Evol Biol (2014) 41:38–51
DOI 10.1007/s11692-013-9244-5

RESEARCH ARTICLE

In and Out the Amazonia: Evolutionary Ecomorphology in Howler and Capuchin Monkeys

Carlo Meloro · Nilton Cáceres · Francesco Carotenuto · Jonas Sponchiado · Geruza Leal Melo · Federico Passaro · Pasquale Raia

Received: 28 March 2013 / Accepted: 26 June 2013 / Published online: 12 July 2013

Abstract The impact of environmental variation on phenotypic diversification is one major issue in evolutionary studies. Environmental variation is thought to be a primary factor in evolution, especially at high latitudes. In contrast, tropical areas are traditionally viewed as the cradle where the long-term effects of biological interactions on phenotypic change reside. We analyse patterns of skull shape variation in two New World monkey groups: capuchins and howlers. These two monophyletic clades are exceptionally similar in terms of the geographic distribution of their species. Yet, their body size and diet are different: howler monkeys are large and almost exclusively folivorous, whereas capuchins are small omnivorous. We found that the size, and direction of vectors of phenotypic changes across South American biomes in those clades are not statistically different. This similarity persists after removing the strong impact of allometry in our data. Additionally, partial least squares and comparative analyses confirm that "allometry free" skull shape is influenced to the same set of environmental variables in both clades. This study remarks the paramount importance of both body size and environmental variation on phenotypic evolution.

Keywords Skull shape · Climatic adaptation · Partial least squares · South America · Amazon forest

Introduction

When climatic change occurs, species respond by adapting, shifting their geographic distribution to track their preferred habitats, or going extinct (Davis et al. 2005; Raia et al. 2012). Adaptation as driven by environmental variation is one of the common mechanisms underlying

---

Electronic supplementary material The online version of this article (doi:10.1007/s11692-013-9244-5) contains supplementary material, which is available to authorized users.

C. Meloro (✉) · F. Carotenuto · F. Passaro · P. Raia
Dipartimento di Scienze della Terra, Università degli Studi di Napoli 'Federico II', L.go San Marcellino 10, 80138 Naples, Italy
e-mail: carlo.meloro@hyms.ac.uk

F. Carotenuto
e-mail: f.carotenuto@ymail.com

F. Passaro
e-mail: federico.passaro@unina.it

P. Raia
e-mail: pasquale.raia@libero.it

N. Cáceres
Department of Biology, Laboratory of Ecology and Biogeography, CCNE, Federal University of Santa Maria, Santa Maria, RS 97110-970, Brazil
e-mail: niltoncaceres@gmail.com

J. Sponchiado
Department of Biology, Programa de Pós-Graduaçao em Biodiversidade Animal, CCNE, Federal University of Santa Maria, Santa Maria, RS 97110-970, Brazil
e-mail: jsponchiado@yahoo.com.br

G. L. Melo
Programa de Pós-Graduação em Ecologia e Conservação, CCBS, Universidade Federal do Mato Grosso do Sul, Cx.P. 549, Campo Grande, MS 79070-900, Brazil
e-mail: geruzalm@yahoo.com.br

phenotypic evolution. The formation of geographic barriers, expansion and contraction of biomes in relation to climatic change, and secular trends in global temperature are some of the many "environmental" factors known to prompt phenotypic diversification (Rundell and Price 2009; Hoffmann and Sgrò 2011; Raia et al. 2013). Within this context, tropical areas always received special attention for the high diversity of species and ecomorphotypes they harbor. As the intensity of environmental variation is directly linked to latitude (Davies et al. 2009) species in the tropics are expected to be less sensitive to climatic change. In fact, the high diversity of species and ecomorphs in the tropics is traditionally attributed to high ecosystem resilience and primary productivity (Currie 1991; Mittelbach et al. 2007), and to the higher survival rate (hence greater age) of tropical lineages (Hawkins et al. 2006; Ricklefs and Renner 1994; Weir and Schluter 2007). This implies that rather than to environmental adaptation, phenotypic evolution in tropical areas is usually attributed to the long-term effects of biological interactions (Van Valen 1973; Stenseth 1984). Recent studies are now challenging this conventional wisdom. One of the most compelling evidence for this concerns the richest Neotropical ecosystem of them all: the Amazonia (Bush and de Oliveira 2006; Hoorn et al. 2010). The high vertebrate richness in the Amazon basin traces back to the Miocene (ca. 20 Ma) and appears to be linked to a large-scale geological event (i.e. the beginning of the Andean uplift) that modified the South American landscapes since (Hoorn et al. 2010), rather than to the great age of the Amazon rainforest (which traces back to the Eocene, Burnham and Johnson 2004).

Recent phylogeographic studies on Amazon mammals provide strong support for the influence of pre-historical environmental changes in shaping species diversification and distribution. This is apparent in patterns in many New World Primates, such as spider monkeys (genus *Ateles*, Collins and Dubach 2000), howler monkeys (genus *Alouatta*, Cortés-Ortiz et al. 2003), squirrel monkeys (genus *Saimiri*, Chiou et al. 2011), and capuchins (Lynch Alfaro et al. 2012a). Since molecular studies provide a strong support for the impact of historical and geographical events on Platyrrhini diversification, we might also expect a strong association between geography and phenotypic differentiation, since phenotypic and taxonomic diversification are often associated to each other both cladewise (Rabosky and Adams 2012) and in time (Raia et al. 2013). This association was in fact reported in at least two groups of New World monkeys: spider monkeys *Ateles*, whose cranial and dental morphometrics differ across parapatric taxa consistently among environments (Froehlich et al. 1991), and capuchins (Cebinae, genera *Cebus* and *Sapajus*) which include allopatric species mostly partitioned into different biomes (Lynch Alfaro et al. 2012a, b). Although these studies support a strong impact of geography on phenotypic variation, they do not take environmental variables explicitly into account. Recent evidences and techniques on how environmental variables can be accounted for in ecogeographical studies primarily come from Old World monkeys (Cardini et al. 2007). Cardini and Elton (2009) investigated skull shape variation in a range of different primates (vervet monkeys and Colobinae) to demonstrate that shape variance within and between species are correlated to the environment (quantified as e.g. annual temperature, precipitation). It is now apparent that environmental variables are a significant factor in explaining skull shape variation in monkeys, although differently among different primate lineages. Indeed, in vervet monkeys environmental shape variance is much higher than in colobines (Cardini et al. 2007; Cardini and Elton 2009).

Since the geographic range of several South American monkeys spans over highly productive territories, such as the Amazon rainforest, to arid, little productive areas like the Caatinga desert, we expect ecogeographical trends and clines in these monkeys, just as they were identified in their African counterparts.

Here, we test for the association between the environment and skull shape at the macroevolutionary scale in two clades of New World monkeys: howlers (*Alouatta* spp.) and capuchins (*Cebus* and *Sapajus* spp.). These two groups are very different in terms of body mass and feeding ecology. *Alouatta* species weigh up to 7–8 kg, and are obliged folivore–fruigivore. Capuchin monkeys are far smaller (between 2 and 3 kg), have a frugivorous-omnivorous diet (Rosenberger 1992), and most notably use tools (Moura and Lee 2004). Whereas they are worlds apart in terms of feeding ecology and size, capuchin and howler monkeys cannot be any more similar in terms of geographic distribution. Species in both genera cover (collectively) most of the Neotropical South America (Cortés-Ortiz et al. 2003; Lynch Alfaro et al. 2012a), exhibit little geographic overlap with each other, and most species are limited to a single biome. This makes these two clades ideal to test for the effect of climatic variation on the phenotype, because they experience almost identical climatic variation, whereas the potentially confounding effects of dietary convergence and interspecific competition on shape are not an issue.

We employ a combination of geometric morphometrics and comparative methods to test the hypothesis that environmentally-driven skull shape changes occur similarly in these two phylogenetically and ecologically distant Plathyrrini clades.

We compute and compare direction, size and shape of evolutionary vectors in skull shape of the two monkey lineages. These vectors describe phenotypic changes along an environmental gradient (Adams and Collyer 2009) that we defined using biomes as discrete ordinal climatic categories.

Since skull shape in Platyrrhini is profoundly influenced by size (the allometric component, Marroig and Cheverud 2004, 2005, 2010) we test also for allometric signal in our skull shape dataset, then we remove it to analyze "allometry free" skull shape components and their association with environmental variables.

Additionally, comparative methods are applied to:

1. Detect phylogenetic signal on species averaged skull shapes (Klingenberg and Gidazwiski 2010); and
2. Test the association between skull shape and environmental variables at the interspecific scale.

Materials and Methods

Sample Size

Our sample includes 535 wild-caught adult specimens belonging to fifteen different species (Table 1, Electronic Supplementary Material): seven *Alouatta* spp., and nine Cebinae (genera *Cebus* and *Sapajus* sensu Lynch Alfaro et al. 2012a). We sampled only South American taxa with the exclusion of species whose range expand into Central America (*Alouatta palliata* and *Cebus capuchinus*). In this way, we focus our attention on evolutionary processes occurred within clades, that started roughly at the same moment, when the uplift of Andean Cordillera was completed. The two clades are monophyletic on their own, whereas the tree we produced is paraphyletic as it excludes non-howler Atelidae. This is not detrimental to our analyses as we compared the clades to each other.

