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RESEARCH ARTICLE

Patterns and Constraints of Craniofacial Variation in Colobine Monkeys: Disentangling the Effects of Phylogeny, Allometry and Diet

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Abstract

Leaf-eating monkeys (colobines) are a highly diversified subfamily with 61 species in ten genera, in which patterns and constraints of morphological evolution are still poorly resolved. In the present study, we measured the skulls of 452 specimens collected from different museums worldwide. Using one of the most extensive samples ever employed, and geometric morphometric techniques, we aimed to elucidate the evolutionary processes that have led to the craniofacial diversification of colobines. Our comprehensive analyses of the colobine cranium demonstrated that phylogeny is the first order signal to emerge, with clear interspecific patterns of differentiation. Allometric trend constrains shape variation for most colobine taxa, but to a lesser degree than phylogeny. We also confirmed that diet is significantly associated with the variation in cranial shape among colobines. In particular, the mechanical advantage of the masseter for biting at the anterior dentition is linked to seed intake. We postulate that such ecomorphological patterns explain, in part, the non-phylogenetic and non-allometric variations in the colobine skull, and indicate the importance of diet in interspecific resource partitioning, allowing for species coexistence.

Keywords Colobinae · Diversification · Diet · Morphometrics

Introduction

Colobinae represents a highly diversified subfamily of Old World monkeys, a sister group of the Cercopithecinae, from which they diverged ~ 19 million years ago (Liedigk et al. 2012). Colobines, also known as "leaf-eating monkeys," are a group that is currently facing extinction (Kamilar and Paciulli 2008), largely owing to the strong anthropogenic pressures exerted upon them (e.g., deforestation and/or hunting). Cercopithecines are considered a model of choice for human evolution, given their complex social structures, and have been extensively studied in the past; colobines, on the contrary, have been largely neglected.

Extant colobines cover a wide geographical range and are distributed from western Africa to eastern Asia (Yan-Zhang et al. 1993; Ting 2008). In the past 12 million years, since the estimated date of their divergence (Liedigk et al. 2012), colobines have experienced a rapid diversification, and the last intergeneric divergence, between *Simias* and *Nasalis*, occurred as recently as ~ 1.5 million years ago. Such evolution is intimately related to the geographical and environmental changes that occurred throughout the Tertiary and Quaternary periods. As an example, Southeast Asia, with its high diversity of biotas, witnessed the radiation of the odd-nosed monkeys (namely members of *Pygathrix, Rhinopithecus, Nasalis*, and *Simias*), which occupy a remarkable range of habitats, from mangrove swamps (Bennett and Sebastian 1988) to high altitude and temperate forests (Li et al. 2002). This variety of habitats highlights the diversity of colobines in terms of behavior, feeding ecology, and social organization (Kirkpatrick and Grueter 2010).

Species in the subfamily Colobinae were long thought to be predominantly folivorous primates, in contrast to cercopithecines (Hylander 1979a; Chivers and Hladik 1980;

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Bouvier 1986; Ravosa 1990; Kamilar and Paciulli 2008). Morphological traits of colobines, such as relatively sharp molar crests, thin tooth enamel, long molar rows, small incisors, robust mandibles, and reduced stiffness of the alveolar bone, have been considered adaptations for masticating leaves (Hylander 1979b; Teaford 1983; Ravosa 1996; Ravosa et al. 2000; Daegling et al. 2011). However, many studies have revealed an unexpectedly high dietary diversity, which has attracted a renewed interest in the study of their morphology (Koyabu and Endo 2009, 2010; McGraw et al. 2016). For example, species-specific diet preferences have been observed in the field, with the intake frequencies of food items such as mature leaves, young leaves, fruits, flowers, lichens, and seeds varying considerably between species (e.g. Fimbel et al. 2001; Chapman et al. 2002, 2004; Teichroeb et al. 2003; Zhou et al. 2006; Anderson et al. 2007; Harris and Chapman 2007; Hanya and Bernard 2012). Overall, most colobines prefer young leaves (Bennett and Davies 1994; Oates 1994) as they are often highly nutritious and contain relatively few secondary compounds (Matsuda et al. 2013). They generally require low masticatory forces compared to more resistant foods such as mature leaves and seeds (Lucas et al. 2012). However, long-term field observations have demonstrated that other food items mostly fill the role of fallback resources during seasons with low food availability (Li et al. 2010; Vandercone et al. 2012; Ehlers Smith et al. 2013; Kibaja 2014). In particular, it is now clear that seed eating is a major aspect of the colobine diet in both Africa (Maisels et al. 1994; Oates 1994; Davies et al. 1999) and Asia (Davies et al. 1988, 1999; Davies 1991; Bennett and Davies 1994; Hanya and Bernard 2015).

Morphologically, colobines show considerable craniodental variation (e.g. Bergmann 1848; Verheyen 1962; Hull 1979; Daegling and McGraw 2001; Nowak et al. 2008; Cardini and Elton 2009), but the evolutionary factors driving this variation are not well understood (Daegling and McGraw 2001; Pan 2006; Wright and Willis 2012). Comparative studies on the craniodental morphology of selected colobines have proposed that the greater mechanical advantage of the masticatory apparatus (the length of the masseter lever arm relative to the load arm of the bite point) found in a handful of Asian (Koyabu and Endo 2010) and African colobines (Koyabu and Endo 2009) is related to seed eating. Among African colobines, it was found that species that are reported to feed frequently on seeds (*Colobus angolensis* and *C. polykomos*) show greater mechanical advantage of the masseter for chewing than do species that feed rarely on seeds (*C. guereza, Pi. badius*, and *Pro. verus*) (Koyabu and Endo 2009). Similarly, among Asian species, seed eaters such as *P. rubicunda* and *T. phayrei* were found to have a greater mechanical advantage for mastication than *P. comata, S. vetulus*, and *T. obscurus*, which rarely exploit seeds (Koyabu and Endo 2010). However, despite this work on a still understudied group, Koyabu and Endo (2009, 2010) were limited to only a few colobines (five species for both studies) and were not able to investigate the relationship between seed-eating and skull form for most of the colobines. In addition, the effect of phylogeny was not addressed in these studies owing to limited taxon sampling. Therefore, in a phylogenetic context, whether cranial variation among the whole clade of colobines actually reflects differences in seed eating remains to be tested.

Although dietary variation is an important driver of craniodental evolution owing to the variation in mechanical constraints occurring during mastication (Wright et al. 2008; Yamashita et al. 2016), non-dietary factors such as allometry should also be considered. Allometry consists of a correlated and proportional variation in shape changes relative to size (e.g., Steudel 1982; Rilling and Seligman 2002; Mitteroecker et al. 2004; Bruner 2007; Zollikofer and Ponce de León 2010), and the mammalian cranium is known to be subject to allometry (Emerson and Bramble 1993; Marroig et al. 2009). In primates, it has been reported that small alterations in size can produce major changes in the shape of the skull (Singleton 2005). For example, allometry is associated with major differences in skull shape between howler monkeys and capuchins (Meloro et al. 2014). It is very likely that skull variation in colobines reflects allometric effects, but to what extent allometric effects have shaped the morphology of colobine skulls is still largely unknown.

The aim of the present study is to test the hypothesis proposed by Koyabu and Endo (2009, 2010) that seed eating explains most of the overall variation in the colobine skull. Covering, for the first time, all ten recognized genera, and 44 of 61 recognized species (Groves 2005), we provide the most extensive analysis of the patterns of morphological differentiation among colobines using geometric morphometric techniques. Furthermore, we examine the patterns of differentiation in relation to non-dietary factors such as phylogeny and allometry. Hence, size and shape variations are discussed in light of both phylogenetic history and local ecology, with a particular focus on seed consumption.

Materials and Methods

Sampling

In this study, 452 colobine specimens from different museums worldwide were analyzed (Table 1), representing 44 species and ten genera from Africa and Asia. The specimens are housed at the Natural History Museum (BMNH), Primate Research Institute of Kyoto University (KUPRI), Smithsonian National Museum of Natural History (USNM), and the Zoological Reference Collection of Lee Kong Chian Natural History Museum at the National University of

Table 1 List of the different genera and species considered in this study with the corresponding number of studied individuals

