

Patterns of cranial shape diversification during the phylogenetic branching process of New World monkeys (Primates: Platyrrhini)

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Abstract

One of the central topics in evolutionary biology is understanding the processes responsible for phenotypic diversification related to ecological factors. New World monkeys are an excellent reference system to investigate processes of diversification at macroevolutionary scales. Here, we investigate the cranial shape diversification related to body size and ecology during the phylogenetic branching process of platyrrhines. To investigate this diversification, we used geometric morphometric techniques, a molecular phylogenetic tree, ecological data and phylogenetic comparative methods. Our statistical analyses demonstrated that the phylogenetic branching process is the most important dimension to understand cranial shape variation among extant platyrrhines and suggested that the main shape divergence among the four principal platyrrhine clades probably occurred during the initial branching process. The phylogenetic conservatism, which is the retention of ancestral traits over time within the four principal platyrrhine clades, could be the most important characteristic of platyrrhine cranial shape diversification. Different factors might have driven early shape divergence and posterior relative conservatism, including genetic drift, stabilizing selection, genetic constraints owing to pleiotropy, developmental or functional constraint, lack of genetic variation, among others. Understanding the processes driving the diversification among platyrrhines will probably require further palaeontological, phylogenetic and comparative studies.

Introduction

High phenotypic diversity is displayed by some phylogenetic groups (Gavrilets & Losos, 2009; Wiens, 2009). One of the central topics in evolutionary biology is understanding the processes responsible for this diversity related to ecological factors (Schluter, 2000; Perez *et al.*, 2009; Losos & Mahler, 2010). Although there have been numerous investigations into these processes, understanding the process of phenotypic diversification is a complicated task because causal relationships are generally complex; the relevant factors vary among evolution-

ary scales; the observed changes mostly took place in the distant past; and we cannot use experimental methods to test these relationships (Schluter, 2000; Gavrilets & Losos, 2009; Wiens, 2009). Studying the associations between axes of morphological variation, such as size and shape, and ecology during the phylogenetic branching process in key phylogenetic groups represents a promising approach for understanding the process of phenotypic diversification at different evolutionary scales (Schluter, 2000; Gavrilets & Losos, 2009; Perez *et al.*, 2009).

Platyrrhines, or New World monkeys (Wilson & Reeder, 2005), are an excellent reference system for investigating processes of diversification at macroevolutionary scales in relation to ecological factors. First, the extant platyrrhines, which currently encompass 125 species (Wilson & Reeder, 2005), are a monophyletic

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group and have a history of nearly 30–40 million years in South America and the Caribbean (Fleagle, 1999; Tejedor, 2008). They evolved as long-stemmed branches of persistent lineages without successive temporal replacements by related primate groups over ca. 15 or 20 million years (Delson & Rosenberger, 1984; Rosenberger, 2002, 2010; Kay *et al.*, 2008; Tejedor, 2008). Second, they underwent a major radiation, exhibiting great variation in body size including species with body weights from ca. 100 g to 10 kg (Smith & Jungers, 1997; Fleagle, 1999). Third, they occupy a large range of dietary niches, consuming food sources including exudates, fruit pulp, seeds, leaves and insects, and they show considerable diversity in many aspects of their life history (Fleagle, 1999; Norconk *et al.*, 2009). Several studies have suggested that ecological axes, such as diet and life history traits, are associated with body size diversity during phylogenetic branching (Fleagle, 1999; Wildman *et al.*, 2009). Recent morphometric studies have focused on cranial size variation, concluding that cranial size mainly diversified allometrically in response to body size (Marroig & Cheverud, 2001, 2005). Overall, previous studies have suggested that body size represents the most important axis to understand platyrrhine morphological diversification (Fleagle, 1999).

Although changes in size are important in understanding the process of platyrrhine morphological diversification, previous studies have not systematically investigated another important axis of this process: shape variation, or changes in the spatial positions of anatomical traits (Bookstein, 1991; Zelditch *et al.*, 2004). Cranial shape, in particular, is related to numerous functions (e.g. the acquisition and initial preparation of food), which have a direct association with ecological axes (Fleagle, 1999; Hall, 2003). Different shape patterns may enhance the fitness of an individual, thus affecting the evolution and diversification of taxa. Consequently, in attempting to understand platyrrhine diversification, we cannot ignore cranial shape variation. Here, we investigate the association of cranial shape with body size and ecological axes during the phylogenetic branching process that led to the diversity of platyrrhines. Based on previous studies that indicated the importance of body size in platyrrhine diversification, we hypothesized that body size is the most important variable to predict cranial shape variation among extant platyrrhine species. Alternatively, because of the functional importance of cranial shape, we hypothesized that ecology could be another important axis to explain cranial shape variation. Finally, cranial shape variation could be merely a by-product (correlate) of the phylogenetic branching process. To evaluate these hypotheses, we (i) estimated phylogenetic relationships between the platyrrhine species based on mitochondrial and nuclear DNA sequences (Lemey *et al.*, 2009); (ii) measured body size as body mass (kg), life history traits as variation in the age of first reproduction, gestational length and age of weaning, and diet as the