For each specimen we photographed skulls in ventral view using a standard protocol: each skull was positioned parallel to a digital camera at a 1 meter distance to avoid parallax and possible image distortion. We used the software tpsDig 2 (Rohlf 2010) to build a database of digital pictures and recorded two-dimensional spatial coordinates of twenty-three homologous landmarks. The landmark configuration was chosen to accurately describe ventral-view skull features shared by *Alouatta* and the Cebinae: the rostrum inclusive of incisor raw, muzzle and palate width, thickness and length of the upper canine, premolars and molars; the occipitalis and the zygomatic arch (Fig. 1). Digital landmarking was computed by one of us (N.C.) in order to avoid inter-observer error. Additionally, we randomly landmarked twice sixteen representative specimens and observed a landmark error much smaller than 5 %. This indicates that intraobserver error is negligible (cf. Cardini and Tongiorgi 2003; Meloro 2011).

Additionally, we recorded the geographical coordinates of specimen collection localities. Geographical coordinates, expressed in decimal degrees, were obtained by using Google Earth and specific reference collection (Silva 2001). Data were imported into EsriArcGis 10 software. Under GIS, we sampled for each locality its biome code (Olson et al. 2001) and bioclimatic variables (www.worldclim.org). Olson et al. (2001) identified 16 biomes to describe categorically environmental complexity of different ecosystems across the earth. The geographic distribution of our sample

Table 1 Sample size of skull order for taxa and biomes of occurrences

Species	No. specimen	No. biome 1	No. biome 2	No. biome 7	No. biome 13	No. biome 14
Cebus albifrons	17	17	0	0	0	0
Cebus olivaceus	16	16	0	0	0	0
Sapajus apella	71	65	0	2	0	4
Sapajus cay	14	1	3	10	0	0
Sapajus libidinosus	21	12	0	8	1	0
Sapajus macrocephalus	12	12	0	0	0	0
Sapajus nigritus	48	36	0	12	0	0
Sapajus robustus	21	21	0	0	0	0
Sapajus xanthosternos	9	1	0	0	5	3
Sub-total Cebinae	229	181	3	32	6	7
Alouatta belzebul	82	67	1	4	1	9
Alouatta caraya	76	31	4	30	9	2
Alouatta guariba	59	50	0	5	0	4
Alouatta macconelli	18	14	0	4	0	0
Alouatta nigerrima	10	10	0	0	0	0
Alouatta seniculus	61	56	0	5	0	0
Sub-total *Alouatta*	306	228	5	48	10	15
Total	535	409	8	80	16	22

Fig. 1 Disposition of 23 landmarks on a skull of *Alouatta caraya* specimen. Landmarks definitions: *1* Prosthion: antero-inferior point on projection of pre-maxilla between central incisors, *2* posterior-most point of lateral incisor alveolus, *3* anterior-most point of canine alveolus, *4* mesial P1: most mesial point on P1 alveolus, projected onto alveolar margin, *5–9* contact points between adjacent pre-molars/molars, projected labially onto alveolar margin, *10* anterior-most point on curvature of the zygomatic process, *11* middle point on curvature of the zygomatic process, *12* posterior-most point on curvature of the zygomatic process, *13* posterior extremity of occipital condyle along margin of foramen magnum, *14* opisthion: posterior-most point of foramen magnum, *15* basion: anterior-most point of foramen magnum, *16* most posterior tip of the palatine, *17* posterior tip onto alveolar margin of M3, *18–23* contact points between adjacent pre-molars/molars, projected lingually onto alveolar margin

covers five of Olson's biomes: 1 = Tropical and Subtropical Moist Broadleaf Forest, 2 = Tropical and Subtropical Dry Broadleaf Forest, 7 = Tropical and Subtropical Grasslands, Savannas and Shrublands, 13 = Deserts and Xeric shrublands, 14 = Mangroves. Because we are interested in understanding evolutionary changes that occurred within and outside the Amazon, we refined Olson classification for biome 1 as split into two other categories: Amazonian and Atlantic Forest (Fiaschi and Pirani 2009). Environmental variables were quantitatively described by 19 different bioclimatic indices (Electronic Supplementary Material). These variables were used to perform a discriminant function analysis, in order to identify a possible climatic gradient between the six biomes we identified.

We anticipate that the chosen biomes could be significantly ordered according to temperature seasonality and annual precipitation from biome "1" (Amazonian forest, category 1), followed by "14" (Mangroves, category 2), then Atlantic Forest (category 3), then biome "7" (Savanna, category 4), by biome "2" (dry forest, category 5) and finally the driest category biome "13" (desert, category 6). This ordinal categorization was necessary for further analyses (see Electronic Supplementary Material for more details).

Geometric Morphometrics

Generalised procustes analysis (GPA) was employed to remove from the original landmark coordinates differences in size and orientation (Rohlf and Slice 1990; Adams et al. 2004). The algorithm applies translation and scaling to unit centroid size (=the square root of the sum of squared distances between each landmark and the centroid, Bookstein 1989) after iterative rotation in order to minimize the summed squared Euclidean distances (procustes distances) among specimens, hence allowing to optimize landmarks superimposition among specimens.

After GPA, original landmark coordinates are transformed into shape variables (the procustes coordinates) while size is obtained as centroid size (Rohlf 2000).

The log transformed centroid size was first analyzed to detect differences between clades, species, biomes and their interaction using standard ANOVA. A test of multivariate allometry was employed using MorphoJ (Klingenberg 2011) in order to identify the strength of allometric signal in shape variables (Klingenberg 1996; Marroig and Cheverud 2005). MANCOVA was applied to test for differences in allometric slope between clades.

Due to the large size differences between Cebinae and *Alouatta* spp., two alternative approaches were applied before summarizing multivariate dimensionality of shape variables with principal components. First, a variance covariance matrix was generated using within-species covariances (Thorpe 1983, 1988; Klingenberg and Spence 1993). Marroig and Cheverud (2001, 2004) consistently demonstrated that the within-group covariance matrices are broadly similar between New World Primates. Consequently, by applying this procedure the interspecific differences are minimized to better detect any ecogeographical signal in the principal component analysis (PCA). MorphoJ (Klingenberg 2011) was used to generate the within-species covariances and then to perform PCA on the latter.

A second approach to reduce size and clade differences was to generate "size-free" shape variables obtained as the residuals of multivariate allometry. These coordinates were then submitted to a second PCA in order to visualize "allometry free" shape differences. Thin plate spline was used to visualize shape changes described by Principal Component scores.

Skull Shape and Environmental Adaptation

Every skull specimen is ascribed to a biome and clade (*Alouatta* or Cebinae either) categories. Before applying the procedure to identify the relative impact of environmental adaptation on skull shape, we performed a MANOVA analysis to test for significant differences in skull shape between "clades" and between "biomes" (with interaction). In spite of the recognized sexual dimorphism in *Alouatta* and Cebinae (Flores and Casinos 2011) we noted no significant interaction between "sex" and either "clades" or "biomes" in skull shape. Consequently, and to retain the original sample size, we did not divide our sample into males and females.

We first used the test of Adams and Collyer (2009) to compare vectors of phenotypic transformations across the environmental gradient between the two monophyletic clades: *Alouatta* versus Cebinae (inclusive of *Cebus* and *Sapajus*). Our environmental gradient (or evolutionary level, sensu Adams and Collyer 2009) is a categorical variable that can be commonly identified in the sample of *Alouatta* and Cebinae. We used biomes as a potential indicator of the environmental complexity (level). The geographic distribution of skull specimens for both clades covers evenly the six tropical biomes (see previous section).

An "averaged" skull shape was computed for each biome within each clade. Averaged skull shapes were obtained first on the principal component shape space of the pooled within-species covariance matrix, then on PCA of "size-free" shape variables. These shapes were used to detect evolutionary vectors (Adams and Collyer 2007). The vector that connects one "averaged" biome shape to the next has three components: direction, size and shape. By employing the algorithm of Adams and Collyer (2009) we compared these three vectors' attributes between the two clades. If biomes represent an evolutionary level with common impact on skull shape changes in *Alouatta* and Cebinae, we expect similarities in all of the three vectors' attributes.

Secondly, in order to increase the resolution of environmental complexity, we also tested for the association between skull shape and 19 standardized bioclimatic variables extracted independently from the collection localities' geographical coordinates. Skull shape coordinates were first averaged by localities for each different species to avoid pseudo-replications (cf. Cardini et al. 2007; Cardini and Elton 2009; Cardini et al. 2013). Two block partial least squares was then applied (Rohlf and Corti 2000) with block 1 being represented by the whole skull shape variables (within-species covariances matrix and "size-free" coordinates), and block 2 by the 19 standardized bioclimatic variables. Here, two block PLS is preferred over linear regression because it does not assume any statistical dependency between variables yet maximizes vector correlation between blocks (Zelditch et al. 2004). Similar examples for geometric morphometrics and climatic datasets can be found in Monteiro et al. (2003), Frost et al. (2003) and Piras et al. (2012). Additionally, to test the hypothesis that a common environmental gradient influences skull shape, we first computed PLS vectors independently for the two clades, than compared the vector angle between these two groups to a random distribution (Klingenberg et al. 2002) under the null hypothesis of independence. If the observed angles are smaller than expected by chance (at $p < 0.05$) then non-independence occurs between vectors.

