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SHAPE AT THE CROSS-ROADS: HOMOPLASY AND HISTORY IN THE EVOLUTION OF THE CARNIVORAN SKULL TOWARD HERBIVORY

Running title: Herbivorous adaptations in carnivores

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ABSTRACT

Patterns of skull shape in Carnivora provide examples of parallel and convergent evolution for similar ecomorphological adaptations. However, although most researchers report on skull homoplasies among hypercarnivorous taxa, evolutionary trends toward herbivory remain largely unexplored. In this study we analyze the skull of the living herbivorous carnivorans to evaluate the importance of natural selection and phylogenetic legacy in shaping the skulls of these peculiar species. We quantitatively estimated shape variability using geometric morphometrics. A principal components analysis of skull shape incorporating all families of arctoid carnivorans recognized several common adaptations toward herbivory. Ancestral state reconstructions of skull shape and the reconstructed phylogenetic history of morphospace occupation more explicitly reveal the true patterns of homoplasy among the herbivorous carnivorans. Our results indicate that both historical constraints and adaptation have interplayed in the evolution toward herbivory of the carnivoran skull, which has resulted in repeated patterns of biomechanical homoplasy.

Key words: Homoplasy, geometric morphometrics, herbivory, Carnivora, adaptation, history, skull shape.

INTRODUCTION

One of the main goals of evolutionary biology is to understand the role and limits of natural selection in sorting phenotypic variation. Adaptive evolution means that natural selection shapes a given trait for a particular function within a determined environment. However, natural selection does not cope with endless variation, as its domain is constrained by shared and stable developmental programs, pleiotropic effects, specific properties of biomaterials and morphogenetic processes (Seilacher, 1970; Gould & Lewontin, 1979; Gould, 1980, 1989; Cheverud, 1982, 1985; Wagner, 1988; Goswami, 2006; Raia *et al.*, in press). Different kinds of evolutionary constraints influence the nature and number of adaptive solutions (Schwenk, 1995; Arthur, 2001; Gould, 2002; Brakefield, 2006). In fact, evolution under similar environmental conditions combined with the effects of historical contingency result in repeated patterns of homoplasy, which define the range of phenotypic possibilities (e.g., Raia *et al.*, in press).

A number of studies on the covariation between function and phylogeny have increased our understanding of the role and limits of natural selection and historical contingency in shaping the mammalian skull (e.g., Cheverud, 1982; Cheverud, 1984; Polly, 1998; Cardini, 2003; Cardini & O' Higgins, 2004; Caumul & Polly, 2005; Cardini & Elton, 2008; Raia *et al.*, in press) and particularly the carnivoran skull (e.g., Goswami, 2006; Wroe & Milne, 2007; Polly 2008; Slater & Van Valkenburgh, 2008; Meloro *et al.*, 2008). In fact, the order Carnivora represents a good choice for exploring homoplasy because over the past 65 Ma different taxa have adapted to different feeding strategies with a limited set of phenotypic variants (Van Valkenburgh, 1999, 2007). This strongly suggests that evolution under similar environmental conditions plus the presence of shared developmental constraints have led to repeated patterns of homoplasy in the evolution of carnivoran skull shape.

Patterns of parallel evolution of skull shape among hypercarnivores have been reported previously by several authors (e.g.,Van Valkenburgh, 1991, 1995, 2007; Holliday & Steppan, 2001; Wroe & Milne, 2007). However, homoplasies toward herbivory remain largely unexplored. This is surprising, as the herbivorous carnivorans represent an excellent choice for examining homoplasy. Taxa from distantly related lineages have repeatedly developed adaptations to a feeding behavior that represents the opposite extreme of the ancestral carnivorous condition (Van Valkenburgh, 2007). Furthermore, few organisms have received as much attention from an evolutionary point of view as the two living pandas, the giant panda (*Ailuropoda melanoleuca*) and the red or lesser panda (*Ailurus fulgens*). Molecular data indicate unequivocally that these species belong to two distinct families (Ursidae and Ailuridae, respectively) that diverged ~40 Ma ago, during early Oligocene times (Goldman *et al.*, 1989; Ledje & Arnason, 1996; Flynn *et al.*, 1998, 2000, 2005; Bininda-Emonds *et al.*, 1999; Ginsburg, 1999), and these emblematical mammals are often cited as one of the best examples of convergent evolution (e.g. Gittleman, 1994). Both feed almost entirely on bamboo, and share a number of morphological specializations to this specialized diet, including broad cheek teeth, well-developed zygomatic arches, a wide temporal fossa, powerful jaws with an extremely enlarged coronoid, and an elongated wrist bone, the radial sesamoid, that functions as an opposable "thumb" (Radinsky, 1981; Endo *et al.*, 2001a; Endo *et al.*, 2001b; Antón *et al.*, 2006; Salesa *et al.*, 2006; Endo *et al.*, 2008).

Given that the feeding adaptations of carnivorans are usually reflected in their skull (e.g., Van Valkenburgh, 1988; Van Valkenburgh *et al.*, 2003; Biknevicius & Ruff, 1992; Palmqvist *et al.*, 1999, 2003, 2007, 2008; Sacco & Van Valkenburgh, 2004;

Christiansen & Adolfssen, 2005; Friscia *et al*., 2006; Goswami, 2006; Christiansen, 2007; Christiansen & Wroe, 2007; Wroe *et al*., 2007; Wroe & Milne, 2007; Figueirido *et al*., 2009, 2010; Slater *et al*., 2009; Slater & Van Valkenburgh, 2009), herbivorous taxa are an excellent choice for investigating how adaptive evolution shaped morphological and ecological diversity.

In this paper we explore the role and limits of natural selection and historical contingency in shaping skull design in herbivorous carnivorans. We investigate the evolutionary dynamics of skull change using *landmark*-based methods of geometric morphometrics and biomechanical models. Specifically, we address the following questions in this study: *(i)* Is there a phylogenetic signal in skull shape? *(ii)* Is there a significant correlation between skull shape and diet? *(iii)* Which are the adaptations toward herbivory in the carnivoran skull? *(iv)* Are there patterns of homoplasy among herbivorous carnivorans in the evolution of skull shape? *(v)* Which are the roles of natural selection and historical contingency in shaping the skull of herbivorous carnivorans? The answers to these questions reveal that phylogenetic legacy and natural selection are both significant causal factors in the evolution of skull shape. Consequently, some adaptations toward herbivory are reflected in the carnivoran skull, but these adaptations are strongly influenced by the particular phylogenetic legacy of the order Carnivora. Thus, it is the combined effects of adaptation and phylogeny which explains the repeated patterns of biomechanical homoplasy in the skull of herbivorous carnivorans.

MATERIAL AND METHODS

Dataset

This study is based on 194 crania and 184 mandibles of adult individuals belonging to 24 species from the infraorder Arctoidea (Table 1). Our analysis included representatives of all terrestrial arctoid families (Ailuridae, Mephitidae, Mustelidae, Procyonidae, and Ursidae) and one aquatic family (Phocidae). We considered specialized herbivores to be those species consuming more than 95% of vegetal matter (fruits and foliage). It is for this reason that we restricted our sample to the infraorder Arctoidea, as no species of Feliformia has these dietary requirements. The percentage of vegetal items consumed by each species studied was taken from published analyses of scat contents (see Table 1 and Appendix 1). For those species for which several estimates were available, the arithmetic means for the percentages of the principal dietary items consumed (i.e., vegetal matter, arthropods and vertebrates) were computed (Appendix 1). As a result, five species were considered as specialized herbivores in this study: the giant panda (*A. melanoleuca,* Ursidae), the red or lesser panda *(A. fulgens,* Ailuridae), the bushy-tailed olingo (*Bassaricyon gabbi,* Procyonidae), the kinkajou (*Potos flavus,* Procyonidae) and the spectacled bear (*Tremarctos ornatus,* Ursidae).

