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Multivariate Craniodental Allometry of Tarsiers

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Abstract Evolutionary allometry describes size and shape differences across taxa matched for developmental stage (e.g., adulthood). Allometric studies can identify subtle differences among species, and therefore help researchers interested in small-bodied, cryptic species such as tarsiers. Recent taxonomic revision has emphasized size differences among three possible tarsier genera inhabiting different island regions: Sulawesi (genus: *Tarsius*), Borneo (genus: *Cephalopachus*), and the Philippines (genus: *Carlito*). We examined seven craniodental measures of 102 museum specimens of adult tarsiers representing these three regions. We found that the allometric patterns within groups do not predict the observable differences among groups. Crania of the largest-bodied genus, *Cephalopachus*, are characterized by *relatively* short skulls and small orbits, with wider palates and molars than predicted by allometric increase from the smaller-bodied *Tarsius*. Overall, we found tarsier skulls stay the same shape as they increase in size. This may reflect shared developmental and biomechanical adaptations across tarsier groups filling an extreme leaping, faunivorous niche with hypertrophied orbits and subtle dietary differences in prey selection. These shared adaptations of tarsiers may severely limit the range of body sizes in tarsiers and impose further constraints on cranial shape. Despite their deep divergence times in the Miocene, living tarsier groups are united by a common craniodental form across a limited size range. Adaptations to extreme niches might result in a hyperconservatism of the cranium. Future primate allometric studies should explore cranial variation in other taxa to determine how adaptations to specific niches affect the size and shape of the cranium.

Keywords *Carlito* · *Cephalopachus* · Evolutionary allometry · Miocene · *Tarsius*

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Introduction

Allometry is the study of organismal variation associated with size and shape (Cheverud 1982; Fleagle 1985; Jungers *et al.* 1995; Klingenberg 1996; Mitteroecker *et al.* 2013). There are three types of allometry: ontogenetic, static, and evolutionary. Ontogenetic allometry is the study of organismal growth stages within a species; static (intraspecific) allometry examines variation among individuals of the same age category and same species; and evolutionary (interspecific) allometry studies the covariation among different traits of different species that belong to a shared phylogeny within the same developmental stage (Cheverud 1982; Fleagle 1985; Klingenberg 1996; Mitteroecker *et al.* 2013). Organisms can be isometric, in which shape is preserved as it increases or decreases in size. Alternatively, measurements may increase more rapidly than overall size (positive allometry) or show less increase than overall size (negative allometry), each resulting in changed proportions or shape (Jungers *et al.* 1995; Klingenberg 1996; Mitteroecker *et al.* 2013).

Allometry has practical applications in biology as a way to interpret limits on the size, ecology, and evolution of an organism (Fleagle 1985; Klingenberg 1996; Mitteroekcer *et al.* 2013). For example, cranial diversity among papionin primates is attributed to large size differences among the genera. Thorough ontogenetic studies found changes in size, particularly in *Papio* and *Mandrillus*, are a result of delayed adult maturation, in which subadult shapes are similar to the smaller sized papionins, and the adult *Papio* and *Mandrillus* exhibit a unique phenotype (Leigh *et al.* 2003; Singleton 2002). In this case, size differences drive shape differences and influence the evolutionary and phylogenetic interpretations of papionin primates (Leigh *et al.* 2003; Singleton 2002). Allometry also explains ecological and adaptive limitations on size. For example, larger primates consume more abundant plant materials, whereas smaller species consume protein rich, but less abundant insects (Fleagle 1985), and size differences correlate with metabolic requirements (West *et al.* 1997). In addition, allometric methods help clarify the relationships between within species static allometries and the evolutionary allometries observed in larger clades (Klingenberg 2016; Mitteroecker *et al.* 2013). Static and evolutionary allometric analyses provide opportunities for researchers to examine allometric variation in minute differences in a closely related phylogeny (Klingenberg 2016; Mitteroecker *et al.* 2013).

Tarsiers are small-bodied primates (60–120 g) (Gursky 2007) that are found on several islands throughout Southeast Asia (Musser and Dagosto 1987; Shekelle *et al.* 2010). All 12 species of tarsiers share morphological, behavioral, and ecological characteristics, and are all sit-and-wait hunting faunivores, consuming both insects and small vertebrates (Crompton and Andau 1987; Gursky 2007; Nietsch 1993; Rosenberger 2010). Their specialized locomotion, vertical clinging and leaping (VCL), contributes to their hunting style, with some species being more specialized for VCL than others. The most specialized species have the longest hind limbs and hands, and the least specialized have the shortest (Musser and Dagosto 1987). Morphologically, tarsiers are distinct from many nocturnal mammals as they lack a *tapetum lucidum* (the reflective eye tissue). Instead they have hypertrophic eyes that are larger, relative to their body size, than in any other mammal (Rosenberger 2010; Rosenberger and Preuschoft 2012). These massive eyes, along with extreme VCL locomotion, influenced modifications to their crania, such as a foramen magnum placed directly below the skull case, which is typically seen in bipedal organisms, such as humans. As

tarsier eye size increased, the skull became lighter, permitting faster movement, and further influencing the construction of the skull, to protect the eyes during foraging and moving, including postorbital flanges and partial postorbital closure (Anemone and Nachman 2003; Rosenberger 2010; Rosenberger and Preuschoft 2012).

Owing to their similar morphologies and behavior, tarsiers are described as monophyletic, and consist of three or four species groups (Table I; Fig. 1). Although morphological differences among the groups appear small (Gursky 2007; Niemitz 1984), differences in limb proportions, craniometrics, and other feature have been used to distinguish species (Groves 1998; Gursky 2007; Musser and Dagosto 1987; Niemitz 1984; Shekelle *et al.* 2008). Taxonomic studies describe a size gradient among the geographic groups in which the Bornean group (*Cephalopachus*) is the largest, followed by the Philippine (*Carlito*) group, and then the Sulawesi group (*Tarsius*). Pygmy or montane tarsiers (*Tarsius pumilus*) are dramatically dwarfed. More subtle cranial and limb length differences show consistent size-related variation among other tarsier groups (Groves 1998; Musser and Dagosto 1987). However, few studies have investigated allometric variation among or within tarsiers alone (*cf.* Anemone and Nachman 2003).

The size gradient among the islands groups, differences in amount and type of vocalizations (i.e., the ultrasonic call of *Tarsius pumilus*), and chromosomal and behavioral differences (Groves and Shekelle 2010; Gursky 2007; Shekelle *et al.* 2010) have incited discussions of tarsier taxonomy. Musser and Dagosto (1987) and Groves (1998) acknowledged at least two groups of tarsiers (Bornean-Philippine and the Sulawesi-pygmy tarsier), and suggested genus-level differences without demarcating any genera. Groves and Shekelle (2010) divided tarsiers into three living genera (Table I): *Cephalopachus*, the Bornean and Sumatran group (*cf. Tarsius bancanus spp.*): *Carlito* (*cf. T. syrichta*), the Philippine species; and *Tarsius*, consisting of the Sulawesi species. For simplicity, we follow Groves and Shekelle's (2010) taxonomy.

