, we found that evolutionary allometry, combined with the adaptation to an underground lifestyle, significantly influenced the evolutionary rates and the morphological disparity through time of the true moles belonging to the subfamily Talpinae. We revealed that different patterns constrained the evolution of the humeral morphology in the Talpini and Scalopini tribes, thus confirming the importance of the taxonomical scale of our investigation (Hopkins and Smith 2015). Moreover, similar to Slater (2015), we highlight the key role of the inclusion of fossils in the phylogeny as we were able to gain new knowledge about the evolutionary dynamics in the highly convergent Talpinae.

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