We must admit that the definition of “herbivory” used here is very broad and, consequently, that the feeding category of “herbivore” does not represent a completely homogeneous group. For example, two herbivorous species can forage differently if they consume quite different kinds of vegetal resources (e.g., bamboo or fruits) or even if they consume the same resource but feed on different parts of the plant (e.g., bamboo stems or leaves). However, we only use the ecological categories of herbivore, omnivore, insectivore and carnivore (Table 1) for interpreting the results obtained in the subsequent morphometric analyses and it should be clear that we do not employ statistical methods such as discriminant analysis or canonical variates analysis that require specifying “a priori” distinct feeding groups. In contrast, we follow an approach based on principal components analysis (PCA) for depicting the distribution of species within the phenotypic space (see below).

Only adult specimens were gathered to avoid effects of ontogenetic variation. Similar numbers of males and females of each species and similar proportions of individuals from different populations were sampled whenever possible. Specimens were sampled at the American Museum of Natural History (New York, USA), the Natural History Museum (London, UK), the Museum für Naturkunde (Berlin, Germany) and the Donald R. Dickey Collection at the University of California (Los Angeles).

Phylogenetic analysis

In order to evaluate phylogenetic structure in our data and to estimate ancestral skull shapes (see below), we developed a time-calibrated phylogenetic hypothesis for the taxa included in the study using gene sequences and fossils. Specifically, we retrieved full length (1140 bp) cytochrome b (*Cytb*) sequences from genbank for all taxa included in this study plus the gray wolf (i.e., *Canis lupus*) as a representative sister taxon to the arctoid carnivores (for accession numbers of sequences, see Appendix 2).We used BEAST v.1.5.2 to produce a time-calibrated phylogeny under a relaxed molecular clock. We identified ten extinct taxa representing the earliest known members of crown clades that were represented in our tree and used these taxa as minimum age calibrations for those nodes. We assigned lognormal prior distributions to all calibrations, with the 95% range spanning the distance from the fossil calibration to the next node to which each fossil could be assigned (Table 2).

There are a number of robust phylogenetic studies on arctoid familial relationships (Flynn *et al.*, 2005; Krause *et al.*, 2008; Koepfli *et al.*, 2007, 2008) and we used them to constrain the tree topology in our dating analysis. We chose this approach instead the one based on unconstrained analysis because the use of *Cytb* sequence data for inferring higher level carnivoran phylogeny can lead to topologies in conflict with those provided by other analyses based on larger and/or less saturated datasets (e.g., Agnarsson *et al*., 2010). We assigned a GTR+I+G model of sequence evolution to three partitions corresponding to codon position, and specified a Yule prior for speciation rates. We ran our analysis for 100,000,000 generations, sampling from the MCMC chain every 10,000 generations. After inspecting the output files to ensure convergence of evolutionary parameters using Tracer v. 1.51, we produced a maximum clade credibility tree for comparative morphological analysis.

Landmark digitization: biomechanical modeling of skull function

All the landmarks (LMs) were digitized on high-resolution digital images into two-dimensional Cartesian coordinates (x, y) of crania (Fig. 1A) and mandibles (Fig. 1B) in lateral view using the program TPSdig v.2.11 (Rohlf, 2008). The digital images were captured using a tripod and following a standardized protocol for avoiding lens distortion and parallax (see Marugán-Lobón & Buscalioni, 2004; Meloro *et al*., 2008).

Feeding adaptations in carnivores involve specific mechanical and physiological requirements for exerting an efficient mastication, which are reflected in craniodental morphology (e.g., Van Valkenburgh, 1988; Biknevicius & Ruff, 1992; Palmqvist *et al*., 1999; Palmqvist *et al*., 2003, 2008; Van Valkenburgh *et al*., 2003; Sacco & Van Valkenburgh, 2004; Christiansen & Adolfssen, 2005; Friscia *et al*., 2006; Anyonge & Baker, 2006; Goswami, 2006; Christiansen, 2007; Christiansen & Wroe, 2007; Wroe *et al*., 2007; Wroe & Milne, 2007; Figueirido *et al*., 2009, 2010; Slater *et al*., 2009; Slater & Van Valkenburgh, 2009). Consequently, the LMs digitized in this study were chosen to capture key functional features of the carnivoran skull (Fig. 1C). The jaw joint was modeled as a third class lever system (Turnbull, 1970; Greaves, 1978, 1982, 1988). In this kind of lever the force exerted by the temporalis and masseter muscles (Fig. 1D) is applied between the area of resistance (i.e., the dentition) and the fulcrum (i.e., the jaw joint) of the lever (Fig. 1E). Therefore, we selected landmarks in the mandible that describe the input moment arms for the temporalis and masseter muscles, the output moment arms to different points of the dentition, as well as other functional traits, including the bending strength of the horizontal ramus at different interdental gaps or the proportions between the molar and premolar teeth (Fig. 1E). Similarly, we selected landmarks in the cranium that capture the output moment arms to different points of the dentition, the area of insertion of the temporalis and masseter muscles in the temporal fossa and the zygomatic arch, respectively, rostral depth and the relative development of the premolar and molar teeth (Fig. 1E).

Geometric morphometrics and principal components analysis

Specimens were aligned using generalized Procrustes fit and projected to the tangent space (e.g., Dryden and Mardia, 1998) separately for each sample (i.e., jaws and crania). The mean shape for each species was then computed by averaging the aligned coordinates and centroid sizes. We averaged the specimens sampled from each species in order to eliminate the effects of static allometry and sexual shape dimorphism. Centroid size, which represents the size of the object, was computed as the square root of the sum of squared distances of the digitized landmarks to the centroid of the object and retained for analysis of interspecific allometry for the size of mandibles and crania (Bookstein, 1991).

In order to investigate the distribution of carnivorans in the phenotypic space, we computed separately principal components analyses (PCA) from the covariance matrix of the aligned coordinates derived from the mean cranial and mandible shapes in the 24 species analyzed. We also explored the major dimensions of shape variation around the mean shape (Zelditch *et al*., 2004) for identifying those traits of skull shape shared by the herbivorous carnivorans. Although skull shape was analyzed here using landmarks instead of semilandmarks, we modeled the interlandmark distances by means of an outline, which allowed us to obtain clearer shape transformation models in PCAs and ancestral skull shape reconstructions (see below). All the geometric morphometric procedures and PCAs were computed with program MorphoJ (Klingenberg, 2008)

Ancestral skull shape reconstruction

The hypothetical morphology of the ancestral nodes in a phylogenetic tree can be reconstructed when the shapes of all terminal taxa are known (e.g., Maddison, 1991; McArdle & Rodrigo, 1994; Martins & Hansen, 1997; Garland *et al*., 1999; Polly, 2001; Rohlf, 2001; Finarelli & Flynn, 2006; Astúa, 2009). These comparative methods can be used for delivering indirect inferences on the paths followed by morphological evolution, which can be tested later with data obtained from the fossil record (e.g., Polly, 2001). Here, we use the squared-change parsimony method (Maddison, 1991), weighted by the respective branch lengths, for reconstructing cranial and jaw shape at the internal nodes of the phylogeny with program MorphoJ (Klingenberg, 2008). It is worth noting that although several limitations have been described for this parsimony method (e.g., the obtaining of wide confidence intervals; Webster & Purvis, 2002), this approach is probably one of the best procedures for working with continuous characters (Astúa, 2009). Finarelli & Flynn (2006) have shown that if all data available (i.e., character observations and temporal information for extinct taxa) are incorporated in the reconstruction of ancestral character states, this improves the results obtained with the weighted squared-change parsimony method. However, the scarcity in the carnivoran fossil record of complete, well-preserved and non-deformed skulls represents a major bias for incorporating shape data that go beyond mere scalar traits, which precludes the use of this information in the reconstruction of ancestral skull shapes. However, special attention was paid in this study to incorporating biochronologic data from the fossil record as well as to increasing taxon sampling. Also, we compared qualitatively the shapes reconstructed for the internal nodes of the phylogeny with the fossil data available.