Table I Trait variations defining the three tarsier groups and pygmy tarsiers

Group/genus	Species	Traits
Borneo, Sumatra, and adjacent islands: *Cephalopachus*	*Ce. bancanus*	Largest body mass (123 g)[a] Solitary sleepers Most faunivorous Little vocalization Most specialized VCL
Philippine islands: *Carlito*	*Ca. syrichta*	Intermediate body mass (132 g)[a] Solitary sleeper Intermediate VCL
Sulawesi and adjacent islands: *Tarsius*	*T. dentatus, T. lariang, T. pelengensis, T. sangirensis, T. tarsius, T. tumpara, T. wallacei*	Small body mass (112 g)[a] Social sleepers Mainly insectivorous Highly vocal Duet calls with mates Least specialized VCL
Sulawesi highlands: *T. pumilus*	*T. pumilus*	Very small body mass (58 g)[a] Ultrasonic vocalizations Ultrasonic duet calls

[a] Body mass averaged from Gursky (2007)

Fig. 1 Adult crania of tarsiers from left to right: *Tarsius*, *Carlito*, *Cephalopachus*, and *T. pumilus* (pygmy tarsier). Photo: Dr. L. Yao. Specimens from American Museum of Natural History, New York, New York.

Another unusual aspect of tarsiers is their morphological similarity to their identified fossil ancestors. Examining these fossil tarsiers reveal the modern-day tarsier synapomorphies. Extinct and extant tarsiers share a suite of dental traits linked to insectivory and enlarged orbits (Beard 1998; Beard *et al.* 1994, Chaimanee *et al.* 2011; Rossie *et al.* 2006; Simons 2003; Zijlstra *et al.* 2013). The large orbit size of extinct tarsiers is inferred primarily from geometric morphometrics based on a partial maxilla of *Tarsius sirindhornae* (Chaimanee *et al.* 2011), and a partial facial fragment of *Tarsius eocaneus* (Rossie *et al.* 2006). Based on the sparse fossil record of tarsiers (*Xanthorhysis tabrumi*, *Tarsius eocaenus*, *T. thailandicus*, and *T. sirindhornae*) for which cranial material

exists, it is speculated extinct tarsiers possessed these large orbits by the Eocene, roughly 45 Ma (Chaimanee *et al.* 2011; Jablonski 2003; Rossie *et al.* 2006).

The dramatically large orbits, specialized dentition, and postcranial specializations for extreme vertical clinging and leaping in tarsiers may explain the minimal variation seen among the insular groups of tarsiers today (Rosenberger 2010; Rosenberger *et al.* 2016). Modern tarsiers are thought to live in forests that resemble those in which their ancestors lived (Jablonski 2003). Inhabiting roughly the same environment has contributed to the minimal morphological variation between ancestral and modern tarsiers, as well as among the modern tarsiers. This pattern, in which modern tarsiers exhibit a phenotype very similar to that of their distant ancestors (Chaimanee *et al.* 2012; Dagasto *et al.* 2003; Rosenberger and Preuschoft 2012; Rossie *et al.* 2006; Simons 2003), could be explained by long-term stabilizing selection in a small-bodied nocturnal, faunivorous, extreme VCL niche.

Molecular phylogenetic studies show that Philippine and Bornean/Sumatran tarsiers are each other's closest relatives, with Sulawesi tarsiers as their sister taxon (Brown *et al.* 2014; Merker *et al.* 2009, 2014). The divergence between Philippine and Bornean/Sumatran tarsiers is estimated from other primate fossil calibration points to be *ca.* 10 Ma in the late Miocene (Driller *et al.* 2015; Merker *et al.* 2009, 2014; Shekelle *et al.* 2010). The last common ancestor of crown tarsiers is estimated to have lived near the Oligocene–Miocene boundary around 22 Ma (Driller *et al.* 2015; Jablonski 2003; Merker *et al.* 2009). Sulawesi tarsiers are thought to be descendants of an initial wave of tarsier dispersal from mainland Asia. Once on Sulawesi, they remained isolated from other tarsier populations, though crown Sulawesi tarsiers date only to 2.5 Ma (Driller *et al.* 2015; Merker *et al.* 2009). A later wave of tarsiers dispersed from mainland Asia to Sumatra, Borneo, and surrounding islands during the Miocene (Dagasto *et al.* 2003), with the Philippine tarsiers likely derived from migrants from Borneo (Brandon-Jones 1998; Dagasto *et al.* 2003). Regardless of the biogeographic scenario of dispersal, there seems to have been ample time for morphological diversification to have taken place.

We tested for allometric differences among the three tarsier island groups (Borneo, Philippines, and Sulawesi). We hypothesize that the deep divergence and ecological differences among the tarsier groups resulted in a detectable size gradient among the island groups. Specifically, we predict that tarsiers increase in size from the small Sulawesi group to the large Bornean tarsiers. Moreover, we hypothesize that any notable shape differences among the groups are largely due to their increase in size (i.e., allometric) (Driller *et al.* 2015; Rychlik *et al.* 2006; Sebastiao and Marroig 2013; Wilson 2013; Zelditch *et al.* 2004). If this is the case, then we predict a common pattern of intragroup covariance among body measurements shared by all groups, of which the major axis of variation distinguishes tarsiers from different island regions (Schluter 1996). Our alternative hypothesis is group differences in shape are largely unrelated to size. In this case, we would predict tarsier groups have differentiated along minor axes of a common intragroup covariance pattern or possibly differences in covariance patterns among groups.

Methods

We refer to the geographic groups by their proposed genus names: *Cephalopachus* (Borneo/Sumatra), *Carlito* (Philippine), and *Tarsius* (Sulawesi). We do not address

taxonomic distinctions among the groups in this article. Shekelle *et al.* (2010) provide more detail regarding the possible genus level differences among the island groups.

We collected craniodental measurements from museum collections of tarsiers representing all four groups described above (Table I). Owing to their rarity in museum collections, sample sizes for *Tarsius pumilus* ($N = 3$) and *Carlito* ($N = 11$) were much smaller than for nonpygmy *Tarsius* ($N = 43$) or *Cephalopachus* ($N = 45$). All individuals examined were adults, based on tag information or skull size. We combined the sexes, as tarsiers exhibit little sexual dimorphism (Gursky 2007). Post-crania of tarsiers are limited, and juveniles are rare in collections, so we could not collect a large sample size for post-crania comparisons or ontogenetic studies. R. H. Dunn collected dental and cranial measurements from the National Museum of Natural History (USNM) to the nearest hundredth of a millimeter using dial calipers under a microscope. We took each measurement three times and recorded the mean of the three measurements. C. Groves provided additional measurements from USNM, the American Museum of Natural History, British Museum of Natural History, Museum Zoologicum Bogoriense, Naturalis Leiden, and the Natural History Museum of Berlin. R. H. Dunn and C. Groves followed collection methods from Musser and Dagosto (1987).