For these analyses we used the R code as published in Adams and Collyer (2009) together with tpsPLS ver. 1.18 (Rohlf 2006) and MorphoJ (Klingenberg 2011) for partial least squares analyses.

Comparative Methods

To validate the results obtained for the whole skull shape sample at the macroevolutionary scale, we re-computed skull shape space based on species averages. Procustes registration was applied separately on each species of *Alouatta* and Cebinae and a new shape space (based on the within-species covariance matrix, see previous section) generated resulting in a dataset with 15 data points (one for species, cf. Meloro et al. 2008). A phylogenetic tree based on the topology and time of divergences obtained independently by Cortés-Ortiz et al. (2003) for *Alouatta* and Lynch Alfaro et al. (2012a) for Cebinae (Electronic Supplementary Material) was mapped within the skull shape space following Klingenberg and Gidazwiski (2010). We tested for a significant phylogenetic signal in skull shape by comparing the observed sum of procustes distances between species averaged shapes and reconstructed ancestral node values, and the distribution of these sums obtained by randomizing tip and node values (cf. Klingenberg and Gidazwiski 2010; Meloro and Jones 2012).

Additionally, we tested for skull shape-environmental correlation at the macroevolutionary scale by applying two block PLS on the averaged skull shape sample versus the averaged 19 bioclimatic values (as obtained by the observed sample). If environmental adaptation occurs also across species we might expect a significant association in the reduced sample. Independent contrasts were applied to both shape and bioclimatic variables to repeat PLS in a phylogenetic context (Garland et al. 2003; Adams 2008; Klingenberg 2011; Bastir et al. 2010).

Results

Skull Size

Skull size differ significantly between clades and species (ANOVA $F_{14,520} = 82.559$, $p < 0.0001$; Fig. 2). Due to heterogeneity of variance ($\text{Levene}_{14,520} = 6.775$, $p < 0.0001$) Dunnett T3 test was applied for post hoc comparisons: all members of *Alouatta* are larger than Cebinae, *Alouatta macconelli* is larger than *Alouatta caraya* and *Alouatta guariba*; within Cebinae *Sapajus macrocephalus* is larger than *Sapajus libidinosus*, *Sapajus cay* (smaller then *Sapajus nigritus*) and *Sapajus apella*.

A full ANOVA model shows that significant size differences between specimens from the same biomes (ANOVA

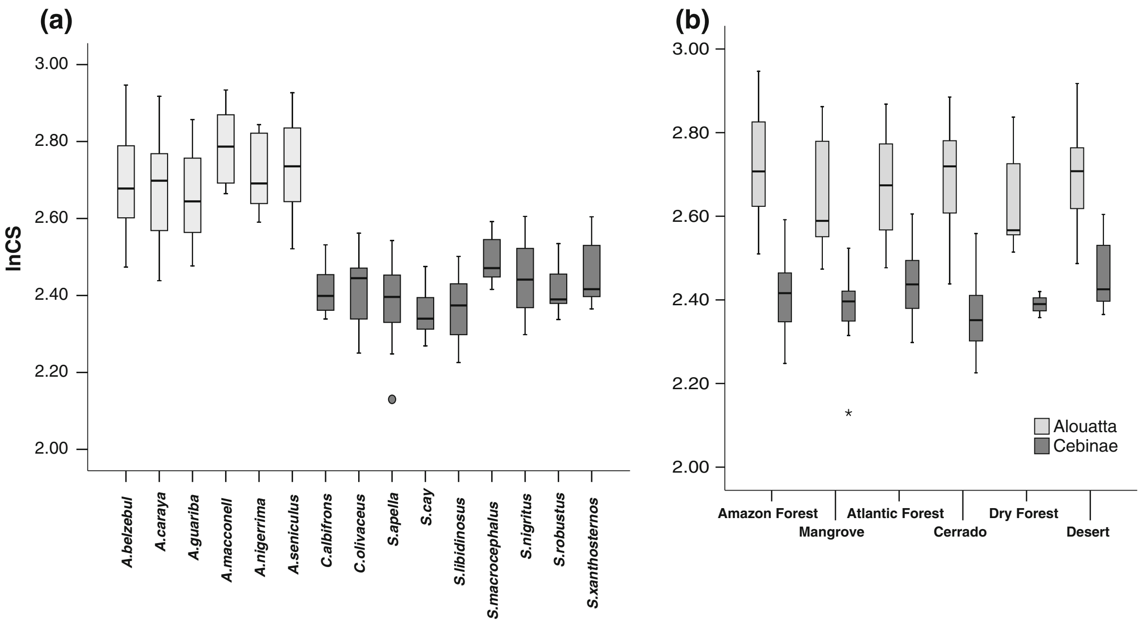


Fig. 2 *Box plot* of natural log transformed centroid size across different taxa (**a**) and biomes (**b**). *Black string* median, *grey box* (*light Alouatta*, *dark* Cebinae): first interquartile, *bar* second interquartile

$F_5 = 3.359$, $p = 0.005$) are due to the interaction with the factor clade (ANOVA $F_5 = 3.432$, $p = 0.005$, Fig. 2). Within *Alouatta* differences between biomes are significant (ANOVA $F_{5,300} = 2.425$, $p = 0.036$) with specimens from Atlantic forest being smaller than those from the Amazon (after Tukey, $\text{Levene}_{5,300} = 0.691$, $p = 0.631$). In Cebinae size per biome differ as well (ANOVA $F_{5,223} = 4.691$, $p < 0.0001$) with specimens from Cerrado being smaller than specimens from the Amazonian and Atlantic forests (after Tukey test, $\text{Levene}_{5,223} = 9.05$, $p = 0.479$).

There is a strong allometric signal in the skull shape of howler and capuchin monkeys (Wilks' $\lambda = 0.0678$, $F_{42,492} = 161.169$, $p < 0.0001$) with size explaining 55.659 % of the sample variance. MANCOVA shows that slope differ significantly between clades (Wilks' $\lambda = 0.654$, $F_{42,490} = 6.176$, $p < 0.0001$) as well as species (Wilks' $\lambda = 0.142$, $F_{588,6,229.9} = 1.068$, $p < 0.0001$).

Skull Shape Variation and Evolutionary Vectors

PCA performed on the pooled within-species covariance matrix allows to summarize the variance of 42 shape variables with the first sixteen vectors explaining together 95.151 % of the total variance.

The plot of the first two PCs shows significant differences in skull shapes of Cebinae and *Alouatta* are. Along the first PC (49.273 %, Fig. 3a) negative scores describe *Alouatta* skulls with relatively short incisors, small premolar and zygomatic arch area, but large molar area. The occipital foramen is relatively short. The opposite occurs in Cebinae that occupies positive scores of PC1 with enlarged rostrum, incisors and premolars, wider zygomatic arch and occipital foramen (Fig. 3a). PC2 (9.93 %) is strongly loaded on shape changes in the zygomatic arch area that is shorter and projected more posteriorly in *Alouatta* (positive scores) while it is larger and projected anteriorly in Cebinae (Fig. 3a).

The "size-free" PC scores clearly show that species differences are no longer apparent (Fig. 3b) even if *Alouatta* specimens still tend to occupy positive PC scores with relatively longer rostrum-molar raw and shorter zygomatic arch while Cebinae are in negative scores.

MANOVA reveals significant differences in skull shape between the two clades both when using within-species covariance matrix and "size-free" PC scores (Table 2). Hotelling pairwise comparisons (based on the first 4 PC scores (=77 %, selected because of sample size restriction) reveal that biomes averages are substantially different between clades (Table 3). However, within clades only the specimens from Amazonian forest are significantly different in skull shape from the Atlantic forest and savanna biomes. When using "size-free" PC scores this same conclusion applies to *Alouatta* only, while in Cebinae also specimens from other biomes (e.g. Mangrove, dry forest) are quite distinct in terms of skull shape.