Genus	Species	*n*	Geographic clade	Mature leaves	Young leaves	Flowers	Fruits	Seeds	References for food items proportions
Colobus	*C. angolensis*	16	African	0.26	0.23	0.05	0.17	0.25	Maisels et al. (1994), Fimbel et al. (2001)
Colobus	*C. guereza*	22	African	0.15	0.70	0.01	0.08	0.02	Chapman et al. (2004), Harris and Chapman (2007)
Colobus	*C. polykomos*	11	African	0.27	0.30	0.03	0.03	0.35	Davies et al. (1999)
Colobus	*C. satanas*	5	African	0.03	0.35	0.09	0.12	0.41	Brugiere et al. (2002)
Colobus	*C. vellerosus*	14	African	0.40	0.34	0.06	0.10	0.10	Teichroeb et al. (2003)
Procolobus	*Pro. verus*	18	African	0.11	0.57	0.09	0.07	0.14	Davies et al. (1999)
Piliocolobus	*Pi. kirkii*	2	African	0.07	0.50	0.08	0.31	0.00	Mturi (1993)
Piliocolobus	*Pi. pennantii*	10	African	0.21	0.51	0.12	0.06	0.00	Clutton-Brock (1975)
Piliocolobus	*Pi. preussi*	2	African	NA	NA	NA	NA	NA	
Piliocolobus	*Pi. rufomitratus*	1	African	0.12	0.52	0.06	0.25	0.01	Marsh (1981)
Piliocolobus	*Pi. temminckii*	2	African	0.12	0.35	0.09	0.42	0.03	Starin (1991)
Piliocolobus	*Pi. tephrosceles*	11	African	0.22	0.57	0.07	0.00	0.08	Rode et al. (2003)
Piliocolobus	*Pi. tholloni*	1	African	NA	NA	NA	NA	NA	
Piliocolobus	*Pi. badius*	14	African	0.23	0.41	0.14	0.06	0.17	Davies et al. (1999), Struhsaker (1975)
Presbytis	*Pr. chrysomelas*	15	Asian	NA	NA	NA	NA	NA	
Presbytis	*Pr. comata*	8	Asian	0.06	0.65	0.06	0.14	0.00	Ruhiyat (1983)
Presbytis	*Pr. femoralis*	5	Asian	0.11	0.24	0.06	0.48	0.08	Curtin (1980)
Presbytis	*Pr. frontata*	4	Asian	NA	NA	NA	NA	NA	
Presbytis	*Pr. hosei*	17	Asian	NA	NA	NA	NA	NA	
Presbytis	*Pr. melalophos*	26	Asian	0.08	0.28	0.12	0.24	0.25	Bennett (1983)
Presbytis	*Pr. potenziani*	7	Asian	NA	NA	NA	NA	NA	
Presbytis	*Pr. robinsoni*	11	Asian	NA	NA	NA	NA	NA	
Presbytis	*Pr. rubicunda*	18	Asian	0.01	0.30	0.05	0.13	0.48	Davies (1991), Hanya and Bernard (2012), Ehlers Smith et al. (2013)
Presbytis	*Pr. siamensis*	23	Asian	NA	NA	NA	NA	NA	
Presbytis	*Pr. thomasi*	4	Asian	NA	NA	NA	NA	NA	
Trachypithecus	*T. auratus*	22	Asian	0.01	0.46	0.14	0.14	0.13	Kool (1993)
Trachypithecus	*T. barbei*	2	Asian	NA	NA	NA	NA	NA	
Trachypithecus	*T. cristatus*	21	Asian	NA	NA	NA	NA	NA	
Trachypithecus	*T. francoisi*	1	Asian	0.14	0.39	0.08	0.17	0.22	Zhou et al. (2006)
Trachypithecus	*T. germaini*	3	Asian	NA	NA	NA	NA	NA	
Trachypithecus	*T. obscurus*	47	Asian	0.23	0.36	0.07	0.32	0.03	Curtin (1976)
Trachypithecus	*T. phayrei*	21	Asian	0.00	0.49	0.21	0.07	0.23	Gupta and Kumar (1994)
Trachypithecus	*T. pileatus*	10	Asian	0.42	0.15	0.07	0.24	0.09	Stanford (1991)
Trachypithecus	*T. poliocephalus*	1	Asian	NA	NA	NA	NA	NA	
Pygathrix	*Py. nemaeus*	8	Asian	NA	NA	NA	NA	NA	
Pygathrix	*Py. nigripes*	8	Asian	0.00	0.40	0.09	0.11	0.40	Rawson (2009)
Rhinopithecus	*R. roxellana*	1	Asian	0.09	0.14	0.01	0.10	0.21	Liu et al. (2013) (including lichen 38%)
Rhinopithecus	*R. avunculus*	2	Asian	NA	NA	NA	NA	NA	
Semnopithecus	*Se. entellus*	2	Asian	NA	NA	NA	NA	NA	
Semnopithecus	*Se. johnii*	4	Asian	0.04	0.44	0.08	0.15	0.19	Sunderraj (2001)
Semnopithecus	*Se. priam*	2	Asian	NA	NA	NA	NA	NA	
Semnopithecus	*Se. vetulus*	8	Asian	0.06	0.19	0.06	0.53	0.04	Dela (2007)
Nasalis	*N. larvatus*	20	Asian	0.03	0.38	0.03	0.41	0.15	Bennett and Sebastian (1988)
Simias	*Si. concolor*	2	Asian	NA	NA	NA	NA	NA	

The geographic clade (Asian or African) is given, as well as the percentage of the different food items (mature leaves, young leaves, flowers, fruits and seeds) observed in their respective diets. The references to compile diet information are also given

Singapore (ZRC) (Online Resource 1). Captive individuals were not included, and all specimens were limited to wild-caught individuals. As a consequence of the sampling process in the wild (males were predominantly shot), too few females were available in the collections. The developmental age of the specimens was unknown; therefore, we were unable to test ontogeny-related variation. To avoid any major effects from this factor, patterns of differentiation were investigated at the interspecific and intergeneric level, and only specimens with fully erupted dentition were considered. Specimens showing any signs of pathology, or supernumerary teeth, were excluded.

Phylogenetic Relationships and Their Projection in the Morphospace

In this study, we compiled a composite tree of the 44 species (Fig. 1) presenting the commonly accepted relationships found in the literature between taxa, based on molecular analyses (Md-Zain et al. 2002; Meijaard and Groves 2004; Karanth et al. 2008; Osterholz et al. 2008; Roos et al. 2008; Ting 2008; Ting et al. 2008; Chatterjee et al. 2009; Meyer et al. 2011; Liedigk et al. 2012; Wang et al. 2015). These studies used different genetic markers, such as mitochondrial DNA alone (e.g. Ting 2008) or in association with nuclear markers (e.g. Karanth et al. 2008; Ting et al. 2008). Divergence dates were given as an indication (Fig. 1) and were obtained from Liedigk et al. (2012) for the Asian clade and Ting (2008) for the African clade. When no clear consensus was reached in the literature (e.g., *Trachypithecus* and *Semnopithecus*), we represented them as unresolved multiple branches. We also included *T. pileatus* in this phylogeny, but the topology here is tentative as this species is considered to be the result of hybridization between *Semnopithecus* and *Trachypithecus* (Wang et al. 2015).

Owing to the composite nature of this tree, and unresolved divergence dates, all terminal nodes were set as equal distances from the root, treating the tree as ultrametric. This allowed us to test for a statistically significant phylogenetic signal in our dataset (using lambda and Blomberg's K estimates), and to project the phylogenetic relationships between taxa in morphological space using the *phylomorphospace* function from the 'phytools' R package (Revell 2012).

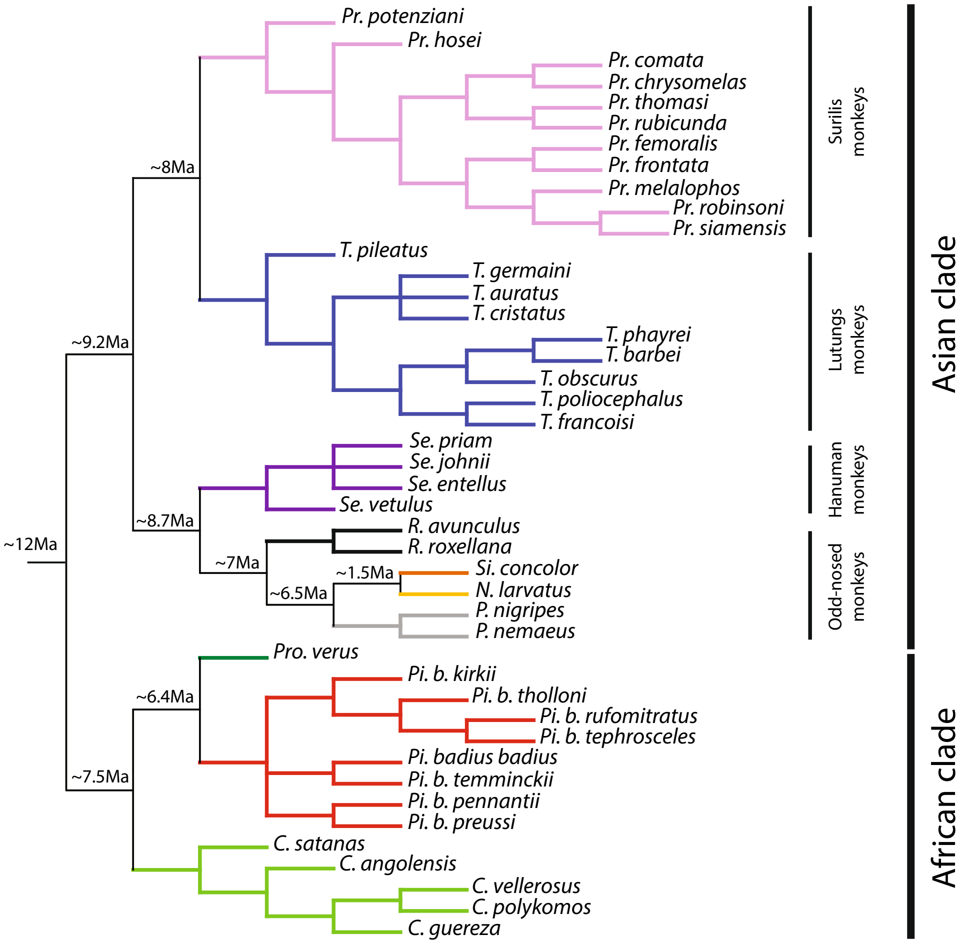


Fig. 1 Composite phylogenetic tree based on studies from the literature and considering multiple markers showing the relationships between the species included in our study. The age of divergence are indicated for selected nodes (Ting 2008; Liedigk et al. 2012). Branch colors represent each genus

Diet

Following Davies et al. (1999), the annual feeding frequencies on five distinct food categories (mature leaves, young leaves, flowers, seeds, and fruits) were considered in this study. Although we used dietary information from long-term observations (available for 26 species, Table 1), diet may vary among localities and observers. Thus, dietary data were averaged across studies when possible, following Koyabu and Endo (2009). Reports of animal matter in the colobine diet are extremely rare, therefore faunivory was assumed to be negligible. Furthermore, because taxonomic names have been confused for some species (*Pr. femoralis, Pr. melalophos*, and *Pi. badius, S. entellus, S. priam*), some previous studies assigned incorrect dietary information to species (e.g. Wright and Willis 2012). Therefore, the localities of field observations and species identity were carefully checked prior to their use in this study.

Mechanical Advantage Analyses and Comparisons with Diet

Coordinates of the muscle attachment positions, bite points, and temporomandibular joint (TMJ) were recorded using a Microscribe 3DX digitizer (Immersion Corp., San Jose, CA) to estimate the mechanical advantage for biting, based on Koyabu and Endo (2010). Landmark positions adopted in this study are illustrated in Fig. 2 and definitions provided in Online Resource 2. The mechanical advantage of the masticatory apparatus was calculated as the ratio between the length of the jaw muscle's moment arm and the length of the moment arm of the corresponding bite point in the occlusal plane. The mechanical advantage of canine biting in *Trachypithecus poliocephalus* was not calculated because the original canine of the only specimen available was lost. Bite-point moment arm lengths were measured from the center of the articular eminence to the center of the trigon basin of M^1 (molar biting) and P^3 (premolar biting), to the tip of the canine (canine biting), and to the point between the central incisors (incisor biting). Moment arm lengths of the masseter, temporalis and medial pterygoid muscles were measured in this study. The moment arm length of the masseter muscle was measured as the distance from the center of the articular eminence to the inferior edge of the malar at the most anterior point of attachment of the superficial masseter muscle. The moment arm length of the temporalis muscle was defined as the distance from the center of the articular eminence to frontotemporale. The moment arm length of the medial pterygoid muscle position was estimated as the distance from the center of the articular eminence to the pterygopalatine suture at the posterior edge of the palatine. In order to adjust for the vertical difference, the measurements described above were taken as projections onto the occlusal plane, defined by the point between the central incisors and the right and left centers of the trigon basin of M^1 (Spencer and Demes 1993; Wright 2005). We evaluated the correlation between the mean mechanical advantage of each species and the dietary information of each species, derived from long-term observations using phylogenetically-independent contrasts (Felsenstein 1985), using the PDAP module of Mesquite (Maddison and Maddison 2011). As noted earlier, because branch lengths were not available for all species, we used an ultrametric tree in this study, i.e. all terminal nodes were considered as equally distant from the root. The alpha level of significance was adjusted using Bonferroni corrections (Sokal and Rohlf 2012).