Analysis

We log-transformed all measurements before analysis to reduce differences in variances among measurements and render them interpretable on proportionate scales, as is traditional in allometric studies (Klingenberg 1996). We visually inspected density plots and z-scores for each single measurement and for all possible measurement pairs in bivariate scatterplots to detect egregious outliers indicative of possible measurement error (z-scores >3 or visual inspection of bivariate plots). We tested interobserver agreement using 20 skulls and limited our analyses to the seven variables with good agreement between the two observers (Pearson's $r > 0.8$ for M^1 length and $r > 0.9$ for all others). These seven measurements are skull length (SkullL), Biorbital breadth (BiOrbB), upper palate width at the third molar (M^3tPalW), maxillary canine to the third molar length (C.M3L), mandibular canine to third molar length (c.m3 L), mesiodistal length of the upper first molar (M^1), buccolingual width of the upper first molar (M^1W). We conducted all analysis in R (v 3.3.1) (R Development Core Team 2013).

We used Bayesian methods implemented in the MCMCglmm package (Hadfield 2010) for most of the analysis. There are distinct advantages to this approach compared to simpler alternatives. First, it allowed flexible modeling of the tarsier group means and intragroup covariances despite the imbalance in sample sizes. Second, the separation into groups roughly accounts for phylogenetic covariance and ensures intragroup covariance matrices are estimated rather than a hybrid of intra- and intercovariance (McCoy *et al.* 2006). Third, Bayesian methods allow propagation of error throughout the analysis from imputation of any missing data through the inference of allometric coefficients. In small data sets this is particularly advantageous because it prevents the false precision of stepwise analysis (Gelman and Weakliem 2009; Ovaskainen *et al.* 2008). The primary output from MCMCglmm was posterior distributions of mean vectors for each of the four tarsier groups and covariance matrices among measurements. Because we had very small samples of *Tarsius pumilus* and *Carlito* we pooled *T. pumilus* with *Tarsius* and *Carlito* with *Cephalopachus* in these models for estimation

of covariance matrices. In other words, each model posterior sample includes an estimate of the mean vector for each of the four groups but only two covariance matrices (all *Tarsius* v. *Cephalopachus* + *Carlito*). We used block diagonal (i.e., a pair of 7×7 blocks) inverse Wishart priors with low variance and degree of belief ($V = 0.02$, $\nu = 8$) on the residual covariance, and normal priors with mean of zero and very large variance (10^8) for the mean vectors. We ran each model for 150,000 iterations, discarded 50,000 as burn-in and retained every 100th thereafter, yielding 1000 samples of the posterior distributions for later analysis. Autocorrelation between successive retained samples was low enough to achieve effective sample sizes close to 1000 (minimum 815). We also extracted posterior means for any missing data points for use in later graphical exploration or analysis where a complete data set was desirable. This amounts to averaging the 1000 imputations of trait-group means.

We applied common principal components analysis implemented in the cpca package (Ziyatdinov *et al.* 2014) to each of the paired posterior covariance matrices. The resulting common principal component posterior distribution describes shared orientation (eigenvectors or principal components) of covariation in multivariate space but allows for group differences in variance/hypervolume (eigenvalues) (Flury 1988; McCoy *et al.* 2006; Phillips and Arnold 1999). Multivariate allometry is described by the principal components (PCs) of the common eigenvector matrix, with the first principal component (PC1) regarded as a metric of overall size and size-related shape variation (Klingenberg 2016). We used the 95% credible interval of the posterior distribution of PC1 and to test against isometry. Isometric PC1 scores are expected to be √7 (i.e., 0.378) (Klingenberg 1996). If the credible intervals did not include the isometric value, then the measurement would either be negatively or positively allometric, indicating relative decrease or increase of the measurement, respectively, with increasing overall size.

Although useful to describe common allometric patterns among groups, the common principal components model may not accurately describe the intragroup covariance matrices. We used a pair of matrix comparison methods to test this assumption using only the data from nonpygmy *Tarsius* and *Cephalopachus* because we had insufficient sample sizes for the other groups. First, we used the Flury hierarchy, which describes a series of comparisons from equal matrices, through common principal components to completely unrelated matrices. We used Phillips CPC program for this test (Bolker and Phillips n.d.; Phillips and Arnold 1999). Different CPC models in the hierarchy are compared to the best model using AIC or Δ_{AIC} from Phillips and Arnold (1999). Because of concerns over the biological interpretation of the CPC hierarchy, we also used random skewers, which tests for overall matrix similarity by computing hypothetical response vectors to directional selection (Cheverud and Marroig 2007). This test relies on randomization for significance testing, for which we used 5000 randomizations with 500 skewers per cycle as implemented in R scripts (Roff *et al.* 2012).

To supplement these numerical results, we plotted the first two common PC scores and compared these with plots of individual craniodental measurements or a ratio of a measurement to the geometric mean against the geometric mean. Discriminant analysis and other clustering statistics were inappropriate given our small sample sizes particularly of *Tarsius pumilus* and *Carlito*. We also calculated a CPC1-adjusted dataset for graphical exploration and *t*-tests comparing nonpygmy *Tarsius* and *Cephalopachus* after this adjustment (Burnaby 1966; Klingenberg 1996). To supplement the allometric analysis, we also built neighbor-joining and hierarchical clustering trees from the

Euclidean distance matrix of all tarsiers. We generated neighbor-joining trees with the nj function in the ape package (Paradis 2010). We used the hclust function with Ward's (1963) minimum variance criterion for agglomerative hierarchical clustering. We use these methods to show information from the distance matrix and do not to attempt to interpret the resulting trees as phylogenies. We used a robust k-means clustering procedure of the pamk function in the fpc package (Hennig 2015) on the logged or logged and z-scored data to identify any additional nonhierarchical patterns. To test for any clustering, we used the Duda–Hart test (H_0 $k = 1$). To test for further clustering ($k >$ 1) we used the average silhouette criterion. We found no difference in results with alternative criteria.

Data Availability The data from this study are available from the corresponding author on request.

Ethical Note

The authors have no conflict of interest regarding the publication of this article.

Results

Although tarsier crania are not dramatically different in overall size, we found clear differences in quantitative comparisons (Fig. 2; Table II). Tarsier crania are not isometrically or allometrically scaled variants; they have different shapes at different sizes and the shape changes are not predicted by intragroup covariance patterns. The departure from isometry is apparent in bivariate plots of ratios of skull dimensions to the geometric mean (Fig. 2). Skulls become relatively shorter with increasing size and palates get relatively wider. Biorbital breadth and canine-third molar lengths have more complex patterns that appear more isometric in aggregate across the tarsier groups. First molars become relatively wider and perhaps shorter across the groups. Although the pygmy tarsier sample is very limited, they do not appear to have unusual shapes for any of these variables. With the exception of skull length, biorbital breadth and first molar length pygmy tarsiers are isometrically shrunken versions of any tarsier group. They have relatively long skulls such as those in nonpygmy *Tarsius*, while their biorbital breadths are most similar to those of the relatively large eyed *Cephalopachus*. Their molar lengths are unusually short with two of the three points outside the shape range of any other tarsier group. *Carlito* are largely intermediate between *Tarsius* and *Cephalopachus* in both size and shape. Although the sample of this group is also small, they fall on the margin of *Tarsius* and are often subsumed in or overlap heavily with *Cephalopachus*.