By plotting averaged skull shapes per biome we observed similarities in vectors of skull shape transformation that are broadly confirmed by statistical tests, no matter if using PC

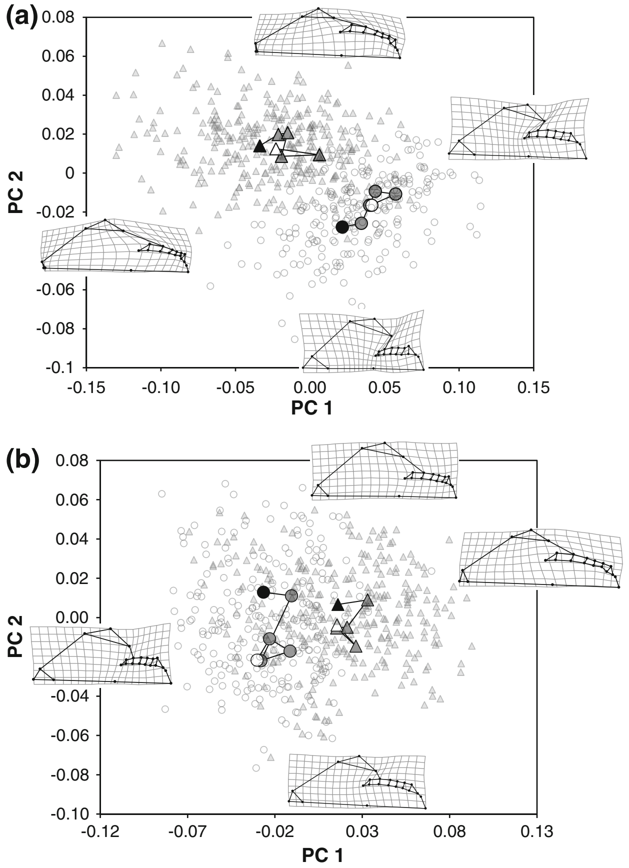


Fig. 3 *Scatter plot* of PC1 versus PC2 based on a sample of 535 specimens of Cebinae (*circles*) and Alouatta (*triangles*). In **a** PC scores are from pooled within-species covariance matrix, in **b** PC scores are *plotted* after removing allometry. Deformation grids are placed as representatives of the extreme scores of each *axis*. *Large circles/triangles* represents averaged phenotype for each biome type (going from *black circle* biome 1—Amazon forest to *white circle* biome 6—desert)

Table 2 Hotelling pairwise comparisons

	Pillai trace	Approx. *F*	*df* 1	*df* 2	*p*
Pooled within-species					
Clade	0.985	770.67	42	482	<0.0001
Biomes	0.877	2.46	210	2,430	<0.0001
Clade × biomes	0.809	2.23	210	2,430	<0.0001
Size-free					
Clade	0.345	6.051	42	482	<0.0001
Biomes	0.877	2.46	210	2,430	<0.0001
Clade × biomes	0.809	2.23	210	2,430	<0.0001

scores of within-group covariance or “size-free” variables (Fig. 3; Table 4). After 10,000 randomizations, we identified non-significant differences in all of the three vectors attributes for both shape datasets: phenotypic trajectory size ($MD_{1,2}$), direction ($\theta_{1,2}$), and shape (D_{Shape}) are the same in the two clades.

Skull Morphology and Environmental Variation

Non-parametric correlation shows that skull centroid size is significantly correlated to a broad range of bioclimatic variables (Table 5). Most bioclimatic variables influence in a similar way size of *Alouatta* and Cebinae (e.g. BIO8, BIO13, BIO18) even if the latter group correlates with a larger number of bio-climes.

Two block partial least squares identifies a significant correlation between skull shape and bioclimatic variables both when using pooled within-species variance or “size-free” across a geographic sample now reduced at 227 data points (Fig. 4a, b). When using within-species variance only the first pair of singular warps (SW) shows a significant association explaining 73.624 % of the total covariation between the blocks. The correlation coefficient for SW1 shape versus SW1 bioclimate is not particularly high ($r = 0.251$) and their plot shows only small overlap between the two separate clusters (*Alouatta* on negative SW1 shape scores and *Cebinae* on positive SW1 scores, Fig. 4a). There is more overlap when “size-free” shape scores are analysed (Fig. 4b) and the first pair of SW1 explains a much higher percentage of covariation (92.338 %). However their correlation is still weak ($r = 0.250$).

PLS repeated on the geographic subsample of *Alouatta* ($N = 134$) and Cebinae ($N = 93$) yields non-significant results when using within-species covariance matrix, but a significant association with “size-free” shape data. Indeed, for *Alouatta* the first pair of SW1 explains a large percentage of covariance (96.629 %) with a high correlation coefficient ($r = 0.666$, Fig. 4c) and the same applies to Cebinae (99.153 % of covariance for SW1, $r = 0.573$, Fig. 4d). From the plots it is evident a strong discrimination of Amazonian specimens (positive PLS scores) from all the other biomes (Fig. 4c, d).

Loading profiles for SW1 bioclimates show that in all the shape datasets analyzed there are very similar patterns with BIO4, BIO12, BIO16 and BIO19 always exhibiting high loadings.

The shape deformations in the overall dataset (Fig. 5a, b) are strongly influenced by differences between *Alouatta* and Cebinae with major deformations related to climate described by zygomatic arch and its position relative to the teeth. In *Alouatta* Amazonian specimens adapted to a less seasonal climate with high and constant rate of rainfall show relatively shorter and thin tooth raw and short zygomatic arch area (Fig. 5c), while in Cebinae there is a more posteriorly projected zygomatic area and shorter rostrum—with smaller molar raw (Fig. 5d).

The angle between SW1 “size-free” shape vectors of Cebinae and *Alouatta* is 64.186, which is significantly different from a right angle ($p < 0.001$) indicating that the two vectors are not independent from each other.

Table 3 Hotelling pairwise comparisons for skull shape of specimens from *Alouatta* and Cebinae belonging to different biome categories

	Alouatta						Cebinae					
	Amazon	Mangrove	Atlantic	Savanna	DryWood	Desert	Amazon	Mangrove	Atlantic	Savanna	DryWood	Desert
Alouatta												
Amazon	0	0.228811	**0.000285**	**0.040156**	0.302463	0.738982	**1.91E−19**	0.228712	**3.93E−21**	**2.20E−09**	**0.022627**	**0.001381**
Mangrove	0.443394	0	0.204019	**0.051451**	0.301087	0.531935	**9.00E−08**	0.146325	**5.40E−08**	**0.000469**	0.108316	**0.009727**
Atlantic	**6.72E−08**	0.524849	0	**0.000776**	0.476655	0.341399	**1.53E−21**	**0.015452**	**8.69E−13**	**3.82E−06**	**0.018286**	**0.004454**
Savanna	**0.000205**	0.094617	**0.000846**	0	0.471282	0.989632	**2.25E−11**	0.269436	**1.57E−10**	**0.000148**	0.1007	**0.017497**
Dry wood	0.061039	0.426971	0.462748	0.487551	0	0.665677	**0.001984**	0.281032	**0.014194**	**0.040496**	0.37563	0.341636
Desert	0.584421	0.678298	0.270707	0.925222	0.56962	0	**0.001218**	0.545595	**0.000732**	0.071302	0.36004	0.139458
Cebinae												
Amazon	**2.72E−148**	**3.98E−48**	**2.95E−97**	**1.79E−78**	**5.33E−22**	**2.72E−38**	0	0.561867	**2.03E−17**	**1.32E−11**	**0.032823**	**0.013356**
Mangrove	**1.51E−35**	**8.00E−09**	**7.51E−23**	**8.91E−18**	**0.001051**	**1.61E−06**	0.814951	0	**0.002637**	0.051012	0.575637	0.227414
Atlantic	**1.99E−107**	**4.38E−32**	**6.32E−67**	**2.10E−53**	**5.32E−16**	**5.04E−26**	**8.37E−14**	0.095887	0	**0.003655**	0.062615	0.861057
Savanna	**1.54E−83**	**2.02E−22**	**6.99E−51**	**1.30E−39**	**9.49E−12**	**3.63E−18**	**5.90E−12**	0.051813	0.326143	0	0.528835	0.163685
Dry wood	**1.01E−21**	**5.09E−06**	**9.68E−15**	**6.77E−12**	0.051391	**0.000341**	0.068463	0.587896	0.688079	0.761157	0	0.430435
Desert	**6.72E−33**	**3.32E−08**	**3.92E−21**	**1.26E−16**	**0.00256**	**5.44E−06**	0.142417	0.813889	0.909363	0.709304	0.921834	0

Below diagonal p values are reported based on a sample of the first four PCs of within-species covariances, above diagonal p values based on the 4 PCs of "allometry free" shape data. Significant differences are reported in bold

Table 4 Summary statistics for differences in phenotypic trajectory size ($MD_{1,2}$), direction ($\theta_{1,2}$), and shape (D_{Shape}) between the clade Cebinae and *Alouatta*

	Parameter	p value
Pooled within-species		
$MD_{1,2}$	0.0152	0.650
$\theta_{1,2}$	125.46	0.815
D_{Shape}	1.0094	0.077
Size-free		
$MD_{1,2}$	0.0026	0.491
$\theta_{1,2}$	56.834	0.799
D_{Shape}	0.996	0.128

Comparative Analyses

The phylomorphospace defined by the 15 species and their ancestral nodes mirrors shape differences observed on the whole sample (Fig. 6). Again, we note on the PC1 (92.15 % of total shape variance explained) a clear separation between the clades *Alouatta* and Cebinae whose distance appears to be proportional to the time of divergence from the tree root. The permutation test evidenced a significant phylogenetic signal in skull shape ($p < 0.0001$ after 10,000 randomizations; observed tree length = 0.0146).