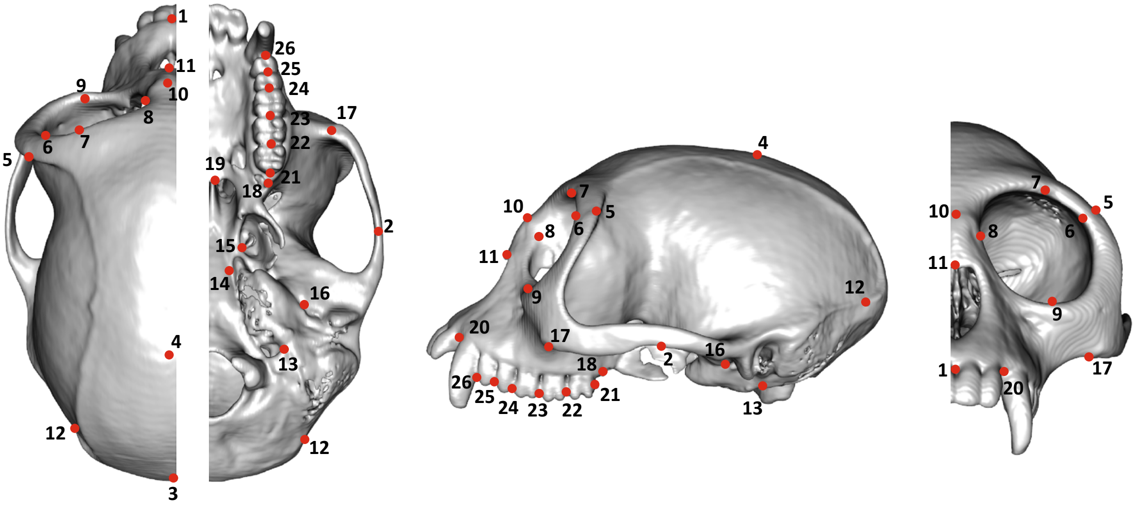


Fig. 2 Positions of the 26 fixed landmarks taken on the left side of the skull. Refer to Supplementary Table 2 in Online Resource 3 for definitions

Morphometric Data Collection and Statistics

For each specimen, three-dimensional Cartesian coordinates of 26 anatomical landmarks were digitized on the left side of the skull (Fig. 2, Online Resource 3) by a single observer (DK) using a Microscribe 3DX digitizer.

Differences in specimen positioning during data acquisition, as well as isometric variations in size, were corrected using a generalized Procrustes analysis (GPA) (Rohlf and Slice 1990). This GPA resulted in a set of standardized Procrustes coordinates, which were then considered for later statistical analyses. The centroid size (i.e., the square root of the sum of squared distances of each landmark from the centroid of the configuration) was also calculated and used as a size estimator. At a large taxonomic scale, in studies focusing on the skull, this parameter is considered to be a reliable proxy for body size (Damuth and MacFadden 1990). Even though we considered a more restricted taxonomic scale (i.e., Colobinae), the results obtained provide a good overview of the size trends existing in this group. However, a one-to-one relationship between centroid size and body mass remains to be tested and we therefore limit our interpretations to the centroid size, referred to hereafter as "size." Centroid size differences between genera and between species were investigated using Kruskal–Wallis tests for pairwise multiple comparisons (as the normality assumption was rejected using a Shapiro–Wilk's test), a nonparametric test for univariate data. All statistics and visualizations were performed in R (R-Core-Team 2016), using the packages *PMCMR* (Pohlert 2016), *ade4* (Dray and Dufour 2007), and *Morpho* (Schlager 2013).

The presence of allometry was first investigated using a linear regression between original Procrustes coordinates and centroid size. Allometric patterns of shape variation were then further studied by calculating the regression score and plotting it against the centroid size following Drake and Klingenberg (2008). The regression score corresponds to the projection of the data points in shape space onto an axis in the direction of the regression vector. It is the shape variable that has the maximal covariation with centroid size. Associated shape changes were visualized using color maps along the regression score by calculating hypothetical configurations at the 0.05 and 0.95 quantiles. The dependence of shape on size was then tested using the 'ffmanova' R package (Langsrud and Mevik 2012), with aligned shape coordinates as the response variable. The residuals of the regression between the aligned coordinates and centroid size were extracted and considered as size-adjusted variables in further analyses.

Patterns of size-adjusted shape differentiation were investigated using these residuals as shape variables, and were visualized using a between-group principal component analysis (bgPCA) with species as the grouping variable (Culhane et al. 2002; Renaud et al. 2015). Among-group differences were tested using permutation tests (Procrustes ANOVA; 9999 permutations) performed on the first 12 axes summarizing 90% of the total variance. Shape differences were visualized using the mean of deformation maps (extrapolated from fixed landmarks) between group means, or along shape axes by calculating hypothetical configurations at known coordinates. For each morphospace the phylogenetic relationships were projected on the bgPC axes using the 'phylomorphospace' function from the 'phytools' package.

Multivariate Regressions

Multivariate multiple regression (MMR) was used to assess the influence of size, phylogeny, and diet on the cranial shape variation. Centroid size was considered as size variable. Phylogeny variable was included as the first four meaningful axes of a principal coordinate analysis performed on the distance matrix of the composite tree using the R 'ape' package (Paradis et al. 2004). The feeding frequencies of five food items were included as diet variable. These three groups of variables were considered as independent variables to explain the dependent variable composed of the first five meaningful principal component axes calculated on the original Procrustes coordinates. The relationship between cranial geometry and diet was also assessed by two-block partial least-squares (2B-PLS) (Rohlf and Corti 2000), using MorphoJ. In 2B-PLS models there is no predictor or predicted variables, and both blocks of variables (here, cranial shape as Block 1 and diet as Block 2) are equally weighted. Then, vectors of maximum covariation between the blocks were extracted.

Results

Mechanical Advantage Analyses and Comparison with Diet

All values of measured moment arms and calculated mechanical advantages of the masseter, temporalis, and medial pterygoid muscles are shown in Online Resource 4. Our results show a strong overall consistency between bite points (incisor, canine, third premolar and first molar). Regarding the mechanical advantage of the masseter, *Pr. rubicunda* (incisor 0.64 and canine 0.67), *T. francoisi* (incisor 0.61 and canine 0.63), *Py. nigripes* (incisor 0.59 and canine 0.62), and *R. avunculus* (incisor 0.61 and canine 0.63) showed high values for anterior dentition. This indicates these species are more mechanically advantageous than other species when biting at the incisor and canine. *Pr. rubicunda* (premolar 0.78 and molar 0.99), *T. francoisi* (premolar 0.77 and molar 0.95), and *T. poliocephalus* (premolar

0.76 and molar 0.97) showed high values for the posterior dentition. These species appear to be more mechanically advantageous than other species when biting at the premolar and molar with the masseter. *Semnopithecus entellus* and members of *Piliocolobus*, such as *Pi. pennantii, Pi. tephrosceles*, and *Pi. tholloni*, exhibited a rather low mechanical advantage of the masseter at all bite points. *Pr. rubicunda* and *Pr. chrysomelas* showed high mechanical advantage of the temporalis at all bite points. On the contrary, *Nasalis, Simias* and *R. avunculus* showed low temporalis mechanical advantage at all bite points. Regarding the mechanical advantage of the medial pterygoid, *Pr. chrysomelas, Pr. robinsoni, T. phayrei*, and *Se. priam* showed high values, whereas *Pi. pennantii, T. francoisi*, and *R. roxellana* presented low values at all bite points.

The results of the correlation analyses between dietary items and mechanical advantages are given in Table 2. Among all food items, seeds showed the strongest level of correlation to mechanical advantages of the masticatory apparatus. Seeds showed a significant strong correlation with the mechanical advantage of the masseter for incisor biting (Fig. 3a, r = 0.64, $P = 0.0004$) and canine biting (Fig. 3b, r = 0.61, $P = 0.0009$). P^3 biting ($r = 0.56$, $P = 0.0026$) and M^1 biting ($r = 0.55$, $P = 0.0035$) showed moderate correlations with seed intake, but these were not statistically significant after Bonferroni corrections. Fruits exhibited a

Table 2 Results of correlation test between dietary items and mechanical advantages

	Mature leaves		Young leaves		Flowers		Fruits		Seeds	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Masseter mechanical advantage										
At incisor	– 0.33	0.0940	– 0.29	0.1547	0.07	0.7503	– 0.10	0.6181	**0.64**	**0.0004**
At canine	– 0.28	0.1716	– 0.32	0.1138	0.14	0.4875	– 0.08	0.6955	**0.61**	**0.0009**
At P3	– 0.27	0.1758	– 0.30	0.1300	0.11	0.5763	– 0.05	0.7962	0.56	0.0026
At M1	– 0.26	0.2046	– 0.30	0.1318	0.11	0.5892	– 0.07	0.7230	0.55	0.0035
Temporalis mechanical advantage										
At incisor	– 0.12	0.5597	– 0.27	0.1813	– 0.03	0.8791	0.11	0.6070	0.05	0.7967
At canine	– 0.09	0.6705	– 0.28	0.1584	0.00	0.9862	0.12	0.5450	0.03	0.9025
At P3	– 0.09	0.6555	– 0.28	0.1666	0.00	0.9696	0.14	0.4889	0.00	0.9816
At M1	– 0.10	0.6352	– 0.29	0.1508	0.00	0.9734	0.13	0.5190	0.02	0.9160
Medial pterygoid mechanical advantage										
At incisor	– 0.40	0.0458	0.02	0.9162	0.07	0.7519	0.43	0.0277	0.02	0.9152
At canine	– 0.34	0.0879	0.00	0.9821	0.12	0.5754	0.44	0.0240	– 0.01	0.9497
At P3	– 0.37	0.0608	0.00	0.9666	0.11	0.5763	0.49	0.0110	– 0.04	0.8566
At M1	– 0.38	0.0532	– 0.02	0.9414	0.13	0.5421	0.48	0.0142	0.00	0.9841