The reconstructed ancestral shapes were plotted into the shape spaces obtained in PCA. Subsequently, the branches of the tree were connected (Klingenberg & Ekau, 1996; Rohlf, 2002; Polly, 2008; Astúa, 2009; Gidaszewski *et al*., 2009; Klingenberg & Gidaszewski, 2010) for assessing the evolutionary history of skull shape changes. This "phylomorphospace" approach provides the possibility of exploring the history of the phylogenetic occupation of skull morphospaces.

Quantifying the effects of size, phylogeny and diet on skull shape

The presence of a phylogenetic structure in our data was evaluated with a permutation test (Laurin, 2004; Klingenberg & Gidaszewski, 2010). The test operates by randomly reassigning the 24 observed mean species shapes to the tips of the phylogenetic tree in each permutation (10,000 in our case). Ancestral shape reconstructions are recomputed for the permuted data by squared-change parsimony (Maddison, 1991), weighting by branch lengths (Gidaszewski *et al*., 2009; Klingenberg & Gidaszewski, 2010). The test provides a *P*-value, which is the proportion of permutations that result in a tree length (calculated as the sum of the squared Procrustes distances between ancestor and descendant for all branches) equal to or less than the observed one. Therefore, if our data have a strong phylogenetic structure, the tree length obtained in each permutation should be greater than the one obtained with the original data (Gidaszewski *et al*., 2009; Klingenberg & Gidaszewski, 2010). We used this *P*-value for assessing the phylogenetic signal present in mandible and cranial shape.

The Procrustes coordinates of each species were regressed on log-transformed centroid size values for assessing the effects of size change on interspecific shape variation (i.e., evolutionary allometry) using multivariate regression analysis (Monteiro, 1999). The statistical significance of the results was tested with a permutation test against the null hypothesis of size independence, following Drake and Klingenberg (2008).

In order to explore the association between skull shape and diet, we computed a multivariate regression analysis (Monteiro, 1999) of skull shape (Procrustes coordinates of crania and mandibles) on the percentage of vegetal matter over the total volume of food items included in the diet of the species studied (see Table 1 and Appendix 1). Statistical significance was tested with a permutation test against the null hypothesis of independence between shape and diet (Drake and Klingenberg, 2008).

However, given that the species analyzed are related phylogenetically, they cannot be treated as statistically independent data points (Felsenstein, 1985). This violates the assumption of independent sampling, which is a variant of the classic Type I error (e.g., Harvey & Pagel, 1991). As a result, the morphological correlations could be attributed to the phylogenetic pattern, which can lead to incorrect interpretations (e.g., MacLeod, 2001). In order to avoiding this bias, we computed phylogenetically independent contrasts analyses (Felsenstein, 1985) of skull shape, size and diet.

Subsequently, the contrast for shape was regressed on the ones for diet and size, which allowed exploring the effects of size and diet on skull shape after accounting for similarity due to shared ancestry. Statistical significance was tested with a permutation test against the null hypothesis of independence (Drake and Klingenberg, 2008). The quantification of the effects of size, phylogeny and diet on skull shape and independent contrast analyses were computed with program MorphoJ (Klingenberg, 2008).

RESULTS

Phylogeny

Figure 2 shows the time-calibrated phylogenetic reconstruction obtained from complete *Cytb* sequences under a relaxed molecular clock. Our divergence time estimates are broadly consistent with dates inferred in other studies. However, divergence times within Mustelidae are uniformly older than those recovered in a recent study based on 22-kbp sequence data from 22 gene segments (Koepfli *et al.*, 2008). It is worth noting that a uniformly older or younger dating of internal nodes is not relevant for the reconstructions of ancestral values by weighted squared-change parsimony because the method uses the relative branch lengths. In spite of this, we manipulated node ages within Mustelidae to mirror the mean node ages reported by Koepfli *et al.*, (2008) to determine if these discrepancies affected our analyses. Results obtained using both trees were similar and, consequently, we report results from analyses using the tree with branch lengths derived from this study.

Principal components analysis

Mandible shape

The first three PCs derived from PCA of mandible shape jointly explain more than 70% of the original variance. Among them, the third PC was significantly correlated with log-transformed centroid size values. Consequently, we only present here the results for the first two axes (>55 % of variance). Figure 3A shows the scores for the species means on the morphospace depicted by these first two PCs.

The first PC describes a shape gradient that goes from the stoutly built mandible of *P. flavus,* to the most slender jaws of *Nasua* (Fig. 3A, *x-axis*). The jaws with negative scores on this axis are characterized by a deep mandibular ramus, a wide coronoid process and a long distance from the jaw condyle to the angular process. In addition, they have a comparatively short premolar tooth row and a small carnassial tooth (Fig. 3B). In contrast, those jaw that take positive scores show a shallow body with a narrow coronoid, a short distance between the condyle and the angular process, and well developed premolars and carnassial teeth (Fig. 3B).

The second PC accounts for the shape change from the mandibles of the giant panda (*A. melanoleuca*) and the red panda (*A. fulgens*) to the jaw of the leopard seal (*Hydrurga leptonyx*) (Fig. 3A, *y-axis*). Mandibles with negatives scores on this axis have a high coronoid process and a deep posterior body. In contrast, those with positive scores have a small coronoid and short post-carnassial molars (Fig. 3B).

Cranial shape

The PCA computed from cranial shape yielded three PCs with significant eigenvalues (>70% of the original variance explained). However, there is a significant correlation between the second vector and the log-transformed centroid size values, which indicates that the shape changes accounted for by this axis are mostly explained by allometric effects. Consequently, we only show here the first and third axes, which summarize 50% of cranial shape variation. Figure 5C shows the morphospace depicted with the scores of the species digitized on PCI and PCIII.

The first PC relates mainly with the morphological gradient between the long-snouted and shallow anterior crania of *Nasua*, and the short-snouted and deep anterior cranium of *P. flavus,* (Fig. 3C, *x*-axis; Fig. 3D). In addition, this component describes also the development of the premolar tooth row (Fig. 3D). Therefore, those crania with positive scores are brachycephalic, have deep snouts, their zygomatic arches and orbits are anteriorly oriented, and have short premolars and enlarged molars. Conversely, the crania that score negatively on this first axis are dolicochephalic and show a shallow snout, well developed premolar teeth and short molars.

The third PC accounts for the shape changes from the crania of *Mustela vison*, *H. leptonyx* and *Ursus ursinus,* among other species that score negatively, to those of *A. melanoleuca* and *A. fulgens*, which take extreme positive scores (Fig. 3C, *y*-axis). The latter have stout, very deep and short neurocrania, well developed zygomatic arches and extremely enlarged molar tooth rows (Fig. 3D). The opposite trend is represented by those crania that take extreme negative scores on this axis, as they are shallow, have a long neurocranium and a shortened molar tooth-row (Fig. 3D).

Ancestral skull shape reconstruction

Phylomorphospaces are shown in Figures 4A and 4B for mandible and cranial shapes, respectively, and the reconstructed shapes at the internal nodes of the phylogeny are depicted in Figure 4C.

The skull shapes of the living herbivores plot in a separate and wider area than the one occupied by the shapes reconstructed for their respective ancestors (Figs. 4A, B). This shows that skull shape leads to different morphological patterns in the living species (Fig. 4C). In general terms, these taxa follow different evolutionary paths for skull shape changes, which means that ancestry was a significant causal factor in shaping skull design. However, it is worth noting that the evolutionary trajectories of skull shape transformation reconstructed for the extant herbivores suggest some common mophological traits (Figs. 4A, B), which indicates also the presence of homoplasy. For example, compared to their ancestral states all these species develop in their mandibles a larger coronoid, a deeper mandibular corpus, a longer distance between the articular condyle and the angular process, and a larger lower molar tooth row (Fig. 4C). In addition, their crania tend to be more brachycephalic and deeper, have a more developed upper molar tooth row and show the orbits more anteriorly placed (Fig. 4C).