Repeating the partial least squares analysis with skull shape as block 1 and averaged bioclimatic variables as block 2 yields non-significant results for the 15 species data (SW1 = 98.3 % of covariation, $r = 0.421$, $p = 0.177$). PLS loadings for Cebinae and *Alouatta* are remarkably similar in SW1 bioclimate being correlated strongly with BIO12, BIO19 and BIO18 (in Cebinae) and BIO12, BIO19, BIO17 and BIO16 in *Alouatta* (Table 5). The test for angle differences in SW1 shape vector between clades is significantly different from 90° (angle = 51.61, $p < 0.0001$).

PLS repeated on the independent contrasts of 15 species data extracts four significant SW pairs (see Electronic Supplementary Material). SW1 explains a high proportion of covariation between shape and bioclimates (95.99 %) and is again loaded on variables which are much the same as with those observed by analyzing Cebinae and *Alouatta* separately (BIO12, BIO18 and BIO19, which is basically precipitation and seasonality Table 5).

Discussion

The evolution of capuchins is characterized by an early split between robust (*Sapajus*) and gracile (*Cebus*) forms during the late Miocene (Lynch Alfaro et al. 2012b). After this major event, capuchins began invading tropical South American habitats, diversifying into different species as

Table 5 Loading coefficients on SW1 block variables based on different PLS analyses with shape as block 1 and bioclimate as block 2

	Alouatta 134 size	Cebinae 93 size	227 pooled	227 size-free	15 size-free	*Alouatta* 134	*Cebus* 93	15 pooled	IC 14
BIO1	−0.122	**−0.257**	0.027	0.009	0.022	0.015	0.014	0.019	0.006
BIO2	−0.122	−0.026	0.019	0.039	0.028	−0.026	−0.022	0.024	0.010
BIO3	0.116	**0.252**	0.001	0.013	−0.009	−0.012	−0.005	−0.009	−0.014
BIO4	**−0.252**	0.062	**0.806**	**0.799**	**−0.298**	**−0.799**	**−0.822**	**−0.317**	**−0.343**
BIO5	−0.187	**−0.278**	0.049	0.028	0.012	0.010	0.005	0.009	−0.005
BIO6	0.157	−0.024	0.005	−0.018	0.035	0.029	0.033	0.033	0.022
BIO7	**−0.212**	−0.072	0.040	0.062	0.019	−0.045	−0.042	0.014	−0.001
BIO8	**−0.203**	**−0.230**	0.034	0.018	0.019	0.003	0.003	0.015	0.002
BIO9	−0.005	**−0.250**	0.016	−0.003	0.027	0.030	0.027	0.024	0.012
BIO10	**−0.173**	**−0.223**	0.036	0.017	0.019	0.007	0.006	0.016	0.003
BIO11	−0.040	**−0.246**	0.016	−0.002	0.026	0.026	0.026	0.024	0.011
BIO12	**0.215**	−0.095	**−0.475**	**−0.501**	−0.024	**0.420**	**0.401**	0.007	**0.117**
BIO13	**0.249**	**−0.234**	−0.070	−0.029	0.046	0.057	0.055	0.006	−0.087
BIO14	0.127	**0.375**	−0.017	−0.014	0.015	−0.028	−0.010	0.037	0.087
BIO15	0.021	0.021	0.009	0.035	−0.009	−0.016	−0.010	−0.027	−0.076
BIO16	0.156	**−0.266**	**−0.197**	**−0.127**	0.050	**0.208**	**0.181**	−0.041	**−0.256**
BIO17	0.137	**0.377**	−0.044	−0.076	−0.048	−0.028	0.003	0.032	**0.229**
BIO18	**−0.196**	**0.189**	0.013	0.029	−0.633	−0.170	−0.156	**−0.598**	**−0.448**
BIO19	**0.274**	0.067	**−0.268**	**−0.280**	0.705	**0.320**	**0.314**	**0.730**	**0.726**

Variables and values generally higher than the others are in bold

new and different biomes were colonized (Lynch Alfaro et al. 2012a). Almost the same partitioning of species per biomes occurred in the evolution of *Alouatta*. Notably, the most recent common ancestors of both howlers and capuchins date to latest Miocene. This means that the two clades have had very similar timelines and geography in the history of their diversification.

In terms of dietary habits, though, howlers and capuchins are very different. This is primarily reflected in skull size that differ considerably between the two clades (Fig. 2) and then in their skull shapes (Figs. 3, 5) with traits evidently related to their different feeding adaptations. A curved rostrum with enlarged incisors and premolars, together with a large zygomatic arch, placed more anteriorly for an efficient bite force, characterize capuchins, which is expected given their omnivorous diet (cf. Rosenberger 1992; Wright 2005; Wright et al. 2009). The opposite applies to *Alouatta* spp. that are homogenously adapted to eat primarily leaves and for this need enlarged molars, and narrower incisors, together with a short zygomatic area positioned more posteriorly (Rosenberger 1992; Rosenberger et al. 2011). Such a broad differentiation in shape between the two clades is clearly the result of the long time past their most recent common ancestor, as revealed by the phylomorphospace analysis (Fig. 6).

In spite of their very distinct ecomorphologies, we identified almost identical directions of skull shape changes across biomes and/or bioclimatic variables in the two clades. Phenotypic trajectory analysis (Adams and Collyer 2009) unequivocally supports non-independent pathways for evolutionary changes in shape of Cebinae and *Alouatta*. This similarity of phenotypic trajectory in New World monkeys is remarkable for we are not aware of any similar results in other studies [e.g. ammonoid cephaloids across time, Monnet et al. (2011); or damselfishes across different dietary strategies, Frédérich et al. 2013]. The similarity in evolutionary vectors in capuchins and howlers supports the idea that the past environmental and geographical variation these clades shared during their diversification set the pace and direction of their morphological change. When both *Alouatta* and Cebinae clades started to diversify in the late Miocene (ca. 6 Ma) the separation between the Amazonian biome (tropical rainforest) and savanna was already existent (Hoorn et al. 2010). This indicates that what today characterizes environmental variation these clades meet was already there when they diversified. It is important to note that the pattern of biome phenotypic changes occurs with or without including allometric component in our analyses. As expected, size represents the major mechanism of phenotypic differentiation between New World Primates species (Marroig and Cheverud 2005, 2010) thus explaining in our sample almost half of phenotypic shape variance. There is a size differentiation also between specimens from different biomes but our analyses support only a strong differentiation of Amazonian species which are generally larger than the others. With "size-free" shape