Those found as statistically significant after Bonferroni correction ($P < 0.05/60$) are given in bold

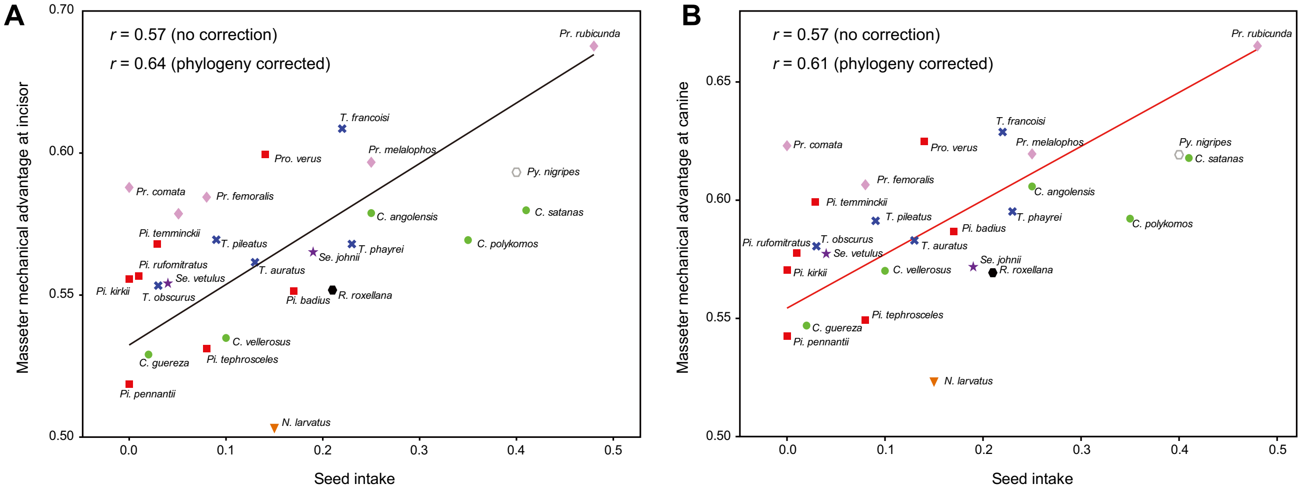


Fig. 3 Plots presenting the frequency of seed intake and mechanical advantage of the masseter at the incisor (**a**) and canine (**b**) of each species. Non-corrected and phylogeny-corrected Pearson's correlation coefficients are also given

weak correlation to the mechanical advantages of the medial pterygoid at all bite points, although not significant under the Bonferroni criterion. The mechanical advantages of the temporalis presented no significant correlation with any of the food items. In addition, all mechanical advantage values were negatively correlated with centroid size (Online Resource 5).

Size Differences

Overall, size was relatively homogeneous within each genus. Some species included in our analysis were rare, and in order to reach a minimum number of specimens for better statistical resolution, we tested for size differences (using Kruskal–Wallis tests) at the genus level (Table 3). However, as inter-generic tests could mask the underlying variation of size between species we chose to plot size differences between species (Fig. 4) and we also provide inter-specific Kruskal–Wallis tests (Online Resource 6). As no significant difference in size was evidenced between species within each genus, we considered that investigating size differences at the genus level was representative.

Among African clades, *Colobus* and *Piliocolobus* presented no difference in size ($P = 0.436$), while *Procolobus* was significantly smaller than the other two ($P < 0.001$).

Among Asian taxa, *Presbytis* was the smallest, being significantly smaller than most of the other genera ($P < 0.001$), with the exception of *Simias* ($P = 0.624$). Its average size is similar to the African *Procolobus* ($P = 0.903$). *Trachypithecus* also displayed a small size on average, with an important intrageneric variation. This variation can be mostly explained by the larger size of *T. pileatus* which lay outside of the range of other *Trachypithecus* species. Because of a high degree of intrageneric variation and low sample size, *Semnopithecus* appeared as significantly different in size only compared to *Presbytis* ($P < 0.001$) and *Procolobus* ($p < 0.001$).

Within odd-nosed monkeys, the two recently diverged sister genera, *Simias* and *Nasalis*, were morphometrically different. Only two *Simias* specimens were available for this study, but given our observations on thirteen additional specimens, which were not included in this study because of partial damage, we assume that our *Simias* specimens were not unusually large or small for this genus (Online Resource 7). Our results are in accordance with previous descriptions of those taxa: *Nasalis* is considered a large species, while *Simias* is considered an average-size species (Jablonski 1998). This contrasts with their close phylogenetic relationship and recent date of divergence. On the contrary *Pygathrix* and *Rhinopithecus*, which are closely related to each other, are similar in size ($P = 0.999$). Overall, with the exception of *Simias*, the odd-nosed monkeys include the largest taxa within the colobines.

Allometric Patterns

Using a multivariate regression of size on the original Procrustes coordinates, we observed a moderate influence of allometry on our dataset ($P < 0.001$, $R^2 = 0.18$). Plotting the regression score (i.e., shape variable) as a function of the centroid size (Fig. 5a, Online Resource 8), we observed that while most of the genera were well-aligned along the regression line ($P < 0.001$, $R^2 = 0.72$), some taxa such as *Procolobus* ($n = 18$), *Colobus* ($n = 68$), and *Simias* ($n = 2$) were slightly shifted above the regression line. Looking at the reconstructions performed along the regression and calculated at the 0.05 and 0.95 size quantiles (Fig. 5b), we saw that the larger the specimen, the less protrusive the orbits and flatter the cranium (evidenced by a downward shift of landmark 4, the bregma). The top posterior part of the parietal was, on the contrary, slightly more rounded. The zygomatic arch was shifted medially in larger taxa. Those changes were associated with an elongation of the anterior region of the maxilla.

Table 3 Inter-generic comparison of size using Kruskal–Wallis tests

	Colobus	*Nasalis*	*Piliocolobus*	*Presbytis*	*Procolobus*	*Pygathrix*	*Rhinopithecus*	*Semnopithecus*	*Simias*
Nasalis	0.4654								
Piliocolobus	0.4357	**0.0095**							
Presbytis	**<0.0001**	**<0.0001**	**<0.0001**						
Procolobus	**<0.0001**	**<0.0001**	**<0.0001**	0.9031					
Pygathrix	0.9999	0.9621	0.6415	**<0.0001**	**<0.0001**				
Rhinopithecus	0.9987	1	0.8872	**0.0005**	**0.0001**	0.9999			
Semnopithecus	0.9999	0.9999	0.9318	**0.0001**	**<0.0001**	1	1		
Simias	0.9944	0.8082	1	0.6236	0.342	0.9853	0.9607	0.9818	
Trachypithecus	**<0.0001**	**<0.0001**	0.0232	**<0.0001**	**<0.0001**	**0.0005**	0.2337	0.2175	0.9999

Significant probabilities (i.e. $P < 0.001$) are in bold

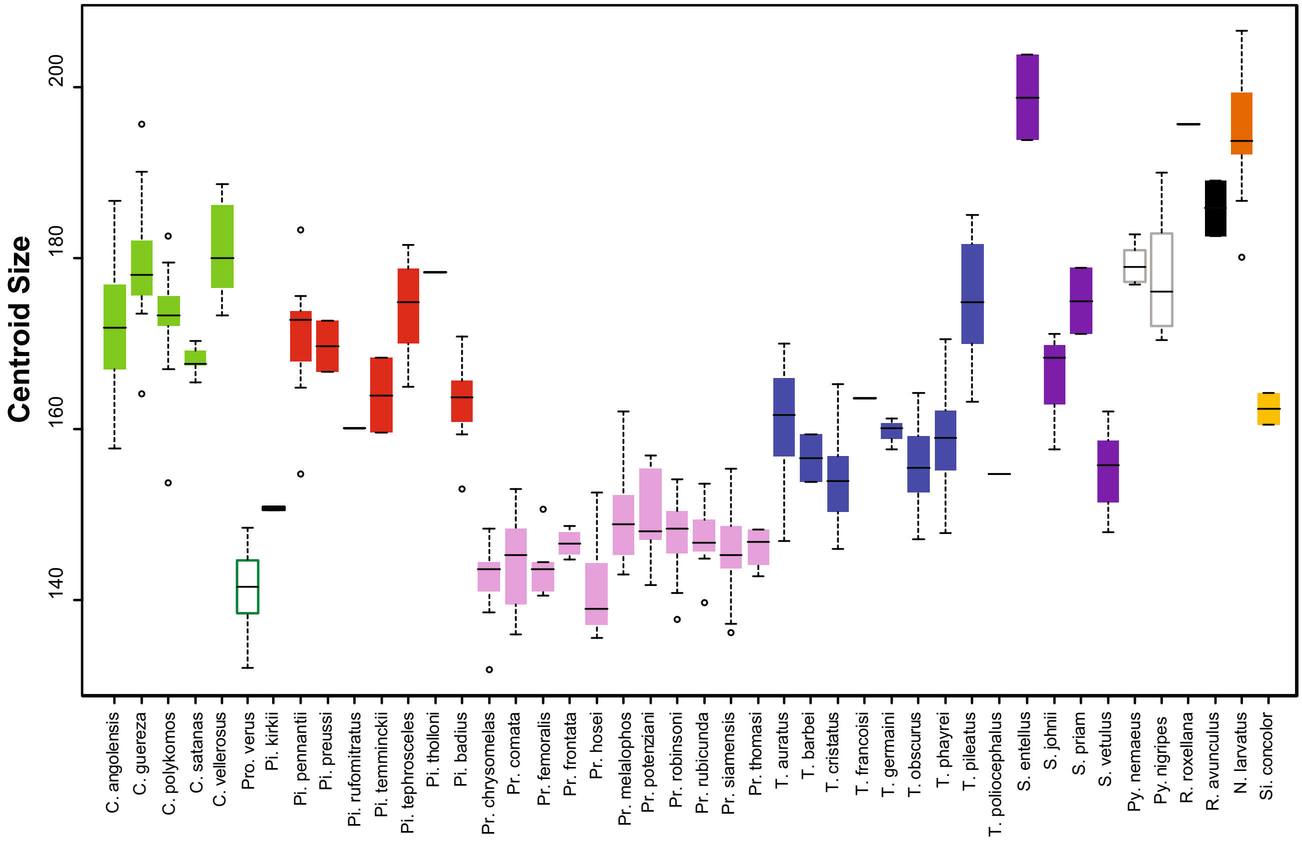


Fig. 4 Histogram showing differences in centroid size between the 44 species considered in this study. The confidence interval is provided, and outliers are represented by empty circles

On the contrary, *Rhinopithecus* displayed a larger size than expected from their morphology and were clearly shifted below the regression line. A similar pattern was observed within *Semnopithecus, S. entellus* being similar in size to *Nasalis*, while its morphology was less extreme along the regression score. We also noted that the extended variation of *Trachypithecus* toward larger sizes was mostly driven by *T. pileatus*, which is an unusually large species among *Trachipithecus*.