Apart from these shared trends, there are also some minor differences in the patterns of mandible shape transformation among the living herbivores (Fig. 4A): for example, the jaws of *Ailurus* and *Ailuropoda*, which are both bamboo feeders, have a longer and narrower ascending ramus, while the mostly frugivorous *Potos* and *Tremarctos* show a wider and shorter ascending ramus (Fig. 4C). These patterns of change from the ancestral states to the living bamboo feeders and frugivores indicate that homoplasy was a significant causal factor for shaping jaw design. Also, it is worth noting that jaw morphology is more similar in both species of pandas than in their reconstructed ancestral shapes (Fig. 4A; Ame-Node2 and Aif-Node4). The case of *Potos* and *Tremarctos* is less evident, however, as the shapes reconstructed for their ancestral stages are closer between them (Fig. 4A; Pof-Node5 and Tor-Node3). However, both species score differently in the mandible morphospace, which most probably reflects that the spectacled bear is less derived morphologically than the kinkajou (the divergence time is only ~10 Ma ago for the former and >25 Ma for the latter; Fig. 1).

The effects of size, phylogeny and diet on skull shape

The multivariate regression of shape on log-transformed centroid size values was significant for both the mandible ($P = 0.0239$) and the cranium ($P = 0.0001$). The total amount of shape variation accounted for size differences was 10.2% in the case of the mandible and 18.6% in the cranium. However, independent contrast analyses showed no significant correlation between the contrast for size and shape in the mandible ($P = 0.4483$) and, to a lesser degree, also in the cranium ($P = 0.0619$).

The multivariate regression of shape on diet was statistically significant for both mandibles ($P = 0.0136$) and crania ($P = 0.0156$), with an amount of 10.7% of the shape variation of mandibles explained by differences of feeding behavior and 11.1 % in the case of crania. Similarly, the multivariate regression of the shape and diet contrasts was statistically significant in both the mandible ($P = 0.0014$) and the cranium ($P = 0.0013$), with 25.7% of the shape variation of the mandibles explained by differences of feeding behavior and 23.7 % in the case of crania. Therefore, the correlation between skull shape and diet remains significant after removing the phylogenetic relationships of the taxa under study.

The permutation tests revealed a statistically significant phylogenetic signal present in the shape data for both mandibles ($P = 0.0006$) and crania ($P < 0.0001$). Our results therefore demonstrate that evolutionary allometry, phylogenetic legacy and feeding behavior are strong causal factors for shaping skull design in arctoid carnivorans

DISCUSSION

Adaptations toward herbivory in the carnivoran skull

Our results demonstrate that skull shape correlates with feeding behavior. Principal components analyses of mandibles and crania suggest that, with the only exception of the bushy-tailed olingo (*B. gabbi*), all herbivorous species of Procyonidae (*P. flavus*), Ailuridae (*A. fulgens*) and Ursidae (*A. melanoleuca* and *T. ornatus*) share a set of common traits in their craniodental anatomy. These features relate to the capacity to exert high bite forces and include a stoutly built mandible with a deep mandibular corpus, an enlarged coronoid and well developed lower postcarnassial molars, as well as a deep brachicephalic cranium with well developed zygomatic arches, a short neurocranium and enlarged upper molars (Fig. 3). On the one hand, an elongated coronoid and an enlarged angular process are indicators of long lever arms for the temporalis and masseter muscles, respectively (Figs. 1C-E), and thus can be used as proxies for the input moment arms of these masticatory muscles (Turnbull, 1970; Van Valkenburgh & Ruff, 1987; Biknevicius & Van Valkenburgh, 1996; Van Valkenburgh *et al*., 2003; Christiansen & Adolfssen, 2005; Palmqvist *et al.,* 2007; Figueirido *et al*., 2010). On the other, a brachycephalic cranium reduces the output moment arms of the masticatory muscles (Figs.1C-E), increasing the load exerted with the carnassials or the canine teeth (Christiansen & Adolfssen, 2005; Wroe *et al*., 2005; Christiansen, 2007; Christiansen & Wroe, 2007). In addition, a high cranium with a deep mandibular body is better suited for dissipating the high torsional forces generated in the parasagittal plane when the herbivorous carnivorans chew on tough foods with their enlarged molar teeth (Buckland-Wright, 1971, 1978; Werdelin, 1989; Biknevicius & Ruff, 1992). These results agree with the findings of Christiansen and Wroe (2007), who showed that the carnivorans that feed on fibrous plants have higher bite forces than those species of similar size that are specialized insectivores, omnivores or even carnivores that take small prey. In fact, only those carnivores that prey upon ungulate prey much larger than themselves and those specialized in the consumption of vertebrate carrion (e.g., the wolverine, *G. gulo*) parallel the bite force of herbivorous carnivorans (Christiansen & Wroe, 2007).

We have identified a set of common functional traits in the carnivoran skull that allow specializing on an herbivorous diet. As a result, we identify these morphological traits as common adaptations toward herbivory in the carnivoran skull, except in the bushy-tailed olingo.

Evolutionary constraints in the skull of herbivorous carnivorans

In this study we have shown that skull shape does not only correlate with feeding behavior but also with phylogenetic legacy. As stated above, all the adaptations of the carnivoran skull toward herbivory have been interpreted as functional traits that allow exerting high bite forces and such traits are similar to those of the hypercarnivorous species that subdue large vertebrate prey (Christiansen & Wroe, 2007). At first sight this fact seems to be counterintuitive if we do not pay attention to historical contingency.

All the herbivorous carnivorans evolved from a generalized carnivorous mammal (Van Valkenburgh, 2007), with a body plan early established in the phylogeny of Carnivora. Therefore, the remarkable morphological resemblance of the skull among the herbivorous carnivorans was most probably driven by extrinsic factors (e.g., natural selection) as well as by intrinsic ones (e.g., a shared developmental pathway) and the latter may have posed constraints (*sensu* Alberch, 1982) on the direction of skull shape evolution toward herbivory. Thus, the adaptations of herbivorous species for exerting high bite forces are constrained functional solutions that were reached in a process driven by natural selection within the set of possibilities allowed by phylogenetic legacy.

The herbivorous carnivorans retain the simple stomach (i.e., without a caecum) and short gastrointestinal tract typical of carnivores, in contrast to the chambered stomachs and complex intenstinal morphologies of ruminants and other mammalian herbivores (Roberts & Gittleman, 1984; Schaller *et al*., 1985; Johnson *et al*., 1988; Bleijenberg & Nijboer, 1989; Reid *et al*., 1991). Microbial digestion only plays a minor role in the digestive strategy of carnivorans, resulting in a low digestibility rate for cellulose and other complex carbohydrates (Wei *et al*., 1999, 2007; Senshu *et al*., 2007). Furthermore, all carnivores have a fixed temporomandibular joint (Ewer, 1973) which, together with their enlarged canine teeth, limits jaw movements to the vertical plane, with some lateral movements for grinding (Davis, 1964). This prevents the teeth from being occluded simultaneously for grinding tough foods, as in ungulates. As a result of these limitations, herbivorous carnivorans must eat huge quantities of food, which forces the development of the main muscles involved in mastication (i.e., masseter and temporalis), hence the capacity for exerting similar bite forces than those of carnivorous mammals that usually hunt prey much larger than themselves. The need in a carnivoran for high input moment arms for the temporalis and masseter muscles translates in the development of a long coronoid and an enlarged angular process, which precludes the development of a wide gape.

Constraints on digestive tract and craniomandibular morphology as a result of a basic body plan adapted to carnivory have resulted in a very different set of solutions in herbivorous carnivorans, compared to ungulates. Skull shape in herbivorous carnivorans evolved toward the capacity of exerting a high bite forces in a process that paralleled the evolution of hypercarnivores. The same adaptive solution (i.e., an increase in bite force) was taken for reaching two opposite feeding strategies (i.e., herbivory and hypercarnivory) through carnivoran evolution. This strongly suggests that not all possible adaptive solutions are under the domain of natural selection, which operates on the variability available by the phylogenetic legacy of each particular group.