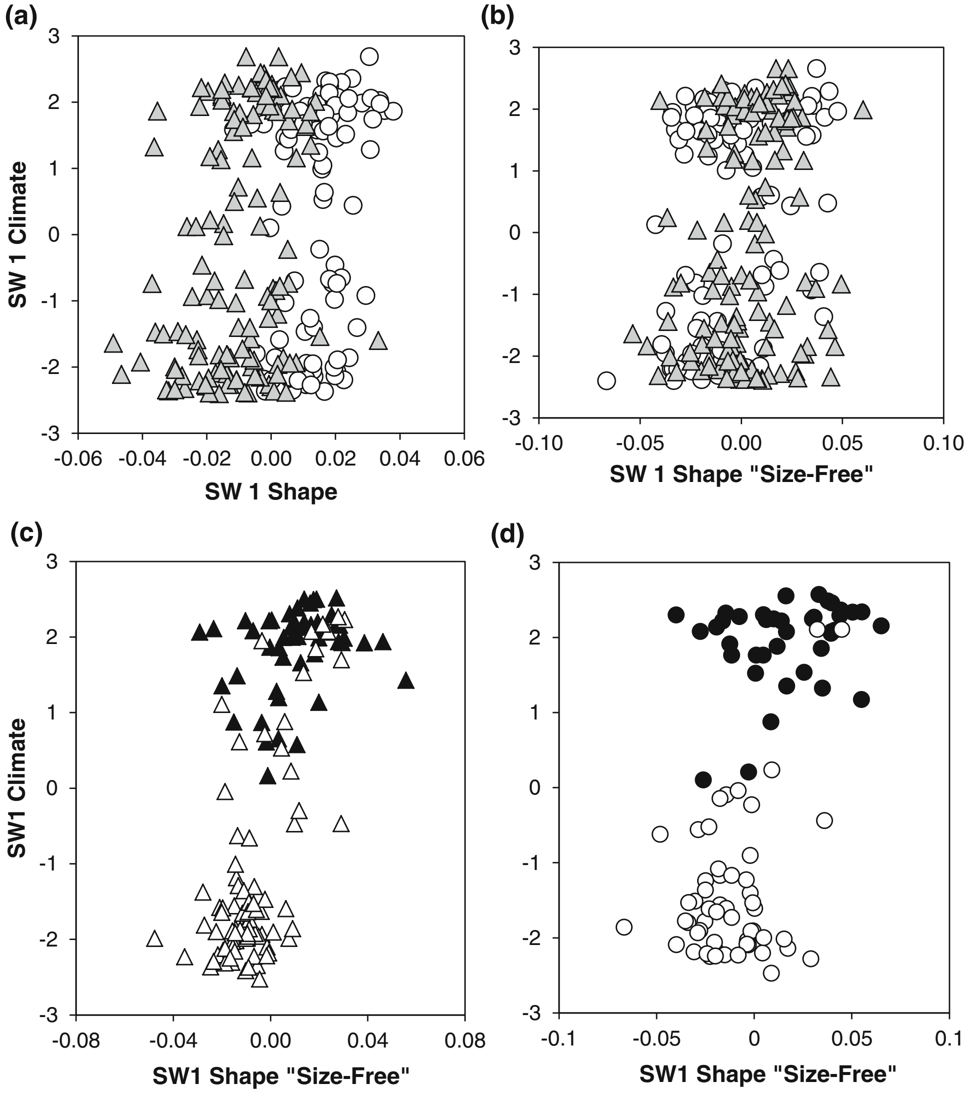
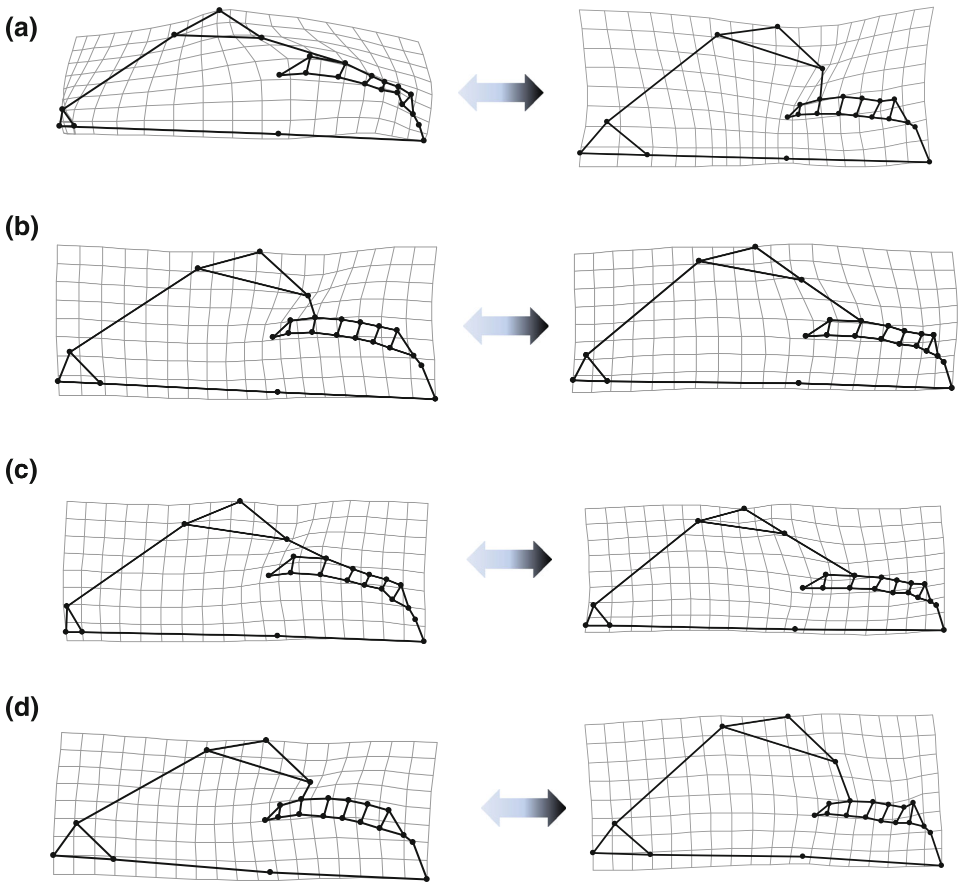


Fig. 4 *Scatter plot* showing the first pair of singular warp axes extracted by *two block* PLS with *skull shape* as block 1 and *bioclimates* as block 2. In **a** the pooled within group PLS was performed on averaged geographic sample of 227 specimen with *Alouatta* as *grey triangles* and Cebinae as *white dots*, in **b** same symbols as in **a** but using "size-free" shape variables, in **c** PLS for a subsample of 134 "size-free" *Alouatta* showing in *black* the Amazonian specimens while in **d** same analyses but using 93 geographically averaged Cebinae

data (Table 3) there is a wider discrimination between specimens of the two clades from different biomes thus suggesting that the adaptation to distinct environments is subtle and act strongly on size-residual shape variance.

This is confirmed by two block partial least squares that indicates a significant impact of environmental variation on skull shape (allometry free) with very similar patterns being noticeable in both clades (the. Frost et al. (2003) also identified a strong association between geography and "size-free" shape data in PLS thus suggesting that the pattern we observe could be found also in Old World Primates. Interestingly, the environmental variables mostly correlated with morphology are precipitation and seasonality (Table 5). Cardini et al. (2007) highlighted the importance of considering precipitation to interpret skull shape variation in Old World monkeys as this variable influences fruit/vegetable availability. In South America precipitation has been very often advocated as a good predictor for primate richness (Lehman and Fleagle 2006). Recent theories about the use of stone tools in *Sapajus* also seems to highlight precipitation as responsible for the spatial distribution of this behavior (Ottoni and Izar 2008). The skull regions mostly correlated with precipitation (and with climatic seasonality) are the dentition, and the zygomatic arch (Fig. 5). Overall, the shape vectors describe changes from more seasonal (lower annual precipitation) to stable (high precipitation) environments. In Cebinae, high seasonality relates to larger zygomatic arch anteriorly placed, and to larger dentition (features typical of the robust *Sapajus* capable to deal with a variety of other food rather than fruit, Wright et al. 2009; Lynch Alfaro et al. 2012b). In *Alouatta* spp. seasonal environments influence again zygomatic area and dentition. Recent studies suggest a higher consumption of mature leaves in Southern/Eastern part of South America for *A. caraya* and *A. guariba* (Martins 2008). It seems likely that the relatively higher

Fig. 5 Shape deformations related to the first singular warp going from the most extreme negative score (*left*) to the most positive scores (*right*). In **a** deformations are from pooled within group PLS of 227 specimens, in **b** deformations from PLS analyses of "size-free" shape components of 227 specimens, in **c** deformation from PLS of a subsample of 134 "size-free" *Alouatta* while in **d** same but form the analysis of 93 geographically averaged Cebinae



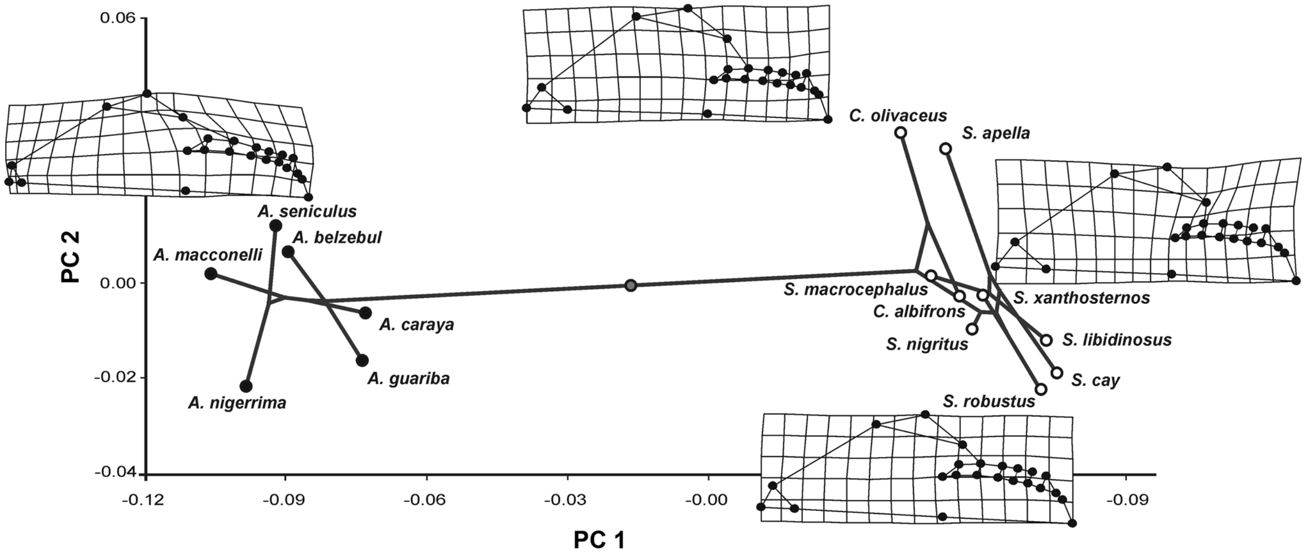


Fig. 6 Phylomorphospace based on species averages (15 data points) for Cebinae and *Alouatta*. Deformation grids are placed as representatives of the extreme scores of each *axis*

productivity of the Amazonian rainforest allows both capuchins and howlers to become less constrained in skull ecomorphology in relation to the annual abundance of energetic fruits that do not demand for high biomechanical performance. We also note that the ecological differentiation between Cebinae and *Alouatta* does not necessarily point to completely opposite feeding strategies. In fact, fruits tend to be most equally represented in all diets of different *Cebus*, *Sapajus* and *Alouatta* species (Rosenberger 1992; Chapman and Fedigan 1990; Galetti and Pedroni 1994; Agostini 2008; Agostini et al. 2010). Such a preference for fruit, together with ecomorphological features generally correlated to feeding on seed, have recently questioned the complete folivory of the howler monkeys that Rosenberger et al. (2011) renamed as "semifolivours". This means that when fruit, as a resource, is available, both howlers and capuchins feed on it. To the South of Amazonia, where fruit is not nearly as abundant as in the rainforest, the consumption of harder food items forces species in both lineages to adapt.

?>It is important to remark once more that allometry is responsible for most shape variation in both capuchins and howler monkeys. Yet, our results also convincingly point to the importance of environmental settings in patterns of shape change in Primates. Despite *Alouatta* spp. and cebids are different in so many regards, they faced the common problem of exploiting hard vegetables (whether they were fruits, leaves, or seeds) as they moved to the more seasonal South. These Primates reacted in very similar ways, with feeding adaptation visible and convergent in the same morphological districts of the skull. The intensity and direction of shape changes are the same in the two groups of species, suggesting that climatic variation was of paramount importance to adaptation in New World Primates. Clearly, capuchins and howlers are only a small fragment of the complex evolutionary Platyrrhini radiation in the New World, and more clades have to be studied to understand if such a pervasive effect of environmental variation of shape differentiation generalizes to New World monkey as a whole.

Conclusion

Macroevolutionary transformations are often advocated as constrained by phylogenetic history, adaptation or biomechanical requirements (Gould 2002). In tropical regions, environmental variation is claimed to be less important than biological interaction in shaping adaptation (Van Valen 1973; Stenseth 1984). We demonstrate that in spite of a deep morphological difference between capuchin and howler monkeys (as a consequence of their broadly distinct ecologies and lifestyles), environmental variation can be considered as the major factor in ecomorphological differentiation after body size. This validates the well established theory of ecogeographical barrier that supports macroevolutionary differentiation of a broad variety of South American mammals crossing the Amazon forest (e.g. rodents and marsupials De Vivo and Carmignotto 2004; monkeys of the genus *Ateles* Froehlich et al. 1991 and see the crab eating fox, *Cerdocyon thous* for an example on intraspecific variation, Machado and Hingst-Zaher 2009). Out of Amazonia, both Cebinae and *Alouatta* evolved with similar direction skull shape to deal with a more seasonal, less stable environmental condition.

Acknowledgments We are grateful curators and staff of the Museu Nacional (MNRJ) (J.A. de Oliveira and S.M. Vaz), Museu Paraense Emílio Goeldi (MPEG) (S.M. Aguiar and J.S. Silva Jr.), Museu de Zoologia da Universidade de São Paulo (MZUSP) (M. De Vivo and J.G. Barros), Museu de História Natural Capão da Imbuia (MHNCI) (G.M. SiqueiraTebet), Coleção Científica de Mastozoologia da UFPR (DZUP) (F.C. Passos and I.P. Bernardi), and Museu de CiênciasNaturais da Fundação Zoobotânica do Rio Grande do Sul (MCN/FZB) (L.S. Hoffmann) for the kindly authorization and support to specimens access. Senior author (Carlo Meloro) is currently supported by TEMASAV/DOTTORI DI RICERCA-ESPERTI/26, Francesco Carotenuto by the program FORGIARE V, and Nilton Cáceres by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) in Brazil (PDE process number 202267/2011-3).

References

Adams, D. C. (2008). Phylogenetic meta-analysis. *Evolution, 62*, 567–572.

Adams, D. C., & Collyer, M. L. (2007). Analysis of character divergence along environmental gradients and other covariates. *Evolution, 3*, 510–515.

Adams, D. C., & Collyer, M. L. (2009). A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution, 5*, 1143–1154.

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: Ten years of progress following the 'revolution'. *Italian Journal of Zoology, 1*, 5–16.

Agostini, I. (2008). *Ecology and behavior of two howler monkey species (Alouatta guariba clamitans and Alouatta caraya) living in sympatry in northeastern Argentina.* PhD dissertation. Rome: University "La Sapienza".

Agostini, I., Holzmann, I., & Di Bitetti, M. S. (2010). Ranging patterns of two syntopic howler monkey species (*Alouatta guariba* and *A. caraya*) in Northeastern Argentina. *International Journal of Primatology, 31*, 363–381.

Bastir, M., Rosas, A., Stringer, C., Cuétara, J. M., Kruszynski, R., Weber, G. W., et al. (2010). Effects of brain and facial size on basicranial form in human and primate evolution. *Journal of Human Evolution, 5*, 424–431.

Bookstein, F. L. (1989). 'Size and shape': A comment on semantics. *Systematic Zoology, 38*, 173–180.

Burnham, R. J., & Johnson, K. R. (2004). South American palaeobotany and the origins of Neotropical rainforests. *Philosophical Transaction of the Royal Society B, 359*, 1595–1610.

Bush, M. B., & de Oliveira, P. E. (2006). The rise and fall of the Refugial Hypothesis of Amazonian speciation: A paleoecological perspective; Apogeu e declínio da Hipótese dos Refúgios para a especiação na Amazônia: Uma perspectiva paleoecológica. *Biota Neotropica*. doi:10.1590/S1676-06032006000100002.

Cardini, A., Dunn, J., O'Higgins, P., & Elton, S. (2013). Clines in Africa: Does size vary in the same way among widespread sub-Saharan monkeys? *Journal of Biogeography, 40*, 370–381.

Cardini, A., & Elton, S. (2009). Geographical and taxonomic influences on cranial variation in red colobus monkeys (Primates, Colobinae): Introducing a new approach to 'morph' monkeys. *Global Ecology and Biogeography, 18*, 248–263.

Cardini, A., Jansson, A. U., & Elton, S. (2007). A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography, 34*, 1663–1678.

Cardini, A., & Tongiorgi, P. (2003). Yellow-bellied marmots 'in the shape space': Sexual dimorphism, growth and allometry of the mandible. *Zoomorphology, 122*, 11–23.

Chapman, C. A., & Fedigan, L. M. (1990). Dietary differences between neighboring *Cebus capucinus* groups: Local traditions, food availability or responses to food profitability? *Folia Primatologica, 54*, 177–186.

Chiou, K. L., Pozzi, L., Lynch Alfaro, J. W., & Di Fiore, A. (2011). Pleistocene diversification of living squirrel monkeys (*Saimiri* spp.) inferred from complete mitochondrial genome sequences. *Molecular Phylogenetics and Evolution, 3*, 736–745.

Collins, C. A., & Dubach, J. M. (2000). Phylogenetic relationships of spider monkeys (*Ateles*) based on mitochondrial DNA variation. *International Journal of Primatology, 21*(3), 381–420.

Cortés-Ortiz, L., Bermingham, E., Rico, C., Rodriguez-Luna, E., Sampaio, I., & Ruiz-Garcia, M. (2003). Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. *Molecular Phylogenetics and Evolution, 26*, 64–81.

Currie, D. J. (1991). Energy and large-scale patterns of animal and plant species richness. *The American Naturalist, 137*, 27–49.

Davies, T. J., Purvis, A., & Gittlemann, J. L. (2009). Quaternary climate change and the geographic ranges of mammals. *The American Naturalist, 174*, 297–307.

Davis, M. B., Shaw, R. G., & Etterson, J. R. (2005). Evolutionary responses to changing climate. *Ecology, 86*, 1704–1714.

De Vivo, M., & Carmignotto, A. P. (2004). Holocene vegetation change and the mammal faunas of South America and Africa. *Journal Biogeography, 31*, 943–957.

Fiaschi, P., & Pirani, J. R. (2009). Review of plant biogeographic studies in Brazil. *Journal of Systematics and Evolution, 47*, 477–496.

Flores, D. A., & Casinos, A. (2011). Cranial ontogeny and sexual dimorphism in two New World monkeys: *Alouatta caraya* (Atelidae) and *Cebus apella* (Cebidae). *Journal of Morphology, 272*, 744–757.

Frédérich, B., Sorenson, L., Santini, F., Slater, G. J., & Alfaro, M. E. (2013). Iterative ecological radiation and convergence during the evolutionary history of damselfishes (Pomacentridae). *The American Naturalist, 1*, 94–113.

Froehlich, J. W., Suprianata, J., & Froehlich, P. H. (1991). Morphometric analyses of *Ateles*: Systematics and biogegraphic implications. *American Journal of Primatology, 25*, 1–22.

Frost, S. R., Marcus, L. F., Bookstein, F. L., Reddy, D. P., & Delson, E. (2003). Cranial allometry, phylogeography and systematics of large-bodied Papionins (Primates: Cercopithecinae) inferred from geometric morphometric analysis of landmark data. *Anatomical Records, 275A*, 1048–1072.

Galetti, M., & Pedroni, F. (1994). Seasonal diet of capuchin monkeys (*Cebus apella*) in a semi-deciduous forest in south-east Brazil. *Journal of Tropical Ecology, 10*, 27–39.

Garland, T., Bennett, A. F., & Rezende, E. L. (2003). Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology, 208*, 3015–3035.

Gould, S. J. (2002). *The structure of evolutionary theory*. Cambridge, MA: Harvard University Press.

Hawkins, B. A., Diniz-Filho, J. A. F., Jaramillo, C. A., & Soeller, S. A. (2006). Post-Eocene climate change, niche conservatism, and the latitudinal diversity gradient of New World birds. *Journal of Biogeography, 33*, 770–780.

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature, 470*, 479–485.

Hoorn, C., Wesselingh, F. P., ter Steege, H., Bermudez, M. A., Mora, A., et al. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science, 330*, 927–931.

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: Plenum Press.

Klingenberg, C. P. (2011). MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources, 11*, 353–357.

Klingenberg, C. P., Barluenga, M., & Meyer, A. (2002). Shape analysis of symmetric structures: Quantifying variation among individuals and asymmetry. *Evolution, 56*, 1909–1920.

Klingenberg, C. P., & Gidazwiski, N. A. (2010). Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Systematic Biology, 59*, 245–261.

Klingenberg, C. P., & Spence, J. R. (1993). Heterochrony and allometry: Lessons from the water strider genus *Limnoporus*. *Evolution, 47*, 1834–1853.

Lehman, S. M., & Fleagle, J. G. (2006). Biogeography and primates: A review. In S. M. Lehman & J. G. Fleagle (Eds.), *Primate biogeography* (pp. 1–36). New York: Springer.

Lynch Alfaro, J. W., Boubli, J. P., Olson, L. E., Di Fiore, A., Wilson, B., et al. (2012a). Explosive Pleistocene range expansion leads to widespread Amazonian sympatry between robust and gracile capuchin monkeys. *Journal of Biogeography, 39*, 272–288.

Lynch Alfaro, J. W., Silva, J. S., & Rylands, A. B. (2012b). How different are robust and gracile capuchin monkeys? An argument for the use of *Sapajus* and *Cebus*. *American Journal of Primatology, 74*, 273–286.

Machado, F. A., & Hingst-Zaher, E. (2009). Investigating South American biogeographic history using patterns of skull shape variation on *Cerdocyon thous* (Mammalia: Canidae). *Biological Journal of the Linnean Society, 1*, 77–84.

Marroig, G., & Cheverud, J. (2001). A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology and ontogeny during cranial evolution of New World monkeys. *Evolution, 55*, 2576–2600.

Marroig, G., & Cheverud, J. (2004). Did natural selection or genetic drift produce the cranial diversification of Neotropical monkeys? *The American Naturalist, 163*, 417–428.

Marroig, G., & Cheverud, J. (2005). Size as a line of least evolutionary resistance: Diet and adaptive morphological radiation in New World monkeys. *Evolution, 59*, 1128–1142.

Marroig, G., & Cheverud, J. (2010). Size as a line of least evolutionary resistance II: Direct selection on size or correlated response due to constraints? *Evolution, 64–5*, 1470–1488.

Martins, M. M. (2008). Fruit diet of *Alouatta guariba* and *Brachyteles arachnoides* in Southeastern Brazil: Comparison of fruit type, color, and seed size. *Primates, 49*, 1–8.

Meloro, C. (2011). Feeding habits of Plio-Pleistocene large carnivores as revealed by their mandibular geometry. *Journal of Vertebrate Paleontology, 31*, 428–446.

Meloro, C., & Jones, M. E. H. (2012). Tooth and cranial disparity in the fossil relatives of *Sphenodon* (Rhynchocephalia) dispute the persistent 'living fossil' label. *Journal of Evolutionary Biology, 25*, 2194–2209.

Meloro, C., Raia, P., Piras, P., Barbera, C., & O'Higgins, P. (2008). The shape of the mandibular corpus in large fissiped carnivores: Allometry, function and phylogeny. *Zoological Journal of the Linnean Society, 154*, 832–845.

Mittelbach, G. G., Schemske, D. W., Cornell, H. V., Allen, A. P., Brown, J. M., Bush, M. B., et al. (2007). Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. *Ecology Letters, 10*, 315–331.

Monnet, C., De Baets, K., & Klug, C. (2011). Parallel evolution controlled by adaptation and covariation in ammonoid cephalopods. *BMC Evolutionary Biology, 11*, 115.

Monteiro, L. R., Duarte, L. C., & dos Reis, S. F. (2003). Environmental correlates of geographical variation in skull and mandible shape of the punarè rat *Thrichomysapereoides* (Rodentia: Echimyidae). *Journal of Zoology, 261*, 47–57.

Moura, A. C. A., & Lee, P. C. (2004). Capuchin stone tool use in Caatinga dry forest. *Science, 306*, 1909.

Olson, R. J., Johnson, K., Zheng, D., & Scurlock, J. M. O. (2001). *Global and regional ecosystem modeling*: *Databases of model drivers and validation measurements.* ORNL/TM-2001/196. Oak Ridge, TN: Oak Ridge National Laboratory.

Ottoni, E. B., & Izar, P. (2008). Capuchin monkey tool use: Overview and implications. *Evolutionary Anthropology, 17*, 171–178.

Piras, P., Marcolini, F., Claude, J., Ventura, J., Kotsakis, T., & Cubo, J. (2012). Ecological and functional correlates of molar shape variation in European populations of Arvicola (Arvicolinae, Rodentia). *Zoologischer Anzeiger, 251*, 335–343.

Rabosky, D. L., & Adams, D. C. (2012). Rates of morphological evolution are correlated with species richness in salamanders. *Evolution, 66*, 1807–1818.

Raia, P., Carotenuto, F., Passaro, F., Piras, P., Fulgione, D., Werdelin, L., et al. (2013). Rapid action in the Palaeogene, the relationship between phenotypic and taxonomic diversification in Cenozoic mammals. *Proceedings of the Royal Society London B, 280*, 20122244.

Raia, P., Passaro, F., Fulgione, D., & Carotenuto, F. (2012). Habitat tracking, stasis and survival in Neogene large mammals. *Biology Letters, 8*, 64–66.

Ricklefs, R. E., & Renner, S. S. (1994). Species richness within families of flowering plants. *Evolution, 48*, 1619–1636.

Rohlf, F. J. (2000). On the use of shape spaces to compare morphometric methods. *Hystrix, Italian Journal of Mammology, 11*, 9–25.

Rohlf, F. J. (2006). *tpsPLS 1.18*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf, F. J. (2010). *tpsDig 2.16*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf, F. J., & Corti, M. (2000). Use of two-block partial least squares to study covariation in shape. *Systematic Biology, 49*, 740–753.

Rohlf, F. J., & Slice, D. E. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology, 9*, 40–59.

Rosenberger, A. L. (1992). Evolution of feeding niches in New World monkeys. *American Journal of Physical Anthropology, 88*, 525–562.

Rosenberger, A. L., Halenar, L., & Cooke, S. B. (2011). The making of platyrrhine semifolivores: Models for the evolution of folivory in primates. *Anatomical Records, 12*, 2112–2130.

Rundell, R. J., & Price, T. D. (2009). Adaptive radiation, non-adaptive radiation, ecological speciation and non-ecological speciation. *Trends in Ecology & Evolution, 24*, 394–399.

Silva, J. S. (2001). *Especiação nos macacos-prego e cairaras, gênero Cebus Erxleben, 1777* (*Primates, Cebidae*). PhD thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Stenseth, N. C. (1984). The tropics: Cradle or museum? *Oikos, 43*, 417–420.

Thorpe, R. S. (1983). A biometric study of the effects of growth on the analysis of geographic variation: Tooth number in green geckos (Reptilia, *Phelsuma*). *Journal of Zoology, London, 201*, 13–26.

Thorpe, R. S. (1988). Multiple group principal component analysis and population differentiation. *Journal of Zoology, London, 216*, 37–40.

Van Valen, L. (1973). A new evolutionary law. *Evolutionary Theory, 1*, 1–30.

Weir, J. T., & Schluter, D. (2007). The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science, 315*, 1574–1576.

Wright, B. W. (2005). Craniodental biomechanics and dietary toughness in the genus Cebus. *Journal of Human Evolution, 48*, 473–492.

Wright, B. W., Wright, K. A., Chalk, J., Verderane, M. P., Fragaszy, D., Visalberghi, E., et al. (2009). Fallback foraging as a way of life: Using dietary toughness to compare the fallback signal among capuchins and implications for interpreting morphological variation. *American Journal of Physical Anthropology, 140*, 687–699.

Zelditch, M. L., Swiderski, D. L., Sheets, H. D., & Fink, W. L. (2004). *Geometric morphometrics for biologists. A primer*. USA: Elsevier.