Phylogenetic Patterns

Patterns of morphological variation between taxa were further investigated based on a between-group analysis performed on size-adjusted variables, with species as the grouping variable. All genera taken together were significantly different ($P < 0.001$, $R^2 = 0.783$), and clear interspecific differences emerged when plotting the first axes of the bgPCA (Fig. 6). This was confirmed using pairwise comparisons between genera with Procrustes ANOVAs performed on the first 12 axes of the bgPCA (summarizing > 90% of the total variance) and displaying highly significant probabilities (Table 4). In addition, the existence of a phylogenetic signal was confirmed by Blomberg's K tests (bgPC1: K = 1.43, $P = 0.001$; bgPC2: K = 1.07, $P = 0.001$; bgPC3: K = 0.78, $P = 0.001$) and Lambda tests (bgPC1: Lambda = 0.79, $P < 0.001$; bgPC2: Lambda = 0.99, $P < 0.001$; bgPC3: Lambda = 0.99, $P < 0.001$).

When considering the first three axes of the bgPCA, the African groups were clearly differentiated according to phylogeny: *Colobus* along bgPC1, and *Procolobus* and *Piliocolobus* along bgPC3 (Fig. 6a–b). Morphologically, for *Colobus* this differentiation involved a more prominent maxilla associated with a smaller neurocranium overall (Fig. 6c). The posterior region of the zygomatic was wider. The orbits were also slightly shifted posteriorly. Concerning *Piliocolobus* and *Procolobus*, the differentiation along bgPC3 corresponded to anteriorly shifted orbital and nasal regions. Similar to *Colobus*, the bregma region was flatter.

Within the Asian group, *Presbytis* and *Trachypithecus* were clearly differentiated along bgPC2 (Fig. 6a–b), moving from, respectively, a more elongated and flattened cranium to a less broad cranium displaying a shorter face and rounder superior part (Fig. 6c, Online Resource 8). *Semnopithecus* appeared to be intermediate between them. Concerning the odd-nosed monkeys, *Rhinopithecus* and *Pygathrix* were

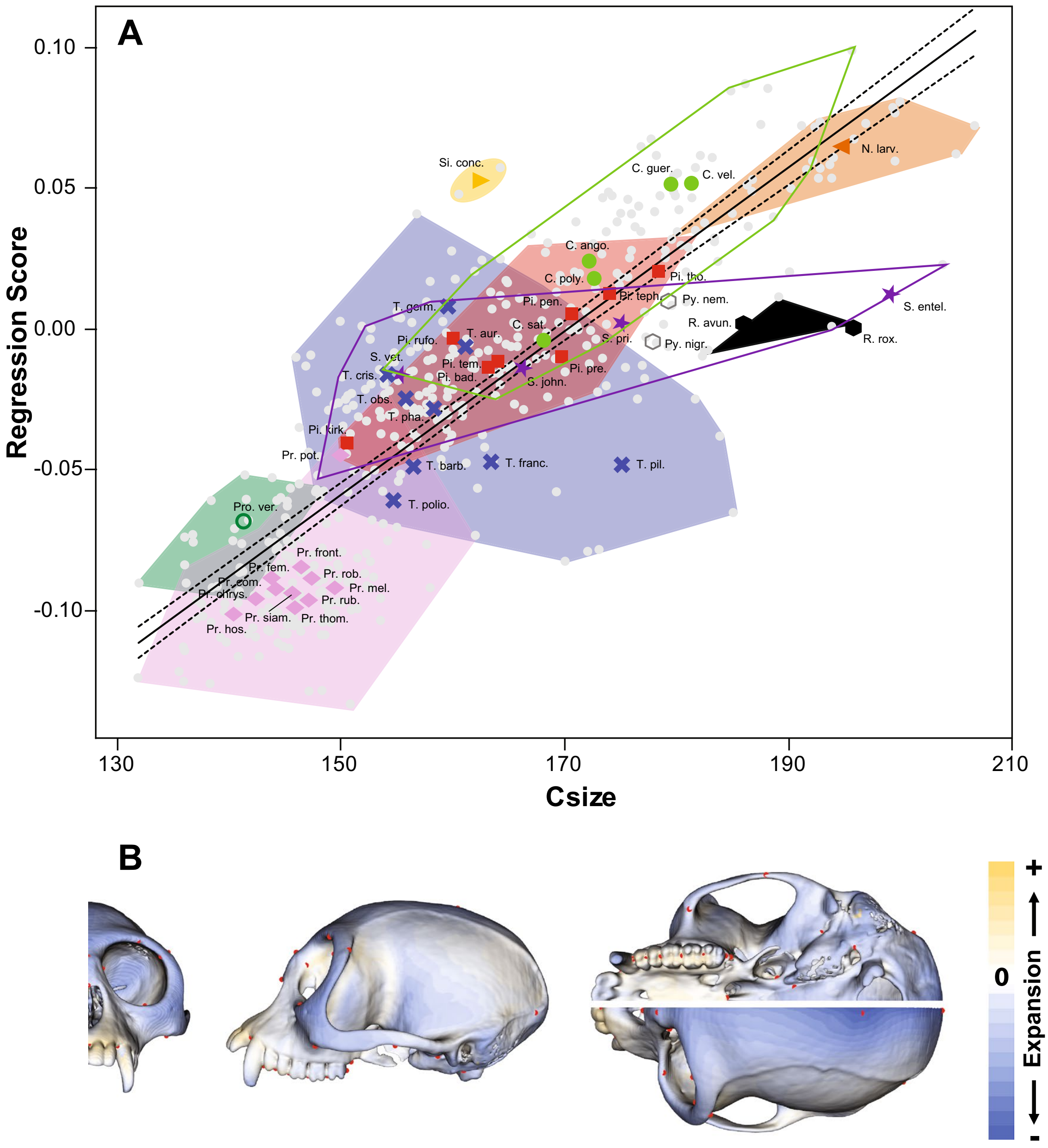


Fig. 5 **a** The regression score (Drake and Klingenberg 2008) is plotted as a function of centroid size. The black line represents the regression line and the two dashed lines the 95% confident interval. Each grey dot represents a single specimen and colored dots of different symbols represent the mean per species. Abbreviated names of the species are given at proximity of their respective mean. **b** Distance map showing shape differences associated to the allometric effect. The original landmarks used to build the distance map are shown as red dots. The color scale ranges from blue (i.e. compression) toward yellow (i.e. expansion). Deformations localized at each landmark, without interpolation, can be found in Online Resource 8. (Color figure online)

opposite to the African genera along bgPC1 (short face, small neurocranium), and were differentiated from one another on the bgPC2-bgPC3 plane. Finally, *Simias* and *Nasalis* genera clearly diverged along bgPC1 despite relatively recent speciation (Fig. 1), and they strongly segregated from other genera in the bgPC2-bgPC3 plane, at the opposite end from other odd-nosed monkeys.

Multivariate Regressions

MMR was built to determine the proportion of cranial shape variation explained by phylogeny, size, and diet. As a result, the model indicated that phylogeny is the first order signal, explaining 28.8% of total variation, while the second and third order signals were respectively diet (4.9%) and size

(3.3%). All three variables were determined to be contributing significantly to cranial shape variation ($P < 0.001$).

A 2B-PLS was performed between the Procrustes coordinates and the matrix composed of the feeding frequencies for each food item. Only specimens for which diet information was available were considered (i.e. 325 specimens representing 26 species). Basic statistics of 2B-PLS are summarized in Table 5. Covariance explained by PLS1 was 53.6%, PLS 2 was 28.3%, PLS 3 was 14.6%, PLS 4 was 2.6%, cumulatively explaining 99.1% of total variance. The correlation coefficient between Block 1 (morphology variable) and Block 2 (diet variable) was $r = 0.50$ ($p = 0.0101$) in PLS1, $r = 0.63$ ($p = 0.0005$) in PLS2, $r = 0.53$ ($p = 0.0053$) in PLS3, and $r = 0.50$ ($p = 0.0001$) in PLS4 (Table 5; Fig. 7). Among the first two major PLS, Block 2 of PLS1 was loaded particularly by mature leaves (0.65) and fruits (− 0.64), and Block 2 of PLS2 was loaded particularly by seeds (− 0.73) and young leaves (0.53) (Table 5).