percentages of food components, using published data (Hartwig, 1996; Smith & Jungers, 1997; Fleagle, 1999; Marroig, 2007; Norconk *et al.*, 2009); (iii) quantified cranial shape using the coordinates of reference points (i.e. landmarks and semilandmarks) and geometric morphometric techniques (Bookstein, 1991; Adams *et al.*, 2004; Zelditch *et al.*, 2004); and (iv) measured the associations of cranial shape with phylogenetic relationships, body size, life history and/or diet using phylogenetic comparative methods (Felsenstein, 1985; Rohlf, 2001; Blomberg *et al.*, 2003; Klingenberg & Gidaszewski, 2010). Additionally, we measured the associations of cranial size with phylogenetic relationships, body size, life history and/or diet to compare the results with the shape results, as well as with the previous size results based on traditional morphometric analyses (Marroig & Cheverud, 2001, 2005).

Materials and methods

Samples

We examined 29 species of the 15 extant genera of platyrrhines from South America (Table S1). The 29 species examined were chosen because they are a representative sample of the phylogenetic, ecological and morphological diversity of the platyrrhine primates (Wilson & Reeder, 2005). Specifically, we sampled ca. 30% extant species, being very careful in selecting almost one species from each genus and several species for the more numerous genera (Table S1). Additionally, there are molecular sequences available for the 29 species selected, so a molecular phylogeny could be estimated. The specimens included in this study were adults defined by the presence of an obliterated basioccipital synchondrosis and completely erupted and functional dentition. Males and females were pooled in the analyses (approximately equal proportions of male and female specimens were selected for each species) to calculate mean shape for each platyrrhine species in order to obtain an adequate sample size for the further statistical analyses. Because this procedure could be problematic, the influence of sexual dimorphism over the further comparative analyses was explored. First, we estimated the correspondence between the patterns of variation in body size for male and female specimens. The selection of this variable is justified by the fact that platyrrhines are mainly dimorphic in size (Materson & Hartwig, 1998; Marroig, 2007), and the information about body mass for each sex is available for all species studied. The correlation between mean body size for male and female was 0.99 and highly significant ($P < 0.0001$). Second, we compared our mean size estimation to the size estimation obtained by an alternative procedure that standardizes the female mean to equal the male mean by adding the male-to-female difference between mean size to the size of females (Cardini *et al.*, 2010). The correlation between

both size estimation was 0.998 and highly significant ($P < 0.0001$). These analyses suggest that our procedure will not affect the further analyses, probably because morphometric variation between sexes is smaller than the variation among platyrrhine species.

Diet, life history, body mass variation and phylogenetic relationships

Data on body mass, diet and life history for each species or genus were collected because these could represent important variables for understanding the cranial shape divergence among New World monkeys (Fleagle, 1999; Marroig & Cheverud, 2001, 2005; Wildman *et al.*, 2009). Body mass (kg; BM) was obtained for each of the 29 species from Smith & Jungers (1997). Data concerning diet for the 15 genera were taken from the studies of Norconk *et al.* (2009) and Fleagle (1999). The available information was classified according to percentages of diet components (i.e. exudates, fruit pulp, flowers, seeds, leaves and insects). Life history (LH) variation was measured using three traits available for all genera: the age of first reproduction, gestational length and age of weaning (Hartwig, 1996; Fleagle, 1999; Marroig, 2007). Because ecological and size variables are highly correlated ($r = 0.80$; Ford & Davis, 1992; Hartwig, 1996), we calculated principal component (PC) scores (ca. 85–90% of total variation) to reduce the number of ecological variables and avoid multicollinearity; these PCs describe broad variation in diet (the first three diet PCs), life history (the first LH PC) and all ecological variables (LH plus diet) plus log body size (the first three EBM PCs).

To study the phylogenetic relationships between the 29 New World primate species, sequences from the $\beta 2$ -microglobulin (B2M), β -globin (HBB), interphotoreceptor retinol-binding protein (IRBP), von Willenbrand factor (vWF) and cytochrome *b* (Cyt B) genes were obtained for the platyrrhine species and outgroups (*Macaca mulatta* and *Homo sapiens*) from GenBank. Sequences of each gene were aligned using ClustalW and manually corrected with BioEdit 7.0.0 software (Hall, 2004). Molecular phylogenetic analyses were performed using the Bayesian Inference (BI) method. We performed a combined analysis based on the combined molecular matrix with a total of 5912 bp, including the mitochondrial gene Cyt B (374 bp) and the nuclear gene sequences B2M (1438 bp), HBB (2985 bp), IRBP (1839 bp) and vWF (905 bp). When the sequence for a given gene was not available for a particular species, it was coded as missing data. This combined matrix of genes allowed the incorporation of a large number of species and the combination of mitochondrial and nuclear genes with high phylogenetic resolution at low and high taxonomic levels. jModelTest 0.1 (Posada, 2008) was employed to determine the most appropriate model of sequence evolution for each analysed gene. The best-fit model for the genes B2M, HBB and vWF was