Eigenvalues from the separate matrices show the first common principal component (CPC1), a general size and size-related shape metric, accounts for 25.99% (CI: 19.00–36.38%, *Tarsius*) or 38.60% (CI: 29.99–48.65%, *Cephalopachus* + *Carlito*) of the variance. Negatively allometric patterns are strongest in skull length and biorbital breadth, while positive allometry is weaker but most notable in the molar dimensions (Fig. 3). Although other variables are less distinguishable from isometry, their values
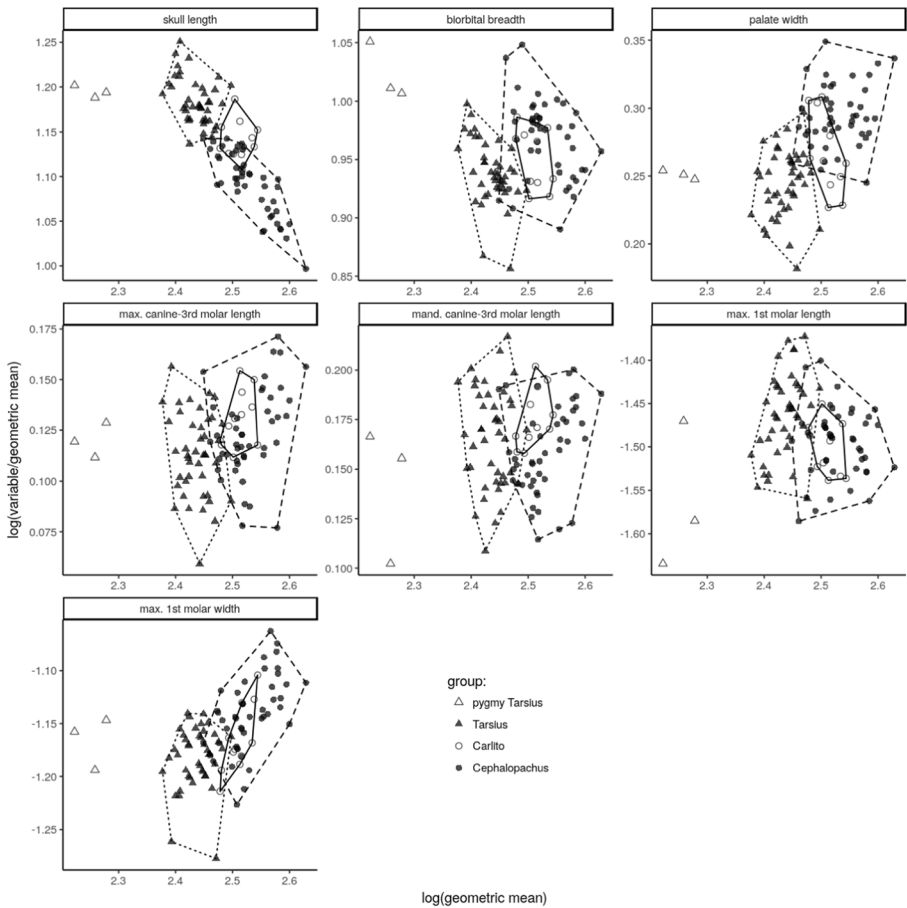
Fig. 2 Departure from isometry in shape change (y-axis) vs. overall size (x-axis) in tarsier craniodental measures from museum specimens. Pygmy tarsiers ($N = 3$) are indicated with open triangles; nonpygmy *Tarsius* ($N = 43$) with filled triangles; *Carlito* ($N = 11$) with open circles; and *Cephalopachus* ($N = 45$) with filled circles.

still imply potentially important size-related shape changes. For example, the weak negative allometry of palate width coupled with strong allometry of skull length implies tarsier skulls within groups should have an increasing ratio of palate width to skull length. This pattern appears to carry over across groups as well in the ratios to the geometric mean described above (Fig. 2). Plots of the first two CPCs show the tarsier groups arrayed in along a size gradient, with the small-bodied pygmy tarsiers well separated from the larger-bodied cluster of *Tarsius*, *Carlito*, and *Cephalopachus* (Fig. 4). There is limited overlap between *Tarsius* and *Cephalopachus* in these plots, and *Carlito* is often subsumed within *Cephalopachus*.

The *t*-tests on the raw and CPC1-adjusted data highlight allometric and nonallometric differences between *Cephalopachus* and *Tarsius* (Fig. 5). *Cephalopachus* is greater than *Tarsius* in all measurements in the raw data and CPC1 adjustment either reduces the difference or changes the sign for all variables except maxillary palate length (C.M3L). For M^1 length and especially skull length, adjustment turns a small difference into a very large difference, with *Tarsius* larger than *Cephalopachus*. The skull and M^1 are shorter

Table II Sample sizes, means, minima, and maxima of tarsier craniodental measurements from museum specimens in millimeters

| Measurement	Total				T. pumilus				Nonpygmy Tarsius				Carlito				Cephalopachus			
	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max
Skull length	91	37.33	30.7	40.40	3	31.43	30.7	32.2	40	37.38	35.20	40.4	11	38.55	36.96	40.30	37	37.40	35.3	39.52
Biorbital breadth	95	31.04	26.3	36.08	3	26.47	26.3	26.7	42	29.23	26.80	30.9	10	31.94	30.50	33.50	40	33.07	28.9	36.08
Palate width at M^3	101	15.68	11.9	19.40	3	12.23	11.9	12.5	43	14.60	13.44	15.9	11	16.08	15.48	16.60	44	16.87	15.0	19.40
Maxillary canine: M^3 length	98	13.52	10.4	16.20	3	10.73	10.4	11.1	40	12.80	12.00	13.6	11	14.05	13.44	14.70	44	14.22	13.2	16.20
Mandibular canine: M_3 length	100	14.11	10.6	16.72	3	10.97	10.6	11.4	41	13.50	12.60	14.6	11	14.69	14.00	15.32	45	14.74	13.7	16.72
Mesiodistal length of M^1	101	2.72	1.8	3.12	3	2.00	1.8	2.2	43	2.65	2.32	3.0	11	2.74	2.64	2.90	44	2.82	2.4	3.12
Buccolingual width of M^1	101	3.75	2.9	4.56	3	2.97	2.9	3.1	43	3.51	3.10	3.8	11	3.84	3.54	4.22	44	4.02	3.6	4.56
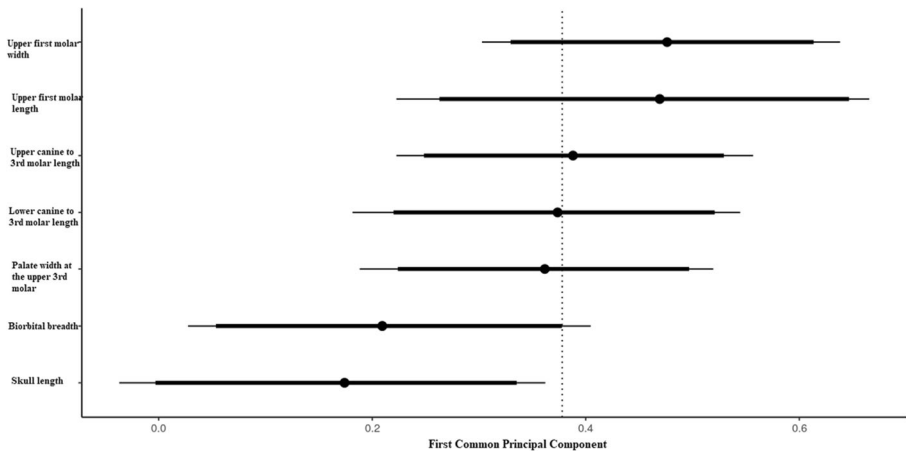
Fig. 3 The first common principal component describing tarsier craniodental allometry, based on log-transformed craniodental measures of museum specimens. Dots indicate posterior modes with 90% (thick) and 95% (thin) credible intervals. The vertical dashed line shows the isometric value.