Discussion

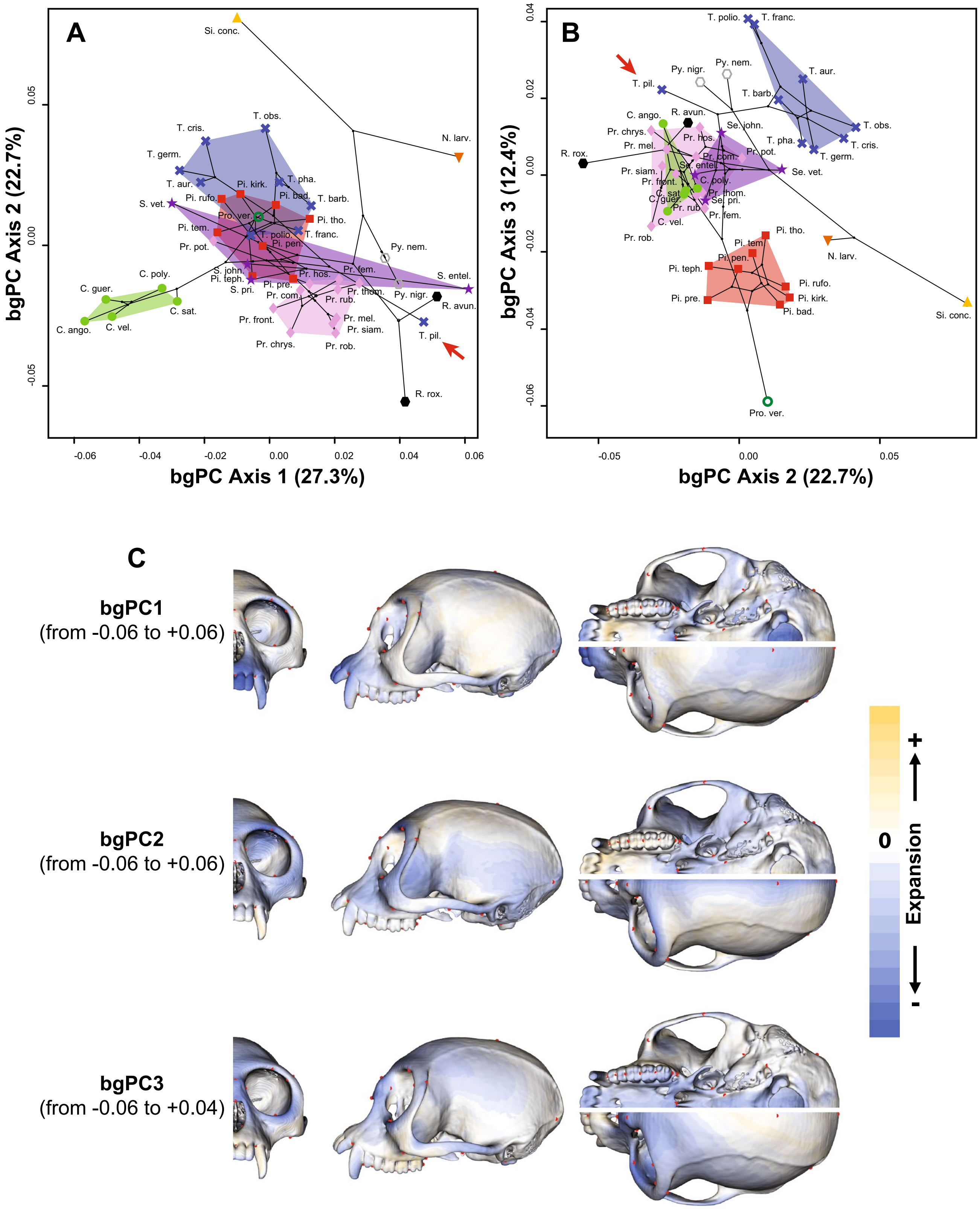
Size Differences Contribute to Interspecific Coexistence Between Closely Related Species

Being univariate and straightforward in its analysis and visualization, size is a parameter routinely investigated in biogeographic studies (Bergmann 1848, see; Meiri and Dayan 2003 for a review concerning birds and mammals). In our study, although intraspecific patterns of size (estimated by the centroid size of the cranium) could not be investigated owing to a lack of information about the precise origins of the specimens, we still observed marked differences at the intergeneric level depending on their geographical origin and local environment.

African colobines are often found in sympatry, which is the case, for example, of *Colobus polykomos, Pi. badius* and *Pro. verus* at Taï (Davies et al. 1999; Teichroeb et al. 2003), *C. angolensis* and *Pi. badius* at Salonga (Maisels et al. 1994), *C. guereza* and *Pi. tephrosceles* at Kibale (Oates 1994), or *C. polykomos*, and *Pi. temminckii* at Cantanhez (Minhós et al. 2015). For sympatric species, several mechanisms aimed at reducing the negative effects of competition have been described, including, for example, behavioral changes (Snaith and Chapman 2008), or morphological modification of the feeding apparatus allowing a differential exploitation of resources (Dayan and Simberloff 1998; Simberloff et al. 2000; Ledevin et al. 2012).

Our results showed a major size difference between *Procolobus* and the two other African genera (*Piliocolobus* and *Colobus*). Phylogenetically, *Colobus* are the most basal African genus, and the *Procolobus-Piliocolobus* divergence occurred ~ 1.1 million years after their differentiation (Ting 2008, Fig. 1). The geographic distribution of *Pro. verus* is similar to that of *Colobus vellerosus*, and their diet is similar, relying on mature and young leaves (Davies et al. 1999; Teichroeb et al. 2003); therefore, the small body size of *Pro. verus* may have facilitated the sympatric coexistence of the two species. Oates (1988) previously pointed out a possible link between the small body size of *Pro. verus* and its high selectivity on young leaves. From 21 months of observations at Tiwai forest in Sierra Leone, Oates (1988) found that the diet of *Pro. verus* is particularly dominated by young leaves, comprising up to 59% of its feeding. It was pointed out that *Pro. verus* (approximately 4 kg body weight at Tiwai) may have evolved in competition with its close, but much larger, relative *Pi. badius* (approximately 8 kg at Tiwai), with both living broadly in sympatry in West Africa. Size differentiation, and consequently niche separation, may have occurred in the two species. Generally, in mammals, larger body size allows for relatively reduced energy requirements, longer passage rates, and thorough digestion, therefore facilitating the use of lower quality forage (Parra 1978). The larger size of *Pi. badius* should therefore facilitate digestion of mature leaves, which generally requires more time to digest and include less protein (Huang et al. 2010; Lucas et al. 2001; Milton 1979). This food item is more frequently observed in its diet than in the smaller *Pro. verus* (Table 1). Members of the small-sized Asian *Presbytis* occupy a similar size range to *Procolobus*. Similar to *Pro. verus*, the annual diet of *Presbytis* appears to include only a small amount of mature leaves, ranging from 1% (*Pr. rubicunda*) to 11% (*Pr. femoralis*) and they feed more frequently on other food items such as young leaves, fruits, or seeds. We postulate that the small size of *Presbytis* constrains it to be highly selective in its choice of food, as with *Pro. verus*.

Insularity can also be responsible for unexpected size differences because of peculiar and local evolutionary processes (Raia and Meiri 2006). Known as the “Island rule” (Van Valen 1973), this effect has been interpreted in different ways, with a combination of many factors governing body size evolution in an insular context. Among these factors, a reduced predation pressure allowing large species to attain smaller sizes (Sondaar 1977; Sinclair et al. 2003), or resource shortage on small islands leading to smaller body sizes (Lomolino 1985; Burness et al. 2001) have been proposed. *Piliocolobus kirkii*, endemic to Unguja Island in Tanzania, is known to present remarkably small size compared to other closely related species, possibly resulting from insularity (Nowak et al. 2008). Although our study included only two *Pi. kirkii* specimens, our results are in agreement with this previous observation. Our study also evidenced a case of a relatively recent, but drastic, diminution in size within the odd-nosed monkeys (i.e. members of *Pygathrix, Rhinopithecus, Nasalis*, and *Simias*). Southeast Asia presents a wide variety of environments, including small



insular ecosystems that were colonized by colobines. Our results show that *Si. concolor* presented a surprisingly small size compared to other odd-nosed monkeys, particularly in comparison to its sister-species *N. larvatus* (divergence ~ 1.5 Ma) (Liedigk et al. 2012). Skulls of *Si. concolor* are extremely rare in collections and have rarely been studied

◂**Fig. 6** **a**–**b** Plots of the first three axes of a between-group principal component analysis (bgPCA) performed on size-adjusted variables with the species as grouping variable. Each dot represents a mean by species, and all species belonging to a single genus are grouped together using manual outlining. **c** Distance map showing shape differences associated along the first three axes of the bgPCA from negative to positive values (bgPC1, bgPC2 and bgPC3). The original landmarks used to build the distance map are shown as red dots. The color scale ranges from blue (i.e. compression) toward yellow (i.e. expansion). Deformations localized at each landmark, without interpolation, can be found in Online Resource 8. (Color figure online)

in a comparative context. We explain the notable difference found between *Simias* and other odd-nosed taxa by the size of their home islands: the first one living in Borneo (area: 743,330 km^2) (Matsuda et al. 2009), while *Simias* is found on the much smaller islands of the Mentawai Archipelago (area of largest island ~ 4000 km^2) (Watanabe 1981). *Nasalis* is known as a foregut fermenter in which regurgitation and remastication (i.e. rumination) were observed in the wild (Matsuda et al. 2014). *Nasalis* appears to have one of the largest skulls among Asian colobines (Fig. 4), and its large body mass is arguably related to its diet and "ruminant-like strategy" (Matsuda et al. 2014). Thus, both insularity in *Simias* and dietary adaptation in *Nasalis* may have resulted in the contrasting size difference between the closely related *Simias* and *Nasalis*.

Allometric Patterns

MMR indicated that size explains 3.3% of whole shape variation. The reconstructions we made to visualize the allometric effect in our dataset showed a relative elongation of the face with increasing cranial size, associated with an overall flattening of the cranium (Fig. 5). This pattern of facial elongation with increasing size has been established for cercopithecines and more generally for cercopithecoids (e.g. Singleton 2002). Although allometric constraints are evident among colobines, it appears that some taxa depart from this trend. For example, two genera (*Rhinopithecus* and *Simias*) deviated notably from the 95% CI of the regression line. Other genera appeared shifted above (e.g., *Procolobus*), or below (*Presbytis*), the regression line describing the allometric constraints. We speculate that such deviation is partly associated with dietary specialization, as discussed later. Strikingly, *N. larvatus*, which is known for their long face (Benefit and McCrossin 1991), does not deviate significantly from the allometric trend line, suggesting that their long face is mostly a result of allometric constraints.

When the allometric pattern of shape variation is interpreted in the light of the results obtained on the mechanical advantage analysis, we observed a negative correlation between size and mechanical advantage for all measurements, suggesting the presence of allometric constraints on mechanical advantage. For instance, large-bodied *N. larvatus* and *C. guereza* showed very low mechanical advantages for biting (Fig. 3, Online Resource 4). These results support the proposition made on cercopithecines that a decrease in mechanical advantage occurs as the face becomes proportionally longer by allometric constraints (Singleton 2005).