The role of natural selection and historical contingency in shaping the skull design of herbivorous carnivorans

The combined effects of phylogenetic legacy and natural selection for shaping cranial design have led to repeated patterns of biomechanical homoplasy in the evolution of the carnivoran skull toward herbivory. In fact, in this study we identify several of these patterns. This is especially the case of jaw shape in the giant panda (*A. melanoleuca*), an ursid, and the red panda (*A. fulgens*), an ailurid, which both feed on bamboo (Table 1; Appendix). This result is also confirmed by the evidence from the fossil record. In fact, the earlier fossil remains of the *Ailuropoda* lineage (e.g., the Miocene *Ailurarctos* from China) have an incipient crushing dentition adapted for a durophagous diet and the late Pliocene *Ailuropoda microta* and *Ailuropoda wulingshanensis* have intermediate tooth morphologies between *Ailurarctos* and the living *A. melanoleuca* (Qiu & Qi, 1989; Hunt, 2004; Qi *et al.*, 2006; Jin *et al.*, 2007). Similarly, the fossil record of the most basal ailurids such as the species of *Simocyon* (Miocene of Europe and North America, Mio-Pliocene of Asia) and *Amphictis* (Oligo-Miocene of Eurasia) do not show the specialized skull morphology of the red panda, which suggests that their common ancestor was a generalized carnivore, not a bamboo feeder (Salesa *et al.*, 2006). Therefore, both the reconstructed evolutionary trajectories as well as the fossil evidence more robustly support the independent evolution towards bamboo feeding between the distantly related *Ailurus* and *Ailuropoda*. As a result, it is striking that two distantly related species that diverged >40 Ma ago (Goldman *et al.*, 1989; Ledje & Arnason, 1996; Flynn *&* Nedbal, 1998; Bininda-Emonds *et al.*, 1999; Ginsburg, 1999; Flynn *et al*., 2000; Flynn *et al*., 2005; Agnarsson *et al.*, 2010) have extremely similar mandibles. This indicates the strong role of the phylogenetic legacy (i.e., the existence of a shared and stable developmental path established early in the evolutionary history of the carnivorans) as well as the consequences of the biomechanical limitations for shaping jaw design. However, this pattern could be also explained by a remarkable evolutionary plasticity and/or a strong effect of natural selection.

Another incipient pattern of jaw homoplasy can be reported for the kinkajou (*P. flavus*), a procyonid, and the spectacled bear (*T. ornatus*), which both are mainly frugivores (Table 1; Appendix). Although in this case the pattern of homoplasy is not as evident as in the case of pandas, again the evidence from the fossil record of *Potos* and *Tremarctos* confirms the independent evolution of similar morphologies. In fact, the earlier tremarctine known from the fossil record is the early Miocene, North American *Plionarctos*, which retains a primitive tremarctine mandible without a premasseteric fossa (Tedford & Martin, 2001). The Tertiary record of procyonids shows that some primitive procyonids like the Miocene *Broiliana* from Europe has a mesocarnivorous dentition (Baskin, 1982, 2004). Furthermore, *Parapotos tedfordii* from the Miocene of North America (Baskin, 2003) shows intermediate mandible features between *Broiliana* and the extant frugivorous *P. flavus* (F.J. Serrano-Alarcón, Per. Obsv.).

Strikingly, the specific patterns of homoplasy reported for the evolution of jaw morphology toward frugivory or bamboo feeding did not arise in the evolution of cranial shape (Fig. 4B). In our opinion, this probably reflects the greater evolutionary plasticity of the mandible compared with the cranium (Barone, 1986). Jaw morphology is mainly related to food acquisition and processing, while cranial shape is the result of conflicting demands between different functions (e.g., feeding, olfaction, vision and brain processing; Figueirido *et al*., 2009). For this reason, although a set of functional cranial traits can be identified as common adaptations toward herbivory in carnivorans, no specific mechanical adaptation toward frugivory or bamboo feeding is evident. In contrast, these adaptations are reflected in the morphology of the mandible, giving rise to repeated patterns of homoplasy among frugivores, on the one hand, and among bamboo feeders, on the other. This strongly suggests that studies of morphological integration between mandible and cranial shape are necessary for understanding the correlated evolution of these two structures that conform the skull.

Patterns of biomechanical homoplasy among carnivorans have been exemplified in a number of studies on the evolution of hypercarnivory (including bone-cracking adaptations) in distantly related lineages (e.g., Werdelin, 1989; Van Valkenburgh, 1991, 2007; Hollyday & Steppan, 2001; Van Valkenburgh *et al*., 2003; Wroe & Milne, 2007; Wroe *et al*., 2007). In spite of the fact that herbivorous carnivorans represent also an excellent choice for exploring the role and limits of natural selection and historical contingency in sorting phenotypic variation, patterns of evolution toward herbivory remain largely unexplored. Our findings show that several skull traits that allow increasing bite force have been shaped in the carnivorans as adaptations for an herbivorous diet. Several evolutionary constraints arising from the peculiar phylogenetic legacy of the carnivoran skull have limited the range of adaptive possibilities that natural selection could follow. This fact indicates that few adaptive solutions allow a carnivoran to behave as a strict herbivore. As a result, phylogenetic legacy and natural selection have interplayed during the evolutionary history of carnivorans, giving rise to repeated patterns of biomechanical homoplasy. To this regard, we have recognized the three types (or levels) of homoplastic patterns proposed by Stayton (2006) whitin our skull shape data. In fact, we have found type II pattern of homoplasy (i.e., partial convergence) in all herbivores: although their shapes are not identical, these species show similar deviations from their sister groups and, as a result, are more similar to other herbivorous carnivorans than to their closest relatives. In contrast, a type I pattern (i.e., complete convergence) has emerged in the nearly identical mandibles of the two bamboo feeders and type III (i.e., parallel evolution) most probably has taken place in the mandibles of frugivores, as they show some parallel changes from their sister groups.

Homoplasy is a key concept in evolutionary theory. Quantitative documentation of homoplasy has provided new advances for the study of adaptation (e.g. Wroe and Milne, 2007), constraints (e.g., Herrel *et al*., 2004) and paleobiology (e.g., Van Valkenburgh, 1991), providing support for a better understanding of the evolutionary history of phenotypes (Stayton, 2006). As a result, the quantification of the homoplastic patterns carried out in this article could ultimately be performed to other taxa and ecological niches. Our findings clearly demonstrate the importance of incorporating historical approaches in morphometric analyses for understanding the role and limits of natural selection and historical contingency in sorting morphological and ecological diversity as key elements of evolutionary theory.

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Table 1. Sample size (*N*, crania/mandibles), diet and percentage of vegetal matter (%VM) consumed by each species included in the morphometric analyses. Species highlighted in gray tone are herbivores. For detailed information on the definition of dietary groups and the studies of scat contents, see Appendix 1.

Family	**Species**	**N**	**Diet**	**%VM**
Ailuridae	*Ailurus fulgens,* red panda	14/13	Herbivore (bamboo feeder)	100%
Mephitidae	*Mephitis mephitis*, striped skunk	6/6	Omnivore	11%
Mustelidae	*Meles meles,* Eurasian badger	12/7	Omnivore	36%
Mustelidae	*Gulo gulo,* wolverine	5/8	Carnivore	0%
Mustelidae	*Eira barbara,* tayra	3/6	Omnivore	21%
Mustelidae	*Neovison vison,* American mink	4/4	Carnivore	0%
Mustelidae	*Martes americana,* American marten	4/4	Carnivore	6%
Phocidae	*Hydrurga leptonyx,* leopard seal	1/1	Carnivore	0%
Procyonidae	*Bassaricyon alleni*, Allen´s olingo	5/4	Omnivore	--
Procyonidae	*Bassaricyon gabbii,* bushy-tailed olingo	3/3	Herbivore (frugivore)	100%
Procyonidae	*Bassariscus astutus,* ringtail	6/5	Omnivore	18%
Procyonidae	*Bassariscus sumichrasti,* cacomistle	5/5	Omnivore	53%
Procyonidae	*Nasua nasua,* coati	14/21	Insectivore / frugivore	41%
Procyonidae	*Nasua narica,* white-nosed coati	1/1	Insectivore / frugivore	50%
Procyonidae	*Potos flavus,* kinkajou	10/10	Herbivore (frugivore)	100%
Procyonidae	*Procyon cancrivorus,* crab-eating raccoon	7/9	Omnivore	88%
Procyonidae	*Procyon lotor,* raccoon	9/7	Omnivore	28.5%
Ursidae	*Ailuropoda melanoleuca,* giant panda	16/13	Herbivore (bamboo feeder)	100%
Ursidae	*Tremarctos ornatus,* spectacled bear	7/8	Herbivore (frugivore)	96%
Ursidae	*Ursus ursinus,* sloth bear	8/10	Insectivore	22%
Ursidae	*Ursus arctos,* brown bear	20/30	Omnivore	55%
Ursidae	*Ursus americanus,* American black bear	8/9	Omnivore	84%
Ursidae	*Ursus maritimus,* polar bear	10/12	Carnivore	0%

