DIFFERENCES AND SIMILARITIES IN SKULL SHAPE BETWEEN THE RED AND GIANT PANDA REVELAED BY GEOMETRIC MORPHOMETRICS

Running title: Differences and similarities between the pandas

Borja Figueirido*, Francisco J. Serrano-Alarcón & Paul Palmqvist

Departamento de Ecología y Geología, Área de Paleontología, Facultad de Ciencias, Universidad de Málaga, Campus Universitario de Teatinos, 29071 Málaga, Spain. E-mail: Francisco.figueirido@uv.es, fra.serrano@estudiante.uam.es, ppb@uma.es

Corresponding author: Borja Figueirido
Área de Paleontología,
Departamento de Ecología y Geología, Facultad de Ciencias,
Universidad de Málaga.
Campus Universitario de Teatinos.
29071-Málaga (Spain)
Phone (all authors): + 34 952 13 2002
Email: Francisco.figueirido@uv.es

ABSTRACT

In this study we develop a comparative study of skull anatomy in the living pandas for evaluating the importance of natural selection and phylogenetic constraints in shaping the convergent morphological adaptations of these peculiar carnivores. Relative warp analysis of landmark data was used in a comparative morphometric analysis across the families Procyonidae, Ursidae and Ailuridae. Skull morphospaces show that major patterns of morphological variation among these carnivorans correlate with differences in feeding behavior. More specifically, this study reveals an extreme convergence in skull shape between the bamboo specialists: the red panda, *Aiulurus fulgens* (Ailuridae), and the giant panda, *Ailuropoda melanoleuca* (Ursidae). Following the most recent molecular and morphological phylogenies and the poor evidence from the fossil record, it seems highly improbable that homology could explain the shared morphology of the two pandas' lineages. On the contrary, most phylogenetic and paleontologic data suggest that convergent or parallel evolution (homoplasy) would be the major evolutionary process shaping the common morphological traits of these species. Therefore, the overall morphological resemblance between the two pandas was probably driven by extrinsic factors (natural selection) as well as by intrinsic ones (shared developmental pathways), which may have posed constraints on the direction of pandas' evolution.

Key words: Convergent evolution, skull morphology, landmark-based morphometrics, *Ailuropoda melanoleuca*, *Aiulurus fulgens*.

INTRODUCTION

Few organisms have received as much attention from an evolutionary point of view as the living pandas (Gittleman 1994). These emblematical mammals belong to two species, the giant panda (*Ailuropoda melanoleuca*) and the red or lesser panda (*Ailurus fulgens*). Both are native to Central Asia, exhibit a highly specialized anatomy and have an unusual trophic behavior among the order Carnivora, as they feed almost entirely on bamboo (Chorn & Hoffmann, 1978; Roberts & Gittleman, 1984; Schaller *et al.* 1989; Pradhan *et al.* 2001). Given the peculiar diet and shared morphological specializations of red and giant panda (Davis 1964; Radinsky 1981; Endo *et al.* 1996; Endo *et al.*1999; Endo *et al.* 2001a; Endo *et al.*, 2001b; Antón *et al.* 2006; Salesa *et al.* 2006a, 2006b; Zhang *et al.* 2007; Endo *et al.* 2008), their systematic position and phylogenetic relationships have been under debate for over a century. For example, several authors have grouped them within the same clade, either in Procyonidae (e.g., Mivart 1885; Lankester & Lydekker 1901; Gregory 1936; Simpson 1945) or in Ursidae (e.g., Segall 1943, Ginsburg 1982). However, with the advent of new approaches for reconstructing phylogenies that combine molecular data with morphologically-based inferences, it has been revealed that both species of pandas diverged during the early Oligocene and belong to different families within the suborder Caniformia: Ailuridae for the red panda and Ursidae for the giant panda (Goldman *et al.* 1989; Ledje & Arnason 1996; Flynn *et al.* 1998; Bininda-Emonds *et al.* 1999; Ginsburg 1999; Flynn *et al.* 2000; Flynn *et al.* 2005).

Despite the phylogenetic position of *Ailuropoda* as a sister lineage to all living ursids is well documented (Davis 1964; Flynn *et al.* 1988; Wyss & Flynn 1993; see also the references in Fig. 1), the evolutionary affinities of *Ailurus* have yet to be resolved: while some authors suggest a close phylogenetic relationship with Ursidae (e.g., Segall

1943; Hunt 1974; Wozencraft 1989), others favor Procyonidae (e.g., Flower & Lydekker 1891; Beddard 1902; Tagle *et al.* 1986; Goldman *et al*. 1989; Wayne *et al*. 1989) or even Mephitidae (Flynn *et al*. 2000; Delisle & Strobeck 2005; Flynn *et al*. 2005). Therefore, the status of *Ailurus* and *Ailuropoda* as distantly related taxa makes their shared morphology one of the most remarkable examples of evolutionary convergence among mammals (e.g., Bardenfleth 1913; Raven 1936; Davis 1964; Gould 1978; Salesa *et al*. 2006b; Zhang *et al*. 2007).

Convergence and parallelism are central concepts in evolutionary theory (Wroe & Milne 2007) and the study of mammalian evolution has provided key elements to this regard (Madsen et al. 2001; Nevo 2001; Winter and Oxnard 2001). However, few comparative studies have addressed the common adaptations of pandas from a functional perspective, despite that only such studies could shed light on the origin of the extreme morphophysiological convergence between them. It is worth noting that Davis (1964) drew Thompsonian diagrams of deformed coordinates for exploring morphological differences between the giant panda and other bears. However, this study was hampered by the lack of technological advances for obtaining mathematical shape descriptors currently available. As a result, Davis (1964) could not develop a multivariate approach based on geometric morphometrics for characterizing the patterns of shape change in arctoid carnivorans. Also, recently, Zhang et al. (2007) have analyzed the mandible morphology of the giant panda relative to other sympatric Chinese carnivores by multivariate approaches. However, the authors do not developed a comparative study of skull morphology between both pandas using geometric morphometrics. Geometric morphometrics (GM) is a powerful tool for multivariate analysis of shape by means of a configuration of *landmarks* which opens the possibility to explore geometrically the variance explained by the eigenvectors graphically (Rohlf & Marcus, 1993; Adams et al., 2004).

In this article, we develop for the first time a comparative analysis of skull shape between *Ailurus* and *Ailuropoda* using *landmark*-based methods of geometric morphometrics. The specific goals of this paper are: *(i)* To quantify skull shape similarities between *Ailurus* and *Ailuropoda* for exploring morphological convergences of feeding behavior between them; *(ii)* To explore skull shape differences between *Ailurus* and *Ailuropoda* in order to reveal skull morphological autoapomorphies of each species; and *(iii)* to debate on the role of evolutionary convergence between *Ailurus* and *Ailuropoda* for shaping the skull design of these emblematical mammals.

MATERIAL AND METHODS

We digitized 13 landmarks on high resolution digital images in direct lateral view (Fig. 1) of XXX crania and XXX mandibles belonging to 18 species within Ursidae, Procyonidae, Ailuridae and Mephitidae (Table 1) using TPSdig v.2.11 (Rohlf, 2008). We collected only adult specimens to avoid influence of ontogenetic variation. The specimens were collected at the American Museum of Natural History (New York, USA), the Natural History Museum (London, UK) and the Museum für Naturkunde (Berlin, Germany).

All the specimens were aligned using the Procrustes superimposition method (e.g., Dryden and Mardia, 1998) for both samples (i.e., mandibles and crania) separately. We computed a Principal component analysis (PCA) from the covariance matrix of the aligned coordinates of cranium and mandible shape separately using all the specimens included in the sample. Also, we computed a discriminant analyses (DA) from the Procrustes coordinates of cranium and mandible shape separately to identify skull shape similarities between both pandas and the differences between them with other related arctoid carnivores (i.e., Procyonidae, Mephitidae and Ursidae). The statistical significance to discriminate between both groups was tested by a permutation test for the Procrustes and Mahalanobis distances between both mean shapes.

In order to report skull shape differences between *Ailurus* and *Ailuropoda,* we conducted a second round of PCA from the covariance matrix of aligned coordinates of cranium and mandible shape using only both species of pandas. Also, a second DA from the aligned coordinates of cranium and mandible shape was computed separately for distinguishing the skull shape between *Ailurus* and *Ailuropoda*. The statistical significance to discriminate between both groups in DA was tested by a permutation test for the Procrustes and Mahalanobis distances between both mean shapes.

All the geometric morphometric procedures and statistical analysis were carried out with MORPHOJ software package (Klingenberg, 2008).

RESULTS

Skull shape similarities

The PCA of the aligned coordinates for the 13 landmarks describing mandible and cranium shape yielded 22 PCs (Supporting information Table S2; Figure S1). The regression analysis between Cs and the scores of the third PC derived from mandible analysis was significant ($P< 0.0001$). For the crania, the regression between Cs and the scores of second PC was also significant ($P< 0.0001$). Therefore, the patterns of shape variation described by the third PC in mandible analysis, and by the second PC in cranial analysis result from allometrics effects (Figure S2). As a result, we only present the results of the first two PCs in the case of the mandible and of the first and third PCs in the case of the cranium, as they contain the most relevant information regarding shape.

Figure 2A show the morphospace depicted from the first two PCs derived from mandible morphology. The first PC explains shape changes accounted from the mandibles of the red panda (*A. fulgens*), giant panda (*A. melanoleuca*) and the kinkajou (*P. flavus*) with negative scores, to the mandibles of other procyonids, ursids and mephitids (Fig. 2A, *x-axis*). The second PC mainly separates the kinkajou, with positive scores, to other carnivores included in the dataset (Fig. 2A, *y-axis*).