Shape Changes Primarily Reflect Phylogeny

Overall, the phylogenetic signal in colobine cranial shape variation was clearly supported by Blomberg's K tests and Lambda tests. Phylogeny was indicated to explain 28.8% of whole shape variation by MMR, being much more influential than diet and size. From their initial radiation ~ 12 million years ago (Liedigk et al. 2012), colobines became a highly diversified subfamily comprising 61 species in ten genera (Groves 2005). The most recent species diverged ~ 1.5 Ma in Southeast Asia and our results showed that even on this timescale, strong morphological differences have emerged. Asian colobines display a much greater craniodental variation compared to African monkeys. Some genera are strongly diverged and stand in the extreme range of phenotypic variation (e.g., *Rhinopithecus* and *Pygathrix* along bgPC1, or *Nasalis* and *Simias* along bgPC1 and bgPC2). This is not surprising as Asian colobines are found in a wider range of habitats, with highly variable environmental conditions such as swamps, tropical or temperate forests, high or low altitude landscapes, mainland or islands (Kirkpatrick and Grueter 2010; Ehlers Smith et al. 2013). Contrary to what one would expect, the first order signal (bgPC1) was not a dichotomy between African and Asian clades. It reflected mostly the within-Asia variation, differentiating *Presbytis, Trachypithecus, Pygathrix*, and *Nasalis*. Although not an Asian clade, the African *Colobus* is also clearly separated from other clades along this axis. *Procolobus* and *Piliocolobus* are not well differentiated from the Asian clade along this axis. bgPC2 separated *Colobus* from other African clades, and bgPC3 also reflected the *Colobus-Piliocolobus-Procolobus* differentiation.

The phylogenetic position of *T.* pileatus has been a focus of dispute in the studies of colobine evolutionary history (Roos et al. 2008; Lavrenchenko 2014; Wang et al. 2015). Recently, a surprising case of intergeneric hybridization between *Semnopithecus* (dispersing in the Indian subcontinent) and *Trachypithecus* (found from mainland southeast Asia to the Sundaland) have been evidenced (Roos et al. 2008; Wang et al. 2015). In particular, it has been proposed that *T. pileatus* originated from a unidirectional introgression hybridization process that occurred between *Semnopithecus* and *Trachypithecus* (Lavrenchenko 2014; Wang et al. 2015). The overlap in their range of geographic distribution in Bhutan, Bangladesh, and northeast India, and an analysis of full-length

Table 4 Tests of shape differences between genera using a Procrustes ANOVA (function procD.lm from the geomorph package) considering the first 12 axes of a between-group principal component analysis performed on the Procrustes coordinates

	Colobus	*Nasalis*	*Piliocolobus*	*Presbytis*	*Procolobus*	*Pygathrix*	*Semnopithecus*	*Trachypithecus*
Colobus		$R^2=0.231$	$R^2=0.113$	$R^2=0.178$	$R^2=0.092$	$R^2=0.128$	$R^2=0.043$	$R^2=0.213$
Nasalis	$P<0.0001$		$R^2=0.102$	$R^2=0.105$	$R^2=0.075$	$R^2=0.065$	$R^2=0.079$	$R^2=0.112$
Piliocolobus	$P<0.0001$	$P<0.0001$		$R^2=0.081$	$R^2=0.023$	$R^2=0.073$	$R^2=0.020$	$R^2=0.089$
Presbytis	$P<0.0001$	$P<0.0001$	$P<0.0001$		$R^2=0.087$	$R^2=0.042$	$R^2=0.030$	$R^2=0.201$
Procolobus	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$		$R^2=0.090$	$R^2=0.045$	$R^2=0.094$
Pygathrix	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$		$R^2=0.046$	$R^2=0.073$
Semnopithecus	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$		$R^2=0.046$
Trachypithecus	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	

The corresponding R^2 is given

Table 5 Basic statistics of two-block partial least-squares

	PLS1	PLS2	PLS3	PLS4	PLS5
Loadings against					
Mature leaves	0.65	− 0.09	0.50	− 0.57	0.08
Young leaves	0.32	0.53	− 0.68	− 0.35	− 0.18
Flowers	− 0.17	0.13	− 0.12	− 0.19	0.95
Fruits	− 0.64	0.40	0.34	− 0.52	− 0.23
Seeds	− 0.23	− 0.73	− 0.39	− 0.50	− 0.09
Covariation explained	53.59%	28.26%	14.57%	2.64%	0.95%
Correlation between blocks (r)	0.49	0.64	0.53	0.69	0.40
P value for the correlation coefficient	0.0101	0.0005	0.0053	0.0001	0.0437

Block 1 was composed of morpholoigcal variables and Block 2 was composed of dietary variables

nuclear mitochondrial DNA segments, estimated this hybridization to be the result of a short-term event that occurred relatively recently ~ 3.5 million years ago (Wang et al. 2015). *Semnopithecus entellus* represents one of the largest species among Asian colobines, and the unusually large size of *T. pileatus* is argued to be a result of genetic inheritance to *Semnopithecus* (Wang et al. 2015). Our results are congruent with this proposition, considering both size and shape parameters. The larger size of *T. pileatus* relative to other *Trachypithecus* species has already been described in previous studies (Delson et al. 2000; Wang et al. 2015); however, our study is the first to reveal the clear divergence of *T. pileatus* from other *Trachypithecus* and its morphometric resemblance to *Semnopithecus*. In the morphological space defined by the bgPC1-bgPC2 axes, this species is more similar to *S. entellus* than to any of the other *Trachypithecus* species. This is in agreement with genetic studies that suggest *S. entellus* played a role in the introgression event (Roos et al. 2008; Wang et al. 2015), highlighting the importance of considering a combination of morphometric and genetic approaches when focusing on hybridization processes.

Diet and Cranial Morphology

MMR indicated that diet explains 4.9% of whole shape variation. Comparing the craniodental morphologies of five species of African and five species of Asian colobines, Koyabu and Endo (2009, 2010) proposed that craniodental configurations in colobines, and more particularly the mechanical advantage of the masseter for biting, are linked to seed eating. We found that species with a high proportion of seeds in their diet exhibited greater mechanical advantage of the masseter muscle for all studied bite points. Biting at the anterior dentition showed a strong and significant correlation with seed eating ($r=0.62$ for the incisor and $r=0.63$ for the canine; Fig. 3). On the other hand, postcanine bite points showed weaker and nonsignificant correlations with seed eating ($r=0.57$ for P^3, $r=0.55$ for M^1; Table 2). However, our 2B-PLS analysis supported the hypothesis that cranial shape is significantly associated with all food items (Table 5; Fig. 7, Online Resources 8). Block 2 of PLS1, which showed a significant correlation to Block 1 of PLS1 ($r=0.50$, $p=0.0101$), was loaded mainly by the frequencies of mature leaves (0.65) and fruits (− 0.64), suggesting that PLS1 indicates the contrast of leaf eaters vs. fruit eaters.

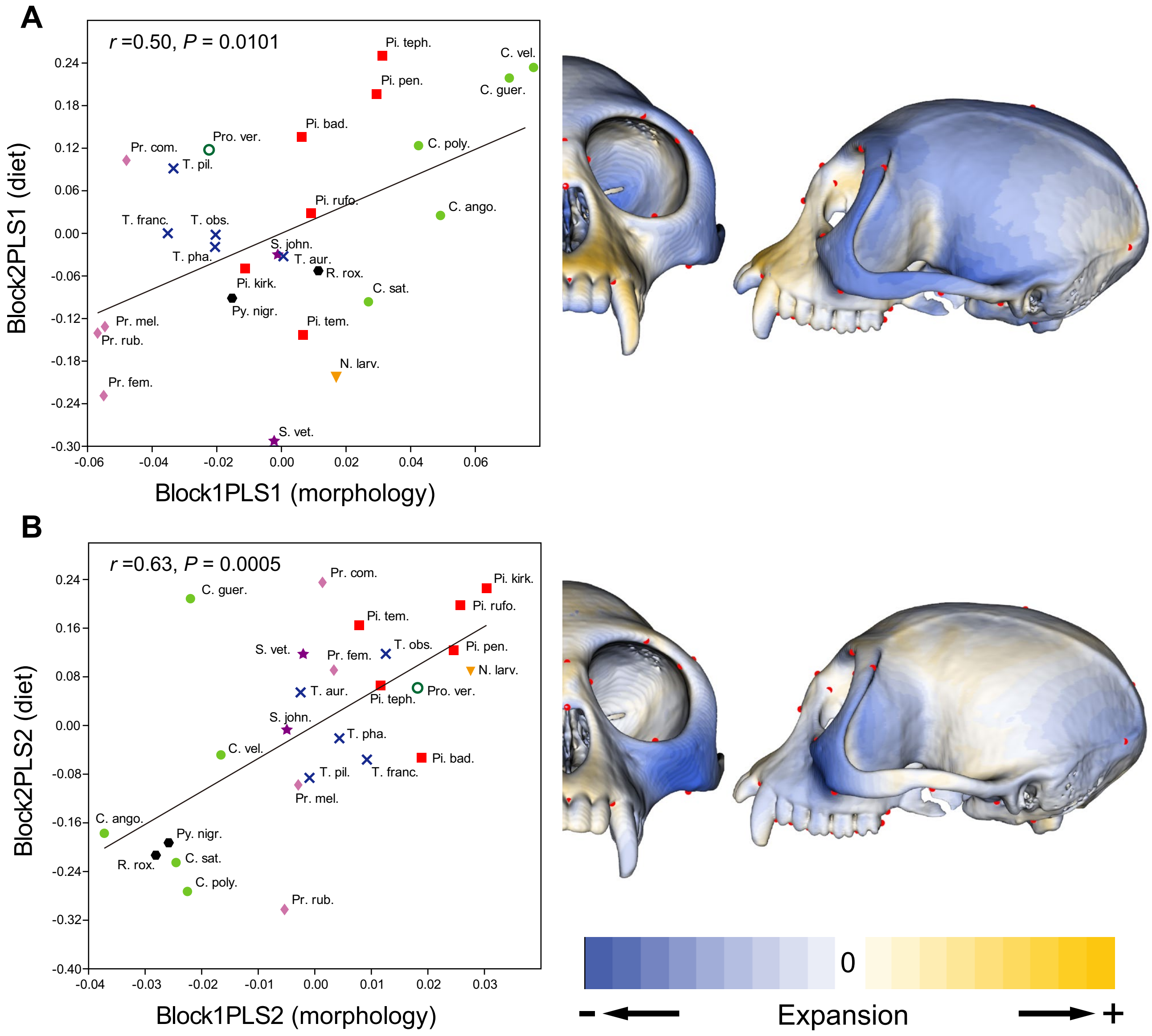


Fig. 7 Plots of the first two pairs of 2B-PLS for morphology and diet (*x*-axis is morphology block, *y* axis is diet block) and distance maps showing shape differences associated along the first two axes of 2B-PLS. **a** Plots and distance map for PLS1. **b** Plots and distance map for PLS2. The original landmarks used to build the distance map are shown as red dots. The color scale ranges from blue (i.e. compression) toward yellow (i.e. expansion). Deformations localized at each landmark, without interpolation, can be found in Online Resource 8. (Color figure online)

Block 2 of PLS2, which showed a significant correlation to Block 1 of PLS2 ($r = 0.63$, $p = 0.0005$), was loaded mainly by the frequencies of seeds (0.73) and young leaves (– 0.53), suggesting that PLS2 reflects the degree of seed eating. Correlation between Block1 (morphology) and Block 2 (diet) was the highest in this PLS2. These results suggest that the overall craniodental shape variation clearly reflects the degree of seed eating but also that correspondence to other food items is also evident. Such ecomorphological patterns for food items possibly explain, in part, the non-phylogenetic and non-allometric variation in the colobine skull. PLS1, which strongly reflected the frugivory vs. folivory ditochomy, indicated that more folivorous species are characterized by a taller dental crown and anteroposteriorly longer postcanine dentition while more frugivorous species tend to exhibit wider face and more anteriorly positioned malar (Fig. 7a, Online Resources 8). PLS2, which strongly reflect seed eating, showed that seed eaters exhibit a more anteriorly protruded alveolar region, wider lower facial region, and more posteriorly shifted dentition relative to the temporomandibular joints (Fig. 7b, Online Resources 8). Specifically, morphological configurations of more posteriorly shifted dentition relative to the temporomandibular joints contribute to enhance the mechanical advantage of the masticatory apparatus and possibly facilitate seed predation (Koyabu and Endo 2009, 2010).