Table 2. Extinct taxa used for calibrating the ages of crown clades in BEAST analysis. References: 1, Bryant,1992; 2, Qiu & Qi, 1989; 3, Hunt, 2004; 4, Qi *et al.*, 2006; 5, Tedford & Martin, 2001; 6, Montoya *et al.*, 2006; 7, Kurtén, 1964; 8, Wolsan & Lange-Badré, 1996; 9, McKenna & Bell, 1997; 10, Koepfli *et al.,* 2007; 11, Baskin, 1998.

Extinct taxa	**Tmrca**	**Age (95% lower limit)**$^{\text{Sources}}$
Hesperocyon sp.	Caniforms + Arctoids	40.0 (55.0)[1]
Ailurarctos sp.	extant Ursidae	11.6 (40.0)[2,3,4]
Plionarctos sp.	Tremarctinae + Ursinae	7.9 (11.6)[5]
Ursus boecki	extant Ursinae	5.3 (7.9)[6]
Ursus maritimus	*Ursus maritimus/U. arctos*	0.1 (5.3)[7]
Pseudobassaris riggsi	Procyonidae/Mustelidae	7.9 (11.6)[8]
Broiliana sp.	Procyonidae	24.0 (28.5)[9,10]
Bassariscus sp	*Bassariscus/Procyon*	11.2 (24.0)
Nasua sp.	*Nasua/Bassaricyon*	3.6 (24.0)[9,10]
Mustela sp.	Mustelidae	5.3 (28.5)[9,11]

Figure 1. Biomechanical modeling of skull function. A, landmarks used in the morphometric analysis of cranial morphology. B, landmarks used for analyzing jaw morphology. C, illustration of key functional features recovered in the morphometric analysis of the carnivoran skull. D, areas of origin and insertion of the main masticatory muscles (i.e., temporalis and masseter). E, the masticatory apparatus modeled as a third class lever system showing the combined effort of the temporalis and masseter muscles as a function of their input moment arms (coronoid and angular process, respectively), which is applied in a point placed between the fulcrum (temporomandibular joint) and the resistance exerted at the carnassials and canines (output force).

Figure 2. Time-calibrated phylogeny of carnivoran species included in this study, based on Cytb sequence data and ten fossil calibration points. Species highlighted in grey tones are herbivores (see Table 1 and Appendix). Numbers of nodes refer to reconstructed shapes in subsequent morphometric analyses.

Figure 3. Principal components analysis derived from skull morphology. A, pairwaise plot of the first two PCs derived from mandible analysis. B, pairwaise plot depicted from the first and third PCs obtained from cranial analysis. C, mandible shape variation accounted for by PCI and PCII. D, cranial shape variation accounted for by PCI and PCIII. For clarity of illustration, only those species that mainly consume animal or vegetal resources are identified. Abbreviations: Ame, *A. melanoleuca;* Aif, *A. fulgens;* Bga, *B. gabbi;* Gul, *G. gulo;* Hyl, *H. leptonyx;* Mam, *M. americana;* Mvis, *M. vison;* Nan, *N. nasua;* Nar, *N. narica;* Uma, *U. maritimus;* Uur, *U. ursinus.*

Figure 4. Patterns of skull shape evolution in caniform carnivorans. A, projection of the tree topology of Figure 2 onto the morphospace depicted with the first two PCs derived from jaw morphology. B, projection of the phylogenetic tree of Figure 2 onto the morphospace developed with PCI and PCIII of cranial analysis. Note that *C. lupus*, used

only for rooting the tree has been omitted for clarity in both phylomorphospaces. C, reconstruction of ancestral skull shapes. For clarity of illustration, only the reconstructed morphologies for the internal nodes of the most direct ancestors of the herbivorous carnivorans are shown. The shape configuration reconstructed for the root of the tree is depicted in grey tone and the shape configurations for each node are drawn in black. Node numbers are represented in the phylogeny of Figure 2. Abbreviations as in Figure 3.