TPM3uf + G; for Cyt B sequences, it was HKY + I + G; and for the gene IRBP, it was K80 + G. The BI method was implemented using Mr. Bayes v3.1.2 (Ronquist & Huelsenbeck, 2003). The models of sequence evolution identified as optimal by jModeltest for the data partitions were implemented for each of the data partitions. Two simultaneous analyses were performed using Markov chain Monte Carlo (MCMC) simulations for 1 000 000 generations. A sample frequency of 100 and a burn-in of 250 were used.

Morphometric analyses

Cranial shape was captured from crania as 3D coordinates for landmarks and semilandmarks (Fig. 1; Table S2 defines the landmarks and semilandmarks). Thirty-five 3D coordinates for landmarks and 67 3D coordinates for semilandmarks were obtained with a Microscribe G2X (Fig. 1; Table S2). The semilandmarks were defined between the neighbouring landmarks from several cranial bones and sutures. Special care was taken to ensure that the semilandmarks were restricted between the locations of fixed landmarks on different specimens to avoid the potential problem with assigning homology to semilandmarks (Wiley *et al.*, 2005; Polly, 2008; Oxnard & O'Higgins, 2009). It is important to note here that landmarks-based morphometric methods make the assumption that the reference points are homologous across specimens. Whereas landmarks have a geometrical homology related to points sited on them, the semilandmarks have geometrical homology related more to the entire curve of a suture or surface of a bone (Gunz *et al.*, 2005; Polly, 2008; Fig. 1; Table S2). In this way, the homology concept behind a landmark or a set of semilandmarks describing a curve is the same. However, because of existing disagreement about the best data to

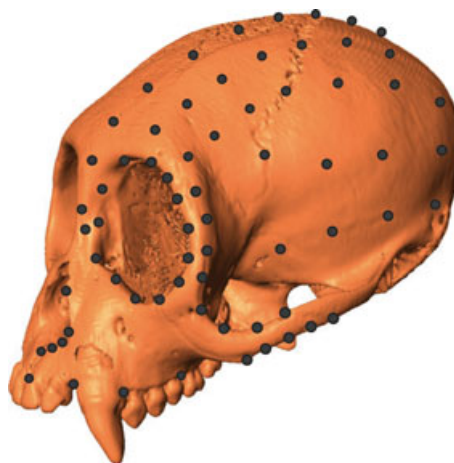


Fig. 1 Cranial landmarks and semilandmarks recorded from New World monkeys using a 3D Microscribe G2X digitiser.

use when studying shape variation (Gunz *et al.*, 2005; Polly, 2008; Oxnard & O'Higgins, 2009), two data sets were chosen to represent the geometry of the platyrrhine crania. First, we used only the set of 35 landmarks (L; Table S2). The second data set was conformed by the 35 landmarks plus 67 semilandmarks (L&SL; Fig. 1; Table S2). Using landmarks plus semilandmarks could be important because they can characterize cranial differences between primate taxa in both regions with (i.e. cranial base) and without landmarks (i.e. cranial vault; Gunz *et al.*, 2005; Wiley *et al.*, 2005). Landmarks and semilandmarks of crania were aligned by means of generalized Procrustes analysis (Rohlf & Slice, 1990; Bookstein, 1991, 1997). This procedure optimally translates, scales and rotates the coordinates of landmarks and semilandmarks (Rohlf & Slice, 1990; Bookstein, 1991; Zelditch *et al.*, 2004). The investigated semilandmarks were analysed as equidistant points along ten curves and three surfaces (Fig. 1; Reddy *et al.*, 2005; Williams & Slice, 2010). Using this procedure, we obtained the superimposed coordinates, referred to as Procrustes shape coordinates, that defined the observed shape variation. The centroid size (CS) of the skull specimens (the square root of the summed squared distances from all landmarks and semilandmarks to the configuration centroid) was measured for each data set and was used to scale the raw coordinates in the Generalized Procrustes Analysis (Rohlf & Slice, 1990). In addition, the logarithm of centroid size (Log CS) was used to explore cranial size variation and compare the results with the previous traditional morphometric analyses (Marroig & Cheverud, 2001, 2005). A principal component analysis (Bookstein, 1991; Zelditch *et al.*, 2004) based on the covariance matrices was performed on the Procrustes shape coordinates (using a full tangent space projection) for all mean samples to reduce the dimensionality of the shape space. The principal component scores of the Procrustes shape coordinates are referred to as relative warps (RW; sensu Mitteroecker & Gunz, 2009) and describe uncorrelated axes of the major trends in shape variation among species (Bookstein, 1991; Zelditch *et al.*, 2004). Visualizations of shape changes at the extremes of the RW scores were performed by warping a 3D surface model created from computed tomography data of a *Cebus apella* skull.