than expected in *Cephalopachus* than if they were allometrically scaled versions of *Tarsius*. In the case of skull length, this implies further shape change beyond negative intragroup allometry to maintain essentially indistinguishable raw lengths across tarsier groups. Similarly, the positive allometry of molar length implies *Cephalopachus* molars are shorter than expected from intragroup allometry. For other measures large raw differences are greatly reduced by CPC1 adjustment. While *Cephalopachus* biorbital breadths are much greater than in *Tarsius*, negative intragroup allometry would predict them to be even larger. Mandibular canine M^3 length is also smaller than expected in *Cephalopachus* despite its approximately isometric scaling. Palate width, molar width, and maxillary canine M^3 length all remain larger in *Cephalopachus*, indicating relative size increase beyond that predicted by their static allometry. In particular, molar width remains larger in *Cephalopachus* after CPC1 adjustment. Although molar widths scale with positive allometry within groups, *Cephalopachus* molars are larger than predicted from this scaling relationship. This result identifies the wide molars of *Cephalopachus* as an important nonallometric group difference.

Matrix comparisons support the CPC model and reveal only subtle differences between *Tarsius* and *Cephalopachus* covariance matrices. Random skewers correlations are high and not significantly different from one ($r = 0.827$, $P = 0.999$ H_0: $r = 1$) suggesting very little difference in response to directional selection for the two groups. AICs for common principal component models in the Flury hierarchy indicate CPC (5) as the best fitting model, but the full CPC model with all eigenvectors shared is nearly as good a model and much better than unrelated matrices (Δ_{AIC} 0.36 v. 7.060). In summary, there are subtle differences between the two genera in covariance matrices, but they should not complicate the common principal components analysis presented earlier.

Neighbor-joining and hierarchical clustering trees show overall craniodental similarity among the tarsiers that capture the size and shape differences described in the allometric analysis and taxonomic descriptions (Fig. 6). In the neighbor-joining tree, pygmy tarsiers are distant outliers on long branches emanating from a cluster of *Tarsius*. The largest crania, from *Cephalopachus*, are the most distant tips from either
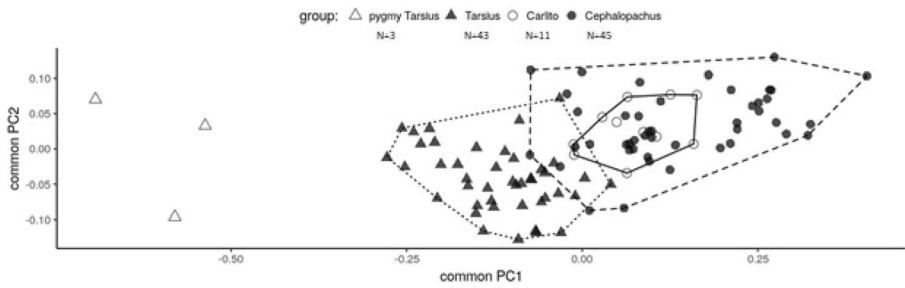
Fig. 4 Scores for four tarsier groups on the first two common principal components of the craniodental measurements of museum specimens.

Tarsius group. The intermediate-sized *Carlito* are in the middle of the tree with *Cephalopachus* as their nearest neighbors. The hierarchical clustering tree has essentially the same pattern, with *Tarsius* and *Cephalopachus* well sorted into two major clades. *Carlito* are scattered within the *Cephalopachus* clade, while pygmy tarsiers form a distinct subclade among *Tarsius*. *k*-means clustering consistently identifies two groups. The first group contains pygmy tarsiers and nearly all *Tarsius*. The second contains *Carlito* and nearly all of *Cephalopachus*. The results are almost identical if measurements are logged or logged and *z*-scored which eliminates size differences among measurements that would weight them differently in the clustering (Table III, only non-*z*-scored results shown). A Duda–Hart test rejects a single cluster ($P < 0.001$) and average silhouette width is maximized by $k = 2$.

Discussion

Our findings do not support the hypothesis that tarsier groups are distinguished primarily by intragroup allometric size and shape differences (Schluter 1996). The
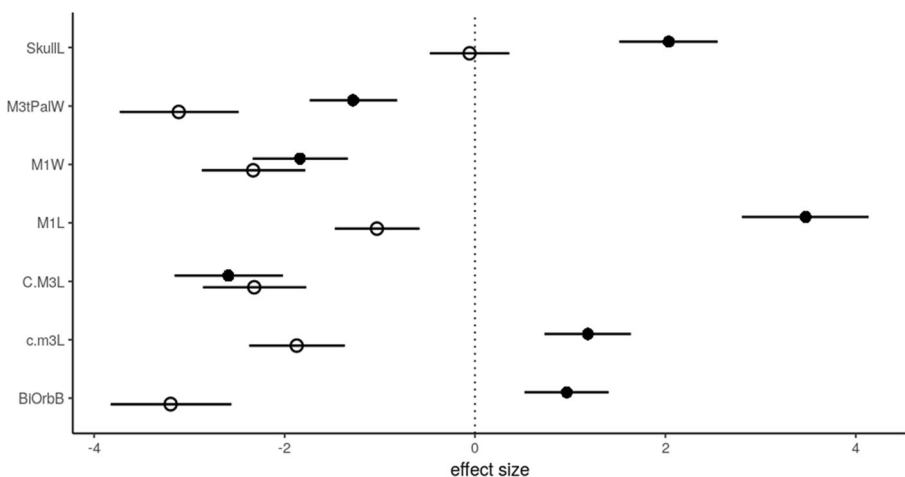