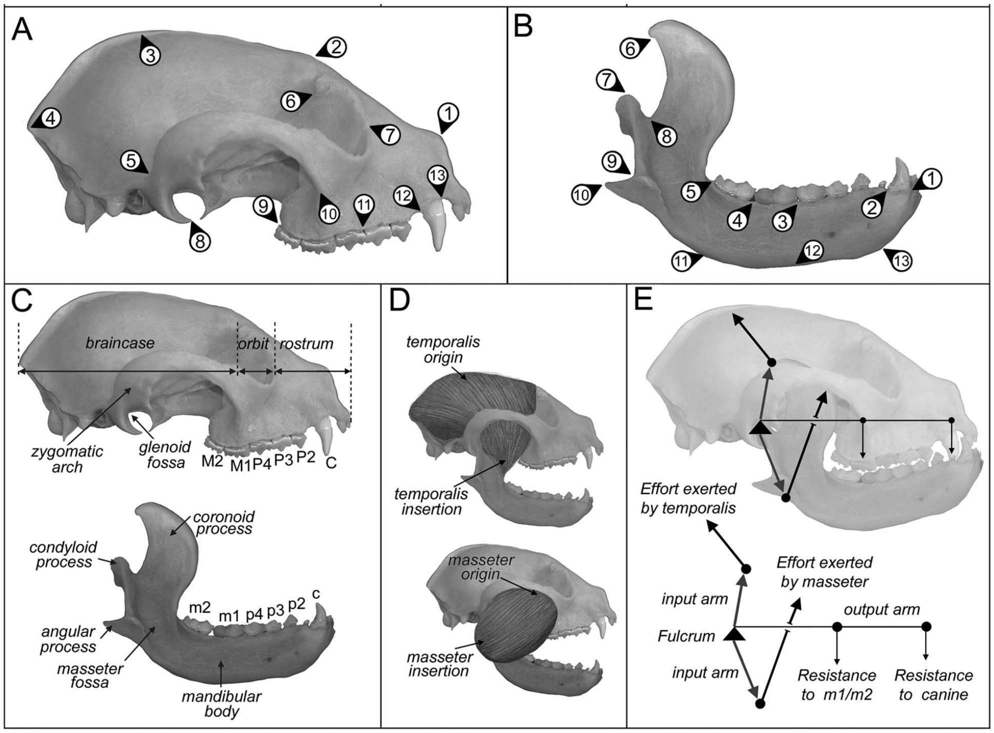

Figure 1. Biomechanical modeling of skull function. A, landmarks used in the morphometric analysis of cranial morphology. B, landmarks used for analyzing jaw morphology. C, illustration of key functional features recovered in the morphometric analysis of the carnivoran skull. D, areas of origin and insertion of the main masticatory muscles (i.e., temporalis and masseter). E, the masticatory apparatus modeled as a third class lever system showing the combined effort of the temporalis and masseter muscles as a function of their input moment arms (coronoid and angular process, respectively), which is applied in a point placed between the fulcrum (temporomandibular joint) and the resistance exerted at the carnassials and canines (output force).
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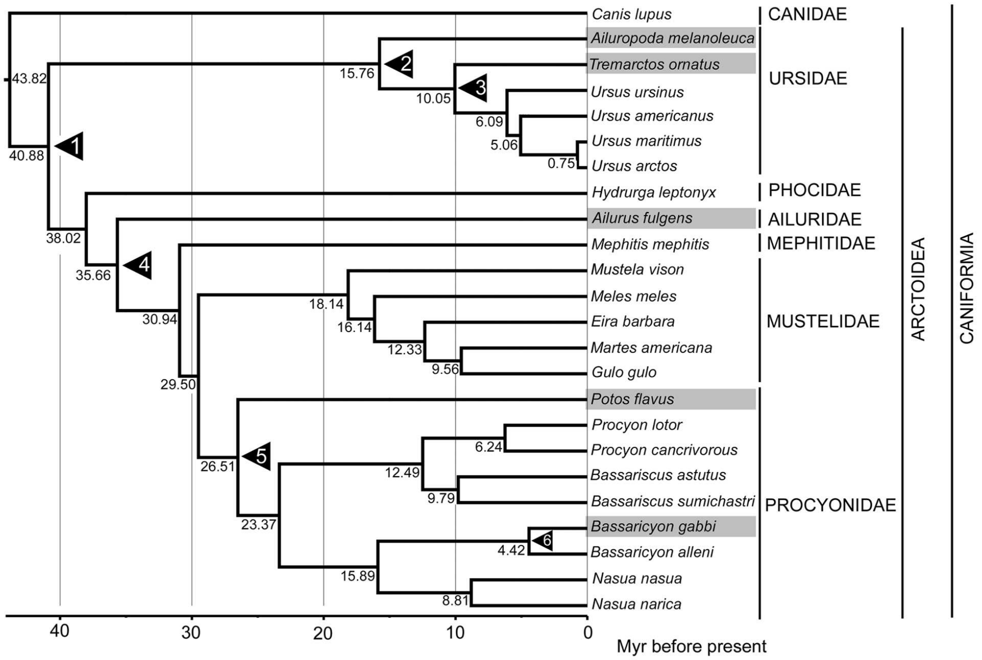

Figure 2. Time-calibrated phylogeny of carnivoran species included in this study, based on Cytb sequence data and ten fossil calibration points. Species highlighted in grey tones are herbivores (see Table 1 and Appendix). Numbers of nodes refer to reconstructed shapes in subsequent morphometric analyses.
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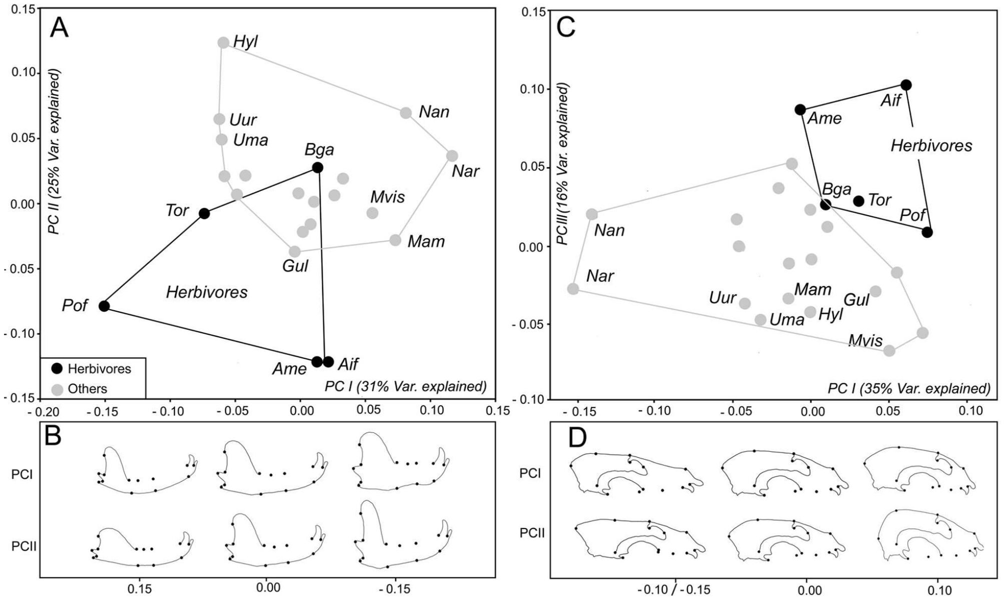

Figure 3. Principal components analysis derived from skull morphology. A, pairwise plot of the first two PCs derived from mandible analysis. B, pairwise plot depicted from the first and third PCs obtained from cranial analysis. C, mandible shape variation accounted for by PCI and PCII. D, cranial shape variation accounted for by PCI and PCIII. For clarity of illustration, only those species that mainly consume animal or vegetal resources are identified. Abbreviations: Ame, A. melanoleuca; Aif, A. fulgens; Bga, B. gabbi; Gul, G. gulo; Hyl, H. leptonyx; Mam, M. americana; Mvis, M. vison; Nan, N. nasua; Nar, N. narica; Uma, U. maritimus; Uur, U. ursinus.
165x99mm (600 x 600 DPI)

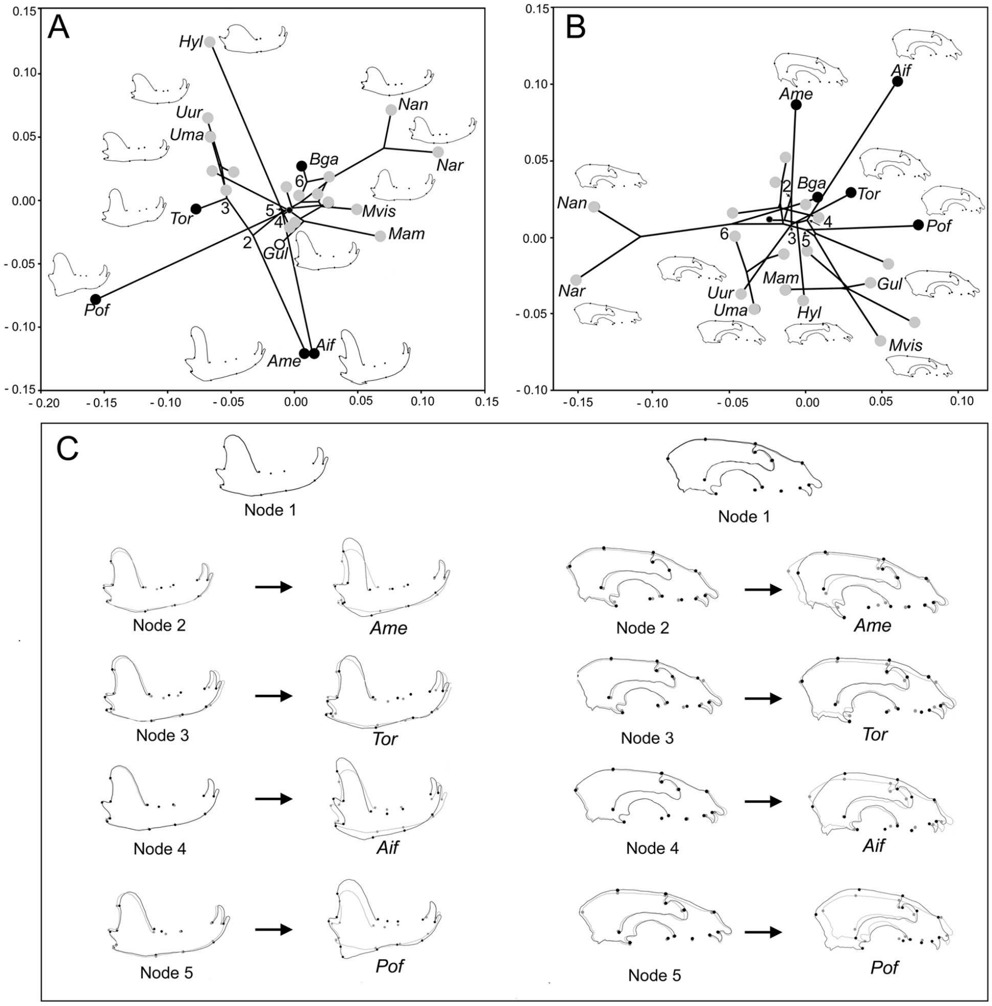

Figure 4. Patterns of skull shape evolution in caniform carnivorans. A, projection of the tree topology of Figure 2 onto the morphospace depicted with the first two PCs derived from jaw morphology. B, projection of the phylogenetic tree of Figure 2 onto the morphospace developed with PCI and PCIII of cranial analysis. Note that C. lupus, used only for rooting the tree has been omitted for clarity in both phylomorphospaces. C, reconstruction of ancestral skull shapes. For clarity of illustration, only the reconstructed morphologies for the internal nodes of the most direct ancestors of the herbivorous carnivorans are shown. The shape configuration reconstructed for the root of the tree is depicted in grey tone and the shape configurations for each node are drawn in black. Node numbers are represented in the phylogeny of Figure 2. Abbreviations as in Figure 3.
165x167mm (600 x 600 DPI)