Ecological studies have suggested that seeds are selectively predated by colobines because they are rich sources of nutritive elements such as lipids, digestible carbohydrates, and protein (McKey 1978; Maisels et al. 1994; Waterman and Kool 1994; Auta and Anwa 2007; Hanya and Bernard 2012, 2015).Thus, seeds can be a desirable food item for animals as long as the defense of the seeds (the pod or pericarp) can be overcome. Seeds can be mechanically resistant whereas young leaves are generally less hard and less tough, therefore requiring less masticatory force to be processed (Lucas 2004; Williams et al. 2005). Although the frequency of seed eating can capture only one aspect of the complex animal diet—and further studies incorporating the mechanical properties of seeds predated by colobines are needed—at least some species, such as *C. polykomos* and *Pr. Rubicunda*, include mechanically challenging seeds in their diet that require relatively stronger masticatory forces to break down (Lucas and Teaford 1994; Lucas et al. 2000; Scott et al. 2012).

Among the Asian species, *Pr. rubicunda, T. francoisi, Py. nigripes*, and *R. avunculus* showed a greater mechanical advantage of the masseter for biting at the anterior dentition. Dietary information on the critically endangered *R. avunculus* and *T. francoisi* is largely lacking, but observations on *Pr. rubicunda* and *Py. nigripes* confirm that these two species frequently predate seeds (Davies 1991; Rawson 2009). Davies (1991) reported that the diet of *Pr. rubicunda* at Sepilok in Northern Borneo can comprise 80% seeds, depending on the season. Similarly, in the Danum Valley, seeds account for more than 80% of its diet in September, confirming the status of seed predator for *Pr. rubicunda* (Hanya and Bernard 2012). Seeds predated by *Pr. rubicunda* are pliant and tough (Lucas and Teaford 1994; Scott et al. 2012), and most seeds are "bitten, chewed, swallowed and digested, leaving no chance for survival" (Davies 1991). Davies (1991) also reported that the resistant arils of fruits such as *Xerospermum internedium, Wallucharia wallichii*, and *Knema laterica*, are chiseled off and removed to access the seeds. While most colobines prefer young leaves over other food items (Bennett and Davies 1994; Oates 1994), Rawson (2009) pointed out that young leaves eaten by *Py. nigripes* were, on the contrary, fallback resources; seeds such as *Sindora siamensis, Peltophorum cf. dasyrrhachis, Terminalia* spp., and *Dracontomelon* appeared to be the most preferred food items, which may even determine group movements and, more particularly, fission–fusion behavior (Rawson 2009). Duc et al. (2009) observed that *Py. nigripes* in southern Vietnam fed more frequently on seeds from unripe fruits than ripe ones, using their canines to gouge out seeds so they could eat those alone. While *Pr. rubicunda* has been a classic model for morphological studies examining the influence of seed eating (Lucas and Teaford 1994; Koyabu and Endo 2010; Scott et al. 2012), the significance of seed eating for *Py. nigripes*' morphology has been overlooked. Its high mechanical advantage for biting (Fig. 3 and Online Resource 4) and the consistent dietary observations (Rawson 2009) place this species as another potential model for seed predation. *Rhinopithecus avunculus* is one of the most endangered mammalian species today, but its diet is largely unknown; a preliminary investigation reported that it predates on tougher food items than other colobines in Vietnam (*R. avunculus* 1393 J/m^2; *Trachypithecus laotum* and *Trachypithecus delacouri* 1156 J/m^2; *Py. nemaeus* and *cinerea* 1238 J/m^2) (Quyet et al. 2007). In line with these observations, *R. avunculus* presents one of the greatest mechanical advantages of the masseter for biting at the anterior dentition among all species considered in our study. Long-term observations of feeding frequencies in *R. avunculus*, and how its dentition is recruited during the feeding process, are of high interest as they could provide useful information on its feeding habits and preferred environments, thereby informing conservation efforts to sustain its populations.

Among African colobines, *C. satanas* is known as a selective seed eater (McKey 1978; McKey et al. 1981; Oates 1994), exhibiting relatively flat molars compared to the more folivorous *C. guereza* and *Pi. badius* (Kay 1975; Ungar 1998). In agreement with this, *C. satanas* showed greater mechanical advantage of the masseter than *C. guereza* and *P. badius* for all bite points, and showed the greatest mechanical advantage of the masseter for most bite points among African colobines. Among *Colobus*, this study further revealed that *C. polykomos* and *C. angolensis* exhibited greater mechanical advantage of the masseter than *C. guereza* and *C. vellerosus*. "Molarized" premolars, a character that is often linked to seed eating in primates (Fleagle and McGraw 1999; Daegling and McGraw 2001), have also been observed in *C. satanas* and *C. polykomos* (Swindler 1976). In Tiwai Forest, Sierra Leone, *C. polykomos* has been observed to laboriously gnaw through the full-sized, thick woody pods of *Pentaclethra macrophylla* and eat the seeds, whereas the sympatric *P. badius* avoids the heavily lignified pods (Maisels et al. 1994). A study on the processing behaviors of *C. polykomos* and *P. badius*, and the material properties of *P. macrophylla* seeds and pods in Tai Forest, has shown that the anterior dentition (incisors and/or canines) is more frequently employed in *C. polykomos* than in *P. badius*, and is particularly recruited to broach the exceptionally tough pods of *P. macrophylla* (mean of 10 603.32 ± 5148.85 J/m^2) for accessing the seeds within (McGraw et al. 2016). Consistent with this observation, *C. polykomos* exhibited greater mechanical advantage of the masseter for biting at the anterior dentition than *P. badius*.

As exemplified by the cases of *Pr. rubicunda* (Davies 1991), *Py. nigripes* (Duc et al. 2009), and *C. polykomos* (McGraw et al. 2016), biting with the anterior dentition (incisors and canines) appears to be highly important for seed eating. Similarly, the Neotropic primate *Chiropotes satanas* is observed to use its anterior dentition to remove the puncture-resistant husks of unripe fruits and gain access to the seeds, a behavior referred as "sclerocarpic harvesting" (Kinzey 1992). It also presents greater mechanical advantage of the masseter for biting at the anterior dentition compared to other closely related primates, which do not conduct this behavior (Wright 2005). The alveolar region of the maxilla is oriented more anteriorly, resulting in procumbent incisors that function as an efficient nipping or cropping device (Kinzey 1992). As exemplified by the morphometric analysis, the oral tip of seed-eating colobines is similarly tilted more anteriorly. Thus, we postulate that the incisor/canine position and masseter insertion are particularly linked for seed eating in some colobines (namely *Pr. rubicunda, Py. nigripes, C. angolensis, C. polykomos*, and *C. satanas*). Although the mechanical advantage of biting is an essential parameter affecting the amount of force production, muscle mass (i.e., physiological cross-sectional area) is another important contributor to the bite forces an animal can generate (Raadsheer et al. 1999). As was previously done for other taxa (e.g., Taylor and Vinyard 2009; Koyabu et al. 2012; Furuuchi et al. 2013; Ito and Endo 2016), muscle mass should be further explored in future studies in order to better characterize the relationship between bite force and seed eating.

In conclusion, our expectation, following Koyabu and Endo (2009, 2010), that seed-eating would be the dominant signal to emerge from variation in the colobine skull, was not fully supported. Phylogeny explains 28.8%, diet explains 4.9%, and allometry explains 3.3% of the overall shape variation, all of which contributing significantly. The mechanical advantage of the masseter for biting at the anterior dentition exhibited correlation to frequencies of seed intake and our multivariate analyses of the colobine cranium demonstrated that cranial variation does reflect dietary variation in seed eating, but seed eating cannot, by itself, explain the overall variation of the skull and other food items also significantly explain the variation. Concerning phylogeny, clear interspecific patterns of differentiation and a robust phylogenetic signal was confirmed. For further studies, integrated phylogenetic analyses including all described taxa of colobines could be of high value in order to test these issues more precisely (including, for example, a correspondence for each specimen between the genetic and morphological data). However, in this study we considered specimens from most of the major collections of colobines worldwide, and the scarcity of additional specimens could be an impediment to further analyses. Size evolution, which is arguably driven by diet, biogeographic effects, and niche displacement, have a strong influence on shape variation for most colobine taxa, as shown by the allometric pattern we described. Size displacement and the diversity of food items consumed by colobines may have facilitated the sympatric coexistence of closely related species following colobine radiations.

Colobines for which diet have been studied in detail and phylogeny is resolved overall, provided us with a unique opportunity to explore patterns of craniodental variation, which aids in further understanding the cranial variation found among mammals. Due to the paucity of female specimens, this study was conducted only on males, but a dedicated study should be performed on females to determine if similar results are observed. Our results also highlight the potential of colobines as a model for future studies testing the relationship between morphology and ecological factors. In the past few decades, more and more studies have revealed the peculiarity of their feeding habits and highly specialized ecological niches, which hopefully will assist with building conservation plans for species such as *R. avunculus* that are currently facing extinction.

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