Fig. 5 Effect sizes and confidence intervals from raw data (open circles) and size-adjusted *t*-tests (filled circles) for differences between *Tarsius* and *Cephalopachus* in craniodental measures. Negative values indicate larger measurements in *Cephalopachus* and positive larger measurements in *Tarsius*.
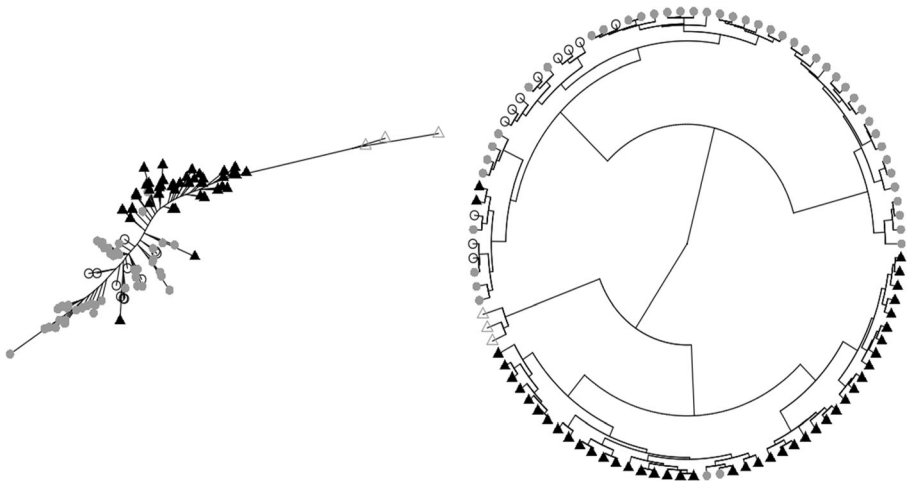
Fig. 6 Unrooted neighbor joining tree (left) and hierarchical clustering (right) of a Euclidean distance matrix of tarsier craniodental measurements. Pygmy tarsiers ($N = 3$) are gray, open triangles; other *Tarsius* ($N = 43$) are black, filled triangles; *Carlito* ($N = 11$) are black, open circles; and *Cephalopachus* ($N = 45$) are gray, filled dots.

most recognizable intergroup differences in tarsier crania are not predicted by intragroup covariance patterns. Previous descriptions have emphasized the relatively shorter skull with wider, flaring orbits and more robust dentition of *Cephalopachus* and *Carlito* (Musser and Dagosto 1987; Shekelle and Groves 2010). Static intragroup allometries do not account for these characterizations. For example, the negative allometry of skull length is far exceeded by the nearly indistinguishable mean lengths of the three groups, and the wide molars of *Cephalopachus* are wider than expected after allometric adjustment. The wide, flaring orbits of *Cephalopachus* are partially accounted for by allometric patterns, but they should be even larger if *Cephalopachus* were a scaled up version of *Tarsius*. These results suggest factors beyond static allometry to explain cranial differences among the groups.

Owing to our limited sample, we did not focus on the taxonomic significance of cranial variation, but did offer some description of their multivariate clustering. In general, we found two readily distinguishable groups of tarsiers: a *Tarsius* group (Sulawesi) and a *Cephalopachus*/*Carlito* (Borneo/Philippines) group. *Carlito* was between *Tarsius* and *Cephalopachus*, but was usually subsumed by *Cephalopachus*.

Table III k-means cluster analysis of tarsier craniodental measurements

Clustering criteria: Duda–Hart H_0: $k = 1$, $P < 0.001$					k-means clustering with $k = 2$:				
Average silhouette criteria					Cluster	*T. pumilus*	*Tarsius*	*Carlito*	*Cephalopachus*
$k = 2$	$k = 3$	$k = 4$	$k = 5$	$k = 6$	A	3	43	0	4
0.430	0.350	0.349	0.321	0.272	B	0	0	11	41

The presence of more than one cluster is confirmed by the Duda–Hart test, while the average silhouette criterion is maximized with two clusters. Assignment of tarsier groups to the two clusters primarily divides *Tarsius* and *T. pumilus* (cluster A) from *Carlito* and *Cephalopachus* (cluster B)

Pygmy tarsiers are most similar to nonpygmy *Tarsius* but are distinct in neighbor-joining and hierarchical cluster analysis. In general, they are most similar to *Tarsius*, but usually have shapes seen in any tarsier group. We hypothesized the limited craniodental variation among tarsiers is maintained because of their unusual adaptations to nocturnal foraging as visually and acoustically reliant predators and extreme vertical clingers and leapers. The observed dental and masticatory differences among groups may reflect specialization on different prey types.

In addition, our allometric results support the hypothesis that hyperconservation of the crania balances and protects the eyes. In particular, this can be seen in the biorbital breadth and skull length, two features that cannot change greatly as tarsiers increase or decrease in size. Both are negatively allometric within groups, reflecting the familiar negative allometry of neural tissues (Howland *et al.* 2004), but the group differences depart from the intragroup CPC1. We hypothesize these group differences reflect shifts either toward maintaining similar shapes at all sizes (biorbital breadth) or enhancing shape change (skull length) with increasing size to reduce anteroposterior forces during leaping or landing. Furthermore, these features are useful in distinguishing among the groups when allometric influences are removed.

Craniodental variation among tarsiers may reflect prey selection differences. It is expected that faunivorous mammals, such as tarsiers, have increased molar size to improve grinding capabilities (Gingerich *et al.* 1982; Gould 1975; Gursky 2007; Jablonski and Crompton 1994; Strait 1993). Yet, insectivores often have narrower molars to effectively break up insects, as the narrower teeth increase bite force to fracture exoskeletons (Evans and Sanson 1998; Strait 1993). These findings may explain why the more insectivorous *Tarsius* (Gursky 2007) has a smaller average molar width than the other two tarsiers, a difference that persists in the maxillary molars even when adjusted for allometry. Field observations indicate *Cephalopachus* favors larger prey, such as small reptiles, amphibians, birds, and occasionally small mammals such as bats (Crompton and Andau 1987; Niemitz 1984), which may explain their absolutely and relatively wider palate and maxillary molars. *Carlito* is thought to be insectivorous, but there are no detailed field studies of this genus (Dagosto *et al.* 2003). *Carlito* may have an intermediate diet that is not as faunivorous as that of *Cephalopachus*, or as insectivorous as that of *Tarsius*. This would be consistent with their intermediate craniodental anatomy, and these dietary differences may explain some of the subtle differences between the groups.

The small-bodied, Neotropical Callitrichidae (*Callithrix*, *Mico*, *Cebuella*, *Callibella*, and *Callimico*) exhibit allometric patterns over a narrow size range that may be related to ecological, behavioral, and dietary differences among taxa. It is speculated that prenatal miniaturization of Callitrichidae is the primary contributor to the observed, predominantly conserved-allometric patterns in Callitrichidae. However, dietary preferences, such as gummivory, and their vertical locomotion may also influence cranial development (Ford 1980; Marroig and Cheverud 2009; Vinyard *et al.* 2009). In addition, similar to tarsiers, Callitrichidae has a pygmy form, the pygmy marmoset (*Cebuella*). Similar to *Cebuella*, which is a scaled-down variant of *Callithrix* (Marroig and Cheverud 2009), we found *Tarsius pumilus* ($N = 3$) to be an allometrically scaled-down variant of *Tarsius*.