APPENDIX SI. Dietary categories used in this study. Those species that consume more than 90% of vegetal matter are considered as herbivores (in grey). The main dietary resources consumed (vegetal matter, **VM**; invertebrates, **I**; vertebrates, **V**) are based on the percentage of these items over the total scat volume; values between brackets are derived from percentages of occurrence. It is worth noting that the diet of *B. gabbi* has only been quantified in a single area (central Panama) of its entire geographic range (from Nicaragua to Bolivia), where Kays (2000) reported a diet of 100% of vegetal matter. However, it is possible that it behaves more as an omnivore in other localities (Nowak, 1991). References: 1, Johnson et al. 1988; 2, Wei et al. 1999; 3, Pradhan et al. 2001; 4, Llewellyn & Uhler 1952; 5, Nowak & Walker 1999; 6, Kays 2000; 7, Taylor 1954; 8, Estrada & Coates-Estrada 1985; 9, Poglayen-Neuwall 1992; 10, Bisbal 1986; 11, Gompper 1996; 12, Valenzuela 1998; 13, Julien-Laferrière 1993; 14, Kays 1999; 15, Gatti et al. 2006; 16, Schoonover & Marshall 1951; 17, Bartoszewicz et al. 2008; 18, Kruuk & Parish 1981; 19, Roper & Mickevicius 1995; 20, Van Dijk et al. 2008; 21, Erlinge 1969; 22, Bartoszewicz & Zalewski 2003; 23, Zielinski et al. 1983; 24, Jedrzejewski et al. 1993; 25, Mattson 1998; 26, Peyton 1980; 27, Joshi et al. 1997; 28, Elgmork & Kaasa 1992; 29, Persson et al. 2001; 30, Beeman & Pelton 1980; 31, Stratman & Pelton 1999; 32, Derocher et al. 2002; 33, Oritsland 1977; 34, Lowry et al. 1988. . Note: no feliform carnivoran shows the same degree of specialization toward folivory or frugivory as the arctoid species studied here. For this reason, we have restricted our analyses to caniform carnivorans. However, it is worth noting that fruit may represent a significant fraction of the diet in a number of viverrids (Muñoz-García and Williams 2005; MacDonald 2009), including the African civet (*Civettictis civetta*), the Asian palm-civet (*Paradoxurus hermaphroditus*), the binturong (*Arctictis binturong*) and the small-toothed palm-civet (*Arctogalidia trivirgata*). This is particularly the case of the African palm-civet (*Nandinia binotata*), in which Charles-Dominique (1978) estimated that fruit constituted 90% of scat volume. However, a subsequent study (Ray and Sunquist 2001) showed a lower percentage of fruit consumption (43.6% of scat volume) for this species.

Family	Species	% VM	% I	% V
Ailuridae	*Ailurus fulgens*	100 [1, 2, 3]	0 [1, 2 ,3]	0 [1, 2, 3]
Mephitidae	*Mephitis mephitis*	11 [4]	53 [4]	36 [4]
Procyonidae	*Bassaricyon alleni* [5]	-	-	-
Procyonidae	*Bassaricyon gabbii*	100 [6]	0 [6]	0 [6]
Procyonidae	*Bassariscus astutus*	18 [7]	45 [7]	37 [7]
Procyonidae	*Bassariscus sumichrasti*	40 [8], 65 [9]	40 [8], 25 [9]	20 [8], 10 [9]
Procyonidae	*Nasua nasua*	41 [10]	58 [10]	2 [10]
Procyonidae	*Nasua narica*	55 [11], 46 [12]	45 [11], 40 [12]	1 [11], 15 [12]
Procyonidae	*Potos flavus*	100 [13, 14]	0 [13, 14]	0 [13, 14]
Procyonidae	*Procyon cancrivorus*	(88) [15]	(61) [15]	(13) [15]
Procyonidae	*Procyon lotor*	55 [16], 2 [17]	32 [16], 12 [17]	6 [16], 85 [17]
Mustelidae	*Meles meles*	33 [18]; 38 [19]	55 [18]; 35 [19]	12 [18]; 20 [19]
Mustelidae	*Gulo gulo*	0 [20]	0 [20]	100 [20]
Mustelidae	Tayra, *Eira barbara*	21[10]	10[10]	69 [10]
Mustelidae	*Mustela vison*	0 [21, 22]	4 [21], 1 [22]	96 [21], 99 [22]
Mustelidae	*Martes americana*	8 [23], 3 [24]	8 [23], 1 [24]	72 [23], 97 [24]
Ursidae	*Ailuropoda melanoleuca*	100 [1], 99 [25]	0 [1, 25]	0 [1, 25]
Ursidae	*Tremarctos ornatus*	96 [26]	3 [26]	1 [26]
Ursidae	*Ursus ursinus*	22 [27]	78 [27]	0 [27]
Ursidae	*Ursus arctos*	54 [28], 82 [25], 29 [29]	11 [28], 7 [25], 7 [29]	35 [28],10 [25], 64 [29]
Ursidae	*Ursus americanus*	81 [30], 90 [25], 80 [31]	11 [30], 5 [25], 7 [31]	0 [30], 4 [25], 1 [31]
Ursidae	*Ursus maritimus*	2 [25], 0 [32]	0 [25, 32]	98 [25], 100 [32]
Phocidae	*Hydrurga leptonyx*	0 [33, 34]	45 [33, 34]	55 [33, 34]

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Family	**Species**	**Number**$^{\text{source}}$
Ailuridae	*Ailurus fulgens*, red panda	AM711897[1]
Canidae	*Canis lupus*, gray wolf	AY598494S06[2]
Mephitidae	*Mephitis mephitis*, striped skunk	X94927[3]
Phocidae	*Hydrurga leptonyx*, leopard seal	AM181026[4]
Procyonidae	*Bassariscus astustus*, ringtail	AF498159[5]
Procyonidae	*Bassariscus sumichrasti*, cacomistle	DQ660301[6]
Procyonidae	*Bassaricyon alleni*, Allen's olingo	DQ660299[6]
Procyonidae	*Bassaricyon gabii*, bushy-tailed olingo	X94931[3]
Procyonidae	*Nasua nasua*, South American coati	DQ660303[6]
Procyonidae	*Nasua narica*, white-nosed coati	DQ660302[6]
Procyonidae	*Procyon lotor*, raccoon	AB297804[7]
Procyonidae	*Procyon cancrivorus*, crab-eating raccoon	DQ660305[6]
Procyonidae	*Potos flavus*, kinkajou	DQ660304[6]
Mustelidae	*Eira barbara*, tayra	AF498154[5]
Mustelidae	*Gulo gulo*, wolverine	X94921[3]
Mustelidae	*Martes americana*, American martin	AY121352[8]
Mustelidae	*Meles meles*, Eurasian badger	AB049794[9]
Mustelidae	*Mustela vison*, North American mink	EF689073[10]
Ursidae	*Ailuropoda melanoleuca*, giant panda	EF212882[11]
Ursidae	*Tremarctos ornatus*, spectacled bear	U23554[12]
Ursidae	*Ursus ursinus*, sloth bear	EF196662[13]
Ursidae	*Ursus americanus*, American black bear	AF303109.1[14]
Ursidae	*Ursus arctos*, brown bear	AF303110[14]
Ursidae	*Ursus maritimus*, polar bear	AF303111[14]

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