In this study, we used Morphologika 2.5 (O'Higgins & Jones, 2009), MorphoJ (Klingenberg, 2011), Landmark (Wiley *et al.*, 2005) and R 2.10.0 (R Development Core Team, 2010) software to perform the morphometric analyses.

Phylogenetically based statistical analyses

The association between the phylogeny (Bayesian tree) and the patterns of cranial shape variation was evaluated by calculating the phylogenetic signal of the first RW and the Procrustes shape coordinates. We calculated the

univariate K statistic proposed by Blomberg *et al.* (2003) and the multivariate *Tree length* measure proposed by Klingenberg & Gidaszewski (2010). The K statistic is computed as $K = \text{observed (MSE}_0/\text{MSE})/\text{expected (MSE}_0/\text{MSE)}$. The numerator MSE_0 is calculated as the mean squared error measured from the phylogenetic mean, and MSE is the mean squared error measured from the phylogenetic mean after first correction for phylogenetic nonindependence, assuming Brownian motion. The denominator is the expected MSE ratio under Brownian motion (Blomberg *et al.*, 2003; Revell *et al.*, 2008). The K statistic provides a univariate measure of the strength of phylogenetic signal data; values near 0 indicate a lack of signal, and values near 1 are expected if the character evolved under a Brownian motion model (Blomberg *et al.*, 2003). The null hypothesis of the absence of a phylogenetic signal was tested calculating whether the observed K value is not greater than expected and whether the values for platyrrhine species were randomized among tips (10 000 replications; Blomberg *et al.*, 2003). We used the K statistic to test the phylogenetic signal of the variation in the Log CS and RW1. The use of the RW1 is justified because it is a linear combination of the Procrustes shape coordinates aligned to the main direction of variation, expected to be a proxy to a phenotypically relevant pattern, and because it is calculated using a rigid orthogonal rotation that ensures that the Procrustes shape space is not deformed and the interobject distances are maintained. We also evaluated the association of phylogeny with the patterns of body mass, diet and life history variation using the K statistic on the natural logarithm of body mass (Log BM), PC scores of diet and PC1 of life history (LH PC1), respectively.

Tree length is a multivariate measure (Klingenberg & Gidaszewski, 2010) and was used to test the phylogenetic signal of Log CS and all shape variation, i.e. the space defined by the Procrustes shape coordinates. This statistic measures the total amount of squared change, summed over all branches of the Bayesian tree. The observed statistic is compared with a distribution of the values obtained by randomly permuting the shape data among the platyrrhine species, i.e. the null hypothesis of absence of a phylogenetic signal among platyrrhines. The significance of the *Tree length* statistic was assessed via permutation tests with 10 000 replications.

Finally, we tested the association of cranial size and shape variation with body size and ecological variables by employing a regression analysis that take into account the expected lack of independence among samples resulting from phylogenetic structure: ordinary least square based on phylogenetic independent contrasts [(OLS-PIC); Felsenstein, 1985; Rohlf, 2001]. We fitted the Log BM, diet PCs and LH PC1, as well as the EBM PCs for all of these variables to the Log CS and Procrustes shape coordinates using the regression model: $\mathbf{S} = \mathbf{XB} + \epsilon$, where \mathbf{S} is the PIC for the Log CS or Procrustes

shape coordinates matrices describing mean differences; \mathbf{X} is the PIC matrix of independent variables (Log BM, diet PCs, LH PC1 or EBM PCs); \mathbf{B} is the matrix of regression coefficients; and ϵ is the error term. In the OLS regression analysis based on PIC, ϵ is assumed to be independent, but the regression analysis is performed on the scores for the independent contrasts for shape, size and ecological variables (Felsenstein, 1985; Rohlf, 2001). We calculated the independent contrast using the Bayesian tree and assuming a Brownian motion model of evolution. The significance of the OLS-PIC was assessed using permutation test (10 000 rounds). We also quantify the amount of variation for which the regression model accounted as a percentage of the total variation, computed using the Procrustes metric (Drake & Klingenberg, 2008).

We also test whether a phylogenetic regression based on an Ornstein–Uhlenbeck model (O-U) of phylogenetic correlation structure fits the data better than a phylogenetic regression based on Brownian motion model (i.e. the PIC-OLS method) using Akaike information criterion (AIC; Paradis, 2006). We fitted the Log BM to the first eight RW scores (ca. 90% of total shape variation) using a phylogenetic generalized least squares [(PGLS); Martins & Hansen, 1997; Rohlf, 2001] regression model: $\mathbf{S} = \mathbf{X}\mathbf{B} + \epsilon$, where \mathbf{S} is the RW scores describing mean differences; \mathbf{X} is the matrix of independent variables (Log BM); \mathbf{B} is the matrix of regression coefficients; and ϵ is the error term that has a covariance matrix derived from the Bayesian phylogenetic tree (Rohlf, 2001). We calculated the two phylogenetic covariance matrices that assume the Brownian motion and the Ornstein–Uhlenbeck models of evolution (Martins & Hansen, 1997; Rohlf, 2001).

The statistical analyses were performed using the packages *picante*, *vegan* and *ape* for R 2.10.0 (R Development Core Team, 2010) and *MorphoJ* software (Klingenberg, 2011).

Results

The Bayesian (Fig. 2) analysis shows that most of the platyrrhine clades are strongly supported with high posterior probabilities (>0.95), and only a small number of terminal clades (i.e. *Pithecia* and *Ateles*) present lower posterior probabilities (between 0.65 and 0.80). The analyses support the previous division of the platyrrhines into three families: Atelidae, Cebidae and Pitheciidae; the analyses also corroborate a closer phylogenetic relationship between Atelidae and Cebidae (Ray *et al.*, 2005; Opazo *et al.*, 2006; Wildman *et al.*, 2009). Within the Atelidae, *Alouatta* is shown as a sister group of a clade including *Ateles*, *Brachyteles* and *Lagothrix*, in which *Brachyteles* and *Lagothrix* are clustered together. Within the Cebidae, there are two major clades that are strongly supported: (i) Cebine, with *Cebus* and *Saimiri* plus *Aotus*, and (ii) Callitrichine, formed by the *Saguinus*, *Leontopi-*

theus, *Callithrix* and *Callimico* genera. Among the Pitheciidae genera, *Callicebus* is the first to branch from the clade that includes *Pithecia*, *Chiropotes* and *Cacajao*, in which *Chiropotes* and *Cacajao* are sister groups (Fig. 2).

The ordination of the 29 platyrrhine species based on shape (RW-L&SL) variation for the crania is shown in Fig. 3. In this figure, each species is represented by its mean value on the first and second RWs. The percentages of variation explained by the first two RWs based on shape variation were 49% and 16% for RW1 and RW2, respectively. The shape ordination generated by the RWs shows some correspondence with the four major phylogenetic clades (Fig. 3). This phylogenetic correspondence is mainly observed in RW1. Because there is no agreement regarding the best criteria to use when studying semilandmarks, we reanalysed the data set using the bending energy criterion to slide semilandmarks (Bookstein, 1997; Gunz *et al.*, 2005), which is implemented in a set of R routines that were written and shared by Dean Adams and Erik Otárola-Castillo. We also analysed shape variation using only landmark coordinates. The results of both landmarks and bending energy criterion are generally similar to the original complete data set ordination (results not shown).

The shape variation along the first and second RWs-L&SL is shown in Fig. 3 by warping the skull surface. RW1 shows changes that consist of forward and downward growth in the facial region, a decrease in vault size and reorientation in the flexion of the cranial base. The Atelidae and Cebidae clades are extreme in this variation (Fig. 3). The facial skeleton is larger in the Atelidae clade, whereas the Cebidae show a more rounded cranial vault. The Atelidae clade also shows a foramen magnum that is backwards in orientation, whereas the Cebidae clade exhibits a re-orientation of the foramen magnum that is placed in a reminiscent position to what is seen in *Homo sapiens* and is associated with the flexion of the cranial base. RW2 shows mainly an increase in orbital size changes and facial elongation, with *Aotus* being extreme in this variation.

The *K* and *Tree length* statistics show significant phylogenetic structure in the cranial size and shape variation (Table 1). These statistical analyses support the importance of phylogeny in understanding the pattern of cranial shape variation observed in the ordination graphs (Fig. 3). The phylogenetic signal is also high for Log BM, diet PC and LH PC1 (*K* values between 3.069 and 2.094, $P = 0.0001$), confirming previous qualitative observations (Fleagle, 1999). OLS-PIC analyses demonstrate that the Log BM is not highly associated with the patterns of shape variation measured with L&SL (Table 2), suggesting that the change in body mass does not explain the major changes in cranial shape. The ecological variables of diet and life history characteristics are not associated with cranial shape (Table 2). In addition, the percentages of shape variation predicted by these variables are low, varying between 5% and 15%. OLS-PIC analyses

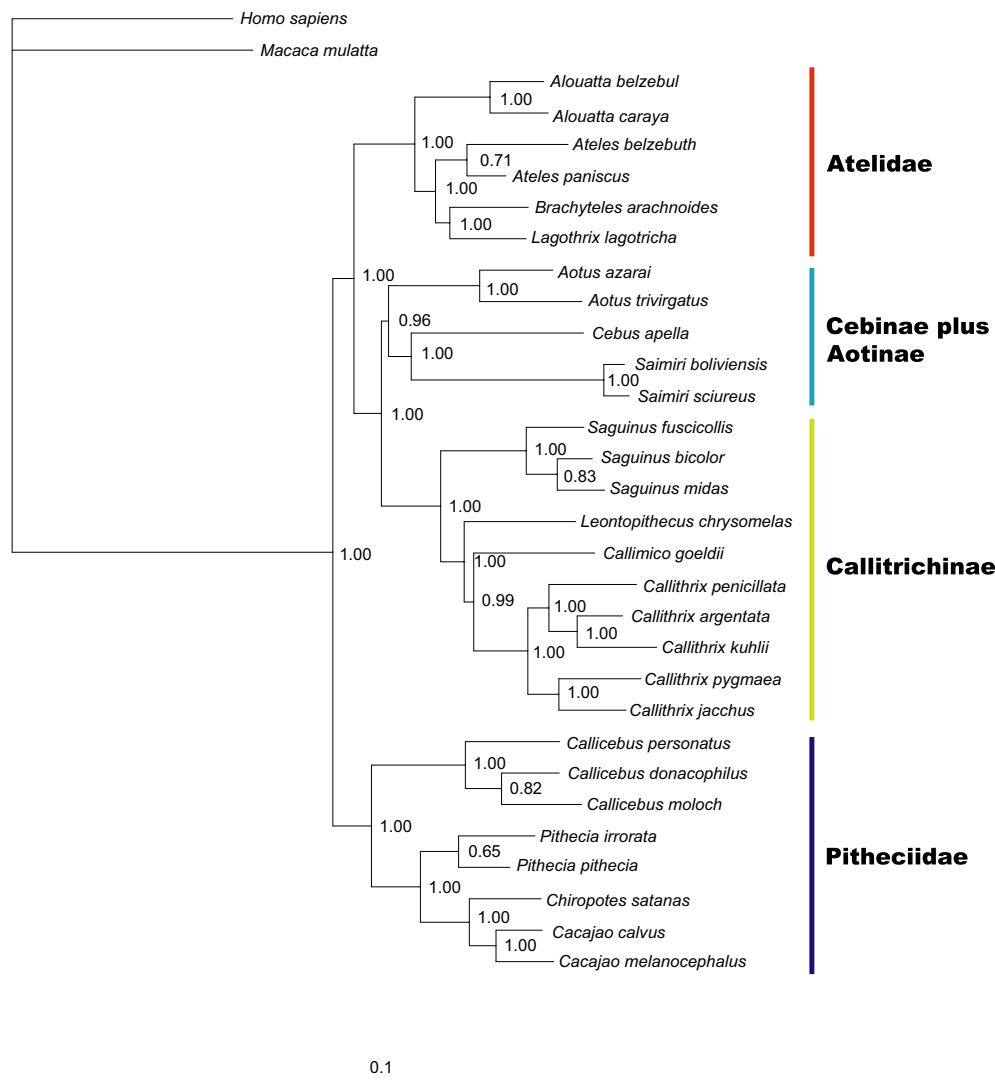


Fig. 2 Phylogenetic tree topology obtained by Bayesian Inference for the New World monkey species studied, combining Cyt B, B2M, HBB, IRBP and vWF sequences. Numbers in the branches represent posterior probability support.

performed using the first eight RWs (ca. 90% of total shape variation) show similar results (results not shown). The AIC value for the fit of the data set to the Ornstein–Uhlenbeck model of phylogenetic correlation structure does not indicate an improvement compared with the Brownian model (AIC for Brownian model = -71.751 and AIC for O-U model = -71.184). The OLS-PIC analyses on L data set support the L&SL results, showing that the shape variation in the L data set is weakly associated with Log BM and ecological variables, explaining between 7% and 18% of shape variation (Table 2). Conversely, the OLS-PIC analyses demonstrated that the Log CS is significantly associated with the Log BM and the PCI of life history characteristics, explaining ca. 85% and 55% of variation, respectively (Table 2), and

corroborating previous traditional morphometric studies. We repeat all these analyses using PGLS (Rohlf, 2001), and the results were similar to the OLS-PIC results (results not shown).

Discussion

Interesting patterns of morphometric changes among platyrrhines were found in this study using 3D coordinate-based techniques that allowed us to measure morphological differentiation as shape variation, i.e. changes in the relative spatial positions of the coordinates describing anatomical points, curves and surfaces of the skull (Bookstein, 1997; Adams *et al.*, 2004; Zelditch *et al.*, 2004). Our cranial shape description of the platyrrhines

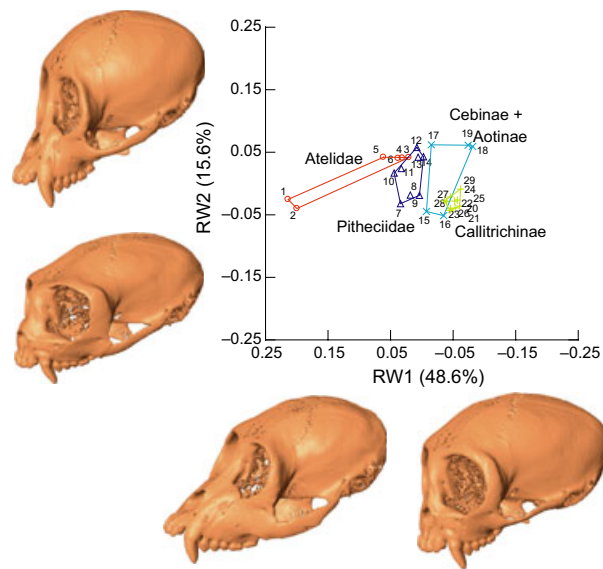


Fig. 3 Ordination of the 29 Platyrrhine species in the space defined by the first two RWs, and cranial shape changes along these RWs shown by surface warps. The dots represent the consensus individual for each species. Species numbers are defined as: 1: *Alouatta belzebul*; 2: *Alouatta caraya*; 3: *Ateles belzebuth*; 4: *Ateles paniscus*; 5: *Brachyteles arachnoides*; 6: *Lagothrix lagotricha*; 7: *Callicebus personatus*; 8: *Callicebus donacophilus*; 9: *Callicebus moloch*; 10: *Pithecia irrorata*; 11: *Pithecia pithecia*; 12: *Cacajao calvus*; 13: *Cacajao melanocephalus*; 14: *Chiropotes satanas*; 15: *Aotus azarai*; 16: *Aotus trivirgatus*; 17: *Cebus apella*; 18: *Saimiri boliviensis*; 19: *Saimiri sciureus*; 20: *Saguinus bicolor*; 22: *Saguinus midas*; 21: *Saguinus fuscicollis*; 23: *Leontopithecus chrysomelas*; 24: *Callithrix argentata*; 26: *Callithrix penicillata*; 25: *Callithrix pygmaea*; 27: *Callithrix jacchus*; 28: *Callithrix kuhlii*; 29: *Callimico goeldii*.

permitted us to show morphometric variation in regions with few landmarks, such as the neurocranial region. Because a large proportion of the shape diversification among platyrrhines involves changes in these regions, the analysis performed in this study sheds light on this important axis of phenotypic variation, which was not previously addressed in morphometric studies. Particularly, the shape variation observed among platyrrhines corresponds to large differences in the relative position of traits of the face, base and cranial vault (Fig. 3). Previous studies have mainly focussed on landmark variation among platyrrhine species based on traditional morphometric methods for measuring cranial size (e.g. Marroig & Cheverud, 2005), which are considered less effective than geometric morphometrics in describing this shape variation (Adams *et al.*, 2004; Zelditch *et al.*, 2004).

Although the OLS analysis shows a correspondence between body size and cranial shape (results not shown), our OLS-PIC analyses – which take into account the phylogenetic structure – demonstrated that body size has little importance in explaining cranial shape variation among extant platyrrhine species, which opposes our main hypothesis. These results are in agreement with

Table 1 Phylogenetic signal measured by the *K* (Blomberg *et al.*, 2003) and *Tree length* (Klingenberg & Gidaszewski, 2010) statistics for cranial size (Log CS) and shape variation for the landmarks (L) and landmarks plus equidistant semilandmarks (L&SL). Shape variation is described using all Procrustes shape coordinates (Shape) and the first relative warp score (RW1).

| | <i>Tree length</i> | <i>K</i> | <i>P</i> |
|-------------|--------------------|----------|----------|
| RW1-L | | 2.571 | 0.0001 |
| RW1-L&SL | | 2.048 | 0.0001 |
| Shape-L | 0.077 | | 0.0001 |
| Shape-L&SL | 0.084 | | 0.0001 |
| Log CS-L&SL | 0.342 | 2.985 | 0.0001 |

Table 2 Ordinary least square on phylogenetic independent contrasts (OLS-PIC) regressions of cranial size (Log CS) and shape variation for the landmarks (L) and landmarks plus equidistant semilandmarks (L&SL) on the log body mass (Log BM), life history (LH PC; the first PC) and diet (diet PC; first three PCs) and all ecological variables plus log body size (EBM PC; first three PCs). Shape variation is described using all Procrustes shape coordinates for PIC-OLS. The analysis assumes a Brownian model and used a multivariate permutation test. We need to consider that increasing the number of predictors from one for Log BM to three for diet PC and EBM PC may lead to overfitting and increasing artificially the percentage predicted.

| | Percentage predicted | <i>P</i> |
|--------------------|----------------------|----------|
| Shape vs. Log BM | | |
| L&SL | 7.904 | 0.0356 |
| L | 11.669 | 0.0072 |
| Shape vs. LH PC1 | | |
| L&SL | 5.708 | 0.1185 |
| L | 7.760 | 0.0360 |
| Shape vs. diet PC | | |
| L&SL | 15.633 | 0.0901 |
| L | 15.650 | 0.0955 |
| Shape vs. EBM PC | | |
| L&SL | 16.335 | 0.0735 |
| L | 18.266 | 0.0309 |
| Log CS vs. Log BM | | |
| L&SL | 84.173 | 0.0001 |
| Log CS vs. LH PC1 | | |
| L&SL | 53.457 | 0.0001 |
| Log CS vs. diet PC | | |
| L&SL | 22.665 | 0.1158 |
| Log CS vs. EBM PC | | |
| L&SL | 79.597 | 0.0001 |

previous observations based on rodents showing that shape measured with geometric morphometric techniques has less dependence on size variation compared with traditional linear measurements (Swiderski, 2003). The statistical analyses also demonstrated that there is not a high association between cranial shape variation and ecological axes after taking into account the non-independence among species resulting from phylogenetic

structure. These results differ from those of previous cranial size studies which suggest that the body size is of considerable importance in explaining the cranial size variation among platyrrhines (Marroig & Cheverud, 2005). Moreover, we performed an OLS-PIC analysis using our morphometric data set, which also shows that the Log BM and life history variables are highly and significantly associated (% predicted = 85 and 55, respectively) with the patterns of cranial size variation calculated with the Log CS.

Cranial shape clearly shows a different pattern of association with body size than the observed association between body size and cranial size, as well as between body size and ecological axes in the extant platyrrhine species (Ford & Davis, 1992; Hartwig, 1996; Fleagle, 1999). The cranial shape variation observed among platyrrhines is strongly associated with their phylogenetic relationships, suggesting that shape diversified in direct association with the phylogenetic branching process. The relative warp analyses of the platyrrhine species based on shape variation for the crania showed the existence of four groups that largely agreed with the four monophyletic clades observed in the Bayesian tree (Figs 2 and 3). The *K* and *Tree length* statistics corroborated the visual interpretation (Fig. 3); the ordination of species based on the RWs showed a strong phylogenetic signal (Table 1), indicating that the phylogenetically related species resemble each other in cranial shape. In particular, the *K* statistic suggests that phylogenetic signal was considerably greater than expected resulting from Brownian motion for the first RW and Log BM variables. The RWs 3–8 show a similar or lower value than the expected resulting from Brownian motion (results not shown). Similar associations between patterns of phylogenetic relationships and shape variation have been reported at different levels of Old World primate phylogenetic divergence (Cardini & Elton, 2008) and for several mammalian groups, e.g. rodents (Polly, 2001; Álvarez *et al.*, 2011).

Contrary to our hypothesis, the phylogenetic branching process was found to be very important to understand shape variation among extant platyrrhines. The existence of a strong association of cranial shape variation with phylogenetic relationships can be understood in the light of molecular clock studies (Opazo *et al.*, 2006; Hodgson *et al.*, 2009) and palaeontological evidence (Rosenberger, 2002, 2010; Kay *et al.*, 2008). These studies suggest that the main clade divergence among platyrrhines happened during the Early Miocene approximately 20–15 million years ago (Opazo *et al.*, 2006; Kay *et al.*, 2008; Hodgson *et al.*, 2009; Rosenberger, 2010). Thus, the main cranial shape divergence among platyrrhines probably also occurred during the Early Miocene. It is possible that only relatively small cranial shape variation associated with random processes characterized the platyrrhine diversification after the initial divergence during the Early Miocene, causing phylogenetically related extant

species to have a high degree of cranial shape similarity. Therefore, the phylogenetic conservatism (Wiens *et al.*, 2010), which is the retention of ancestral traits over time within the four principal platyrrhine clades, could be the most important characteristic of platyrrhine cranial shape diversification. Different factors might have driven the early shape divergence and posterior conservatism, including genetic drift, stabilizing selection, genetic constraints owing to pleiotropy, developmental or functional constraint, lack of genetic variation, among others (Gavrilets & Losos, 2009; Wiens *et al.*, 2010). However, if the main cranial shape divergence occurred deeper in the platyrrhine branching process, the factors driving the posterior shape divergence could make it difficult to know the process responsible for the early divergence using only extant species (Glor, 2010). Understanding the processes driving the divergence in cranial shape among platyrrhines will probably require further studies (Gavrilets & Losos, 2009; Glor, 2010; Wiens *et al.*, 2010) using additional palaeontological evidence, testing alternative models of the time and mode of the phylogenetic branching process (Kay *et al.*, 2008; Rosenberger, 2010) and estimating the fitness values of the shape differences among the main platyrrhine clades.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Names of the 29 species used in the study, together with the number used to abbreviations, sample sizes (*n*) and phylogenetic clades in the Fig. 2.

Table S2 Definition of 3-dimensional landmarks and semilandmarks (SL) for each cranial region and bone.

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