In summary, tarsier craniodental variation reflects their extreme locomotor and dietary specialization. Given our limited sample size, we cannot confidently address

tarsier taxonomy, but they suggest there are at least two groups: a western tarsier group (*Cephalopachus/Carlito*) and an eastern tarsier (*Tarsius*) group that, although united by a common craniodental form, can be readily distinguished. Although there are important size and shape differences between these groups, they are not simply extensions of the allometric patterns seen within groups. Future studies should investigate how these differences emerge through ontogeny and their relationship with postcranial morphology.

References

Anemone, R. L., & Nachman, B. A. (2003). Morphometrics, functional anatomy, and the biomechanics of locomotion among tarsiers. In P. C. Wright, E. L. Simons, & S. Gursky (Eds.), *Tarsiers: Past, present and future* (pp. 97–120). New Brunswick: Rutgers University Press.

Beard, K. C. (1998). A new genus of Tarsiidae (Mammalia: Primates) from the Middle Eocene of Shanxi Province, China, with notes on the historical biogeography of tarsiers. *Bulletin of Carnegie Museum of Natural History, 34*, 260–277.

Beard, K. C., Qi, T., Dawson, M. R., Wang, B., & Li, C. K. (1994). A diverse new primate fauna from middle Eocene fissure-fillings in southeaster China. *Nature, 368*, 604–609.

Bolker, B., Phillips, P. C. (n.d.). Common principal components/back-projections analysis. cpcbp package version 0.3.3.

Brandon-Jones, D. (1998). Pre-glacial Bornean primate impoverishment and Wallace's line. In R. Hall & J. D. Holloway (Eds.), *Biogeography and geological evolution of SE Asia* (pp. 393–404). Leiden: Backhuys.

Brown, R. M., Weghorst, J. A., Olson, K. V., Duya, M. R. M., Barley, A. J., et al (2014). Conservation genetics of the Philippine tarsier: Cryptic genetic variation restructures conservation priorities for an island archipelago primate. *PLoS One, 9*(8), e104340.

Burnaby, T. P. (1966). Growth-invariant discrimination functions and generalized distances. *Biometrics, 22*, 96–110.

Chaimanee, Y., Chavasseau, O., Beard, K. C., Kyaw, A. A., Soe, A. N., *et al.* (2012). Late Middle Eocene primates from the Myanmar and the initial anthropoid colonization of Africa. *Proceedings of the National Academy of Sciences of the USA, 109*, 10293–10297.

Chaimanee, Y., Lebrun, R., Yamee, C., Jaeger, J. J. (2011). A new Middle Miocene tarsier from Thailand and the reconstruction of its orbital morphology using a geometric-morphometric method. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20102062.

Cheverud, J. M. (1982). Relationships among ontogenetic, static, and evolutionary allometry. *American Journal of Physical Anthropology, 59*, 139–149.

Cheverud, J. M., & Marroig, G. (2007). Comparing covariance matrices: Random skewers method compared to the common principal components model. *Genetics and Molecular Biology, 30*(2), 461–469.

Crompton, R. H., & Andau, P. M. (1987). Ranging, activity rhythms, and sociality in free-ranging *Tarsius bancanus:* A preliminary report. *International Journal of Primatology, 8*(1), 43–71.

Dagosto, M., Gebo, D. L., & Dolino, C. N. (2003). The natural history of the Philippine tarsier (*Tarsius syrichta*). In P. C. Wright, E. L. Simons, & S. Gursky (Eds.), *Tarsiers: Past, present and future* (pp. 237–259). New Brunswick: Rutgers University Press.

Driller, C., Merker, S., Perwitasari-Farajallah, D., Sinaga, W., Anggraeni, N., & Zischler, H. (2015). Stop and go-waves of tarsier dispersal mirror the genesis of Sulawesi island. *PLoS One, 10*(11), e141212.

Evans, A. R., & Sanson, G. D. (1998). The effect of tooth shape on the breakdown of insects. *Journal of Zoology London, 246*, 391–400.

Fleagle, J. G. (1985). Size and adaptations in primates. In W. L. Jungers (Ed.), *Size and scaling in primate biology* (pp. 1–19). New York: Plenum Press.

Flury, B. (1988). *Common principal components and related multivariate models*. New York: John Wiley & Sons.

Ford, S. M. (1980). Callitrichids as phyletic dwarfs, and the place of the Callitrichidae in Platyrrhini. *Primates, 21*, 31–43.

Gelman, A., & Weakliem, D. (2009). Of beauty, sex and power: Too little attention has been paid to the statistical challenges in estimating small effects. *American Scientist, 97*, 310–316.

Gingerich, P. D., Smith, B. H., & Rosenberg, K. (1982). Allometric scaling in the dentition of primates and prediction of body weight from tooth size in fossils. *American Journal of Physical Anthropology, 58*, 81–100.

Gould, S. J. (1975). On the scaling of tooth size in mammals. *American Zoologist, 15*, 353–362.

Groves, C. (1998). Systematics of tarsiers and lorises. *Primates, 39*, 13–27.

Groves, C., & Shekelle, M. (2010). The genera and species of Tarsiidae. *International Journal of Primatology, 31*, 1071–1082.

Gursky, S. (2007). Tarsiiformes. In C. Campbell, A. Fuentes, K. MacKinnon, M. Panger, & S. K. Bearder (Eds.), *Primates in perspective* (pp. 73–85). Oxford: Oxford University Press.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software, 33*(2), 1–22.

Hennig, C. (2015). Package 'fpc'.

Howland, H. C., Merola, S., & Basarab, J. B. (2004). The allometry and scaling of the size of vertebrate eyes. *Vision Research, 44*(17), 2043–2065.

Jablonski, N. G. (2003). The evolution of the Tarsiid niche. In P. C. Wright, E. L. Simons, & S. Gursky (Eds.), *Tarsiers: Past, present and future* (pp. 35–49). New Brunswick: Rutgers University Press.

Jablonski, N. G., & Crompton, R. H. (1994). Feeding behavior, mastication, and tooth wear in the Western tarsier *(Tarsius bancanus). International Journal of Primatology, 5*(1), 29–59.

Jungers, W. L., Falsetti, A. B., & Wall, C. E. (1995). Shape, relative size, and size-adjustments in morphometrics. *Yearbook of Physical Anthropology, 38*, 137–161.

Klingenberg, C. P. (1996). Multivariate allometry. In L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, & D. E. Slice (Eds.), *Advances in morphometrics* (pp. 23–49). New York: Springer-Verlag.

Klingenberg, C. P. (2016). Size, shape, and form: Concepts of allometry in geometric morphometrics. *Development Genes and Evolution, 226*, 113–137.

Leigh, S. R., Shah, N. F., & Buchanan, L. S. (2003). Ontogeny and phylogeny in papionin primates. *Journal of Human Evolution, 45*, 285–316.

Marroig, G., & Cheverud, J. M. (2009). Size and shape in callimico and marmoset skulls: allometry and heterochrony in the morphological evolution of small anthropoids. In S. M. Ford, L. M. Porter, & L. C. Davis (Eds.), *The marmoset/callimico radiation* (pp. 331–354). New York: Springer Science+Business Media.

McCoy, M. W., Bolker, B. M., Osenberg, C. W., Miner, B. G., & Vonesh, J. R. (2006). Size correction: Comparing morphological traits among populations and environments. *Oecologia, 148*, 547–554.

Merker, S., Driller, C., Perwitasari-Farajallah, D., Pamungkas, J., & Zischler, H. (2009). Elucidating geological and biological processes underlying the diversification of Sulawesi tarsiers. *Proceedings of the National Academy of Sciences of the USA, 106*, 8459–8464.

Merker, S., Thomas, S., Volker, E., Perwitasari-Farajallah, D., Feldmeyer, B., et al (2014). Control region length dynamics potentially drives amino acid evolution in tarsier mitochondrial genomes. *Journal of Molecular Evolution, 79*(1–2), 40–51.

Mitteroecker, P., Gunz, P., Windhager, S., & Schaefer, K. (2013). A brief review of shape, form, and allometry in geometric morphometrics, with applications to human facial morphology. *Hystrix, 24*, 59–66.

Musser, G. G., & Dagosto, M. (1987). The identity of *Tarsius pumilus*, a pygmy species endemic to the montane mossy forests of central Sulawesi. *American Museum Novitates, 2867*, 1–53.

Niemitz, C. (1984). *The biology of tarsiers*. New York: Gustav Fischer Verlag.

Nietsch, A. (1993). *Beitrage zur Biologie von* Tarsius spectrum *in Sulawesis-Zur morphometric, Entwicklung sowie zum Verhalten unter halbfreien und unter Freilandbedingungne.* PhD thesis, Free University of Berlin.

Ovaskainen, O., Cano, J. M., & Merilä, J. (2008). A Bayesian framework for comparative quantitative genetics. *Proceedings of the Royal Society of London B: Biological Sciences, 275*, 669–678.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics, 26*, 419–420.

Phillips, P. C., & Arnold, S. J. (1999). Hierarchical comparison of genetics variance-covariance matrices. I. Using the Flury hierarchy. *Evolution, 53*(5), 1506–1515.

R Development Core Team (2013). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Roff, D. A., Prokkola, J. M., Krams, I., & Rantala, M. J. (2012). There is more than one way to skin a G matrix. *Journal of Evolutionary Biology, 25*(6), 1113–1126.

Rosenberger, A. L. (2010). The skull of *Tarsius*: Functional morphology, eyeballs, and the nonpursuit of predatory lifestyle. *International Journal of Primatology, 31*, 1031–1054.

Rosenberger, A. L., & Preuschoft, H. (2012). Evolutionary morphology, cranial biomechanics and the origins of tarsiers and anthropoids. *Palaeobiodiversity and Palaeoenvironments, 92*(4), 507–525.

Rosenberger, A. L., Smith, T. D., DeLeon, V. B., Burrows, A. M., Schenck, R., & Halenar, L. B. (2016). Eye size and set in small-bodied fossil primates: A three-dimensional method. *The Anatomical Record, 299*, 1671–1689.

Rossie, J. B., Xijun, N., & Beard, K. C. (2006). Cranial remains of an Eocene tarsier. *Proceedings of the National Academy of Sciences of the USA, 103*(12), 4381–4385.

Rychlik, L., Ramalhinho, G., & Polly, P. D. (2006). Response to environmental factors and competition: Skull, mandible and tooth shapes in Polish water shrews (Neomys, Soricidae, Mammalia). *Journal of Zoological Systematics and Evolutionary Research, 44*(4), 339–351.

Schluter, D. (1996). Adaptive radiation along genetic lines of least resistance. *Evolution, 50*(5), 1766–1774.

Sebastiao, H., & Marroig, G. (2013). Size and shape in cranial evolution of 2 marsupial genera: Didelphis and Philander (Didelphimorphia, Didelphidae). *Journal of Mammalogy, 94*(6), 1424–1437.

Shekelle, M., Groves, C., Gursky, S., Neri-Arboleda, I., & Nietsch, A. (2008). A method for multivariate analysis and classification of tarsier tail tufts. In M. Shekelle, I. Maryanto, C. P. Groves, H. Schulze & H. Fitch-Snyder (Eds.), *Primates of the oriental night* (pp. 71–84). Cibinong: Indonesian Institute of Sciences.

Shekelle, M., Meier, R., Wahyu, W. I., & Ting, N. (2010). Molecular phylogenetics and chronometrics of Tarsiidae based on 12S mtDNA haplotypes: Evidence for Miocene origins of crown tarsiers and numerous species within the Sulawesian Clade. *International Journal of Primatology, 31*, 1083–1106.

Simons, E. L. (2003). The evolution of the Tarsiid niche. In P. C. Wright, E. L. Simons, & S. Gursky (Eds.), *Tarsiers: Past, present and future* (pp. 9–34). New Brunswick: Rutgers University Press.

Singleton, M. (2002). Patterns of cranial shape variation in the Papionini (Primates: Cercopithecinae). *Journal of Human Evolution, 42*(5), 547–578.

Strait, S. G. (1993). Differences in occlusal morphology and molar size in frugivores and faunivores. *Journal of Human Evolution, 25*(6), 471–484.

Vinyard, C. J., Wall, C. E., Williams, S. H., Mork, A. L., Armfield, B. A., et al (2009). The evolutionary morphology of tree gouging in marmosets. In S. M. Ford, L. M. Porter, & L. C. Davis (Eds.), *The marmoset/callimico radiation* (pp. 395–409). New York: Springer Science+Business Media.

Ward Jr., J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association, 58*(301), 236–244.

West, G. B., Brown, J. H., & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science, 276*, 122–126.

Wilson, L. A. B. (2013). Allometric disparity in rodent evolution. *Ecology and Evolution, 3*(4), 971–984.

Zelditch, M. L., Lundrigan, B. L., & Garland, T. (2004). Developmental regulation of skull morphology. I. Ontogenetic dynamics of variance. *Evolution & Development, 6*(3), 194–206.

Zijlstra, J. S., Lawerence, J. F., & Wessels, W. (2013). The westernmost tarsier: A new genus and species from the Miocene of Pakistan. *Journal of Human Evolution, 65*, 544–550.

Ziyatdinov, A., Kanaan-Izquierdo, S., Trendafilov, N. T., Perera-Lluna, A. (2014). cpca: Methods to perform common principal component analysis (CPCA). R package version 0.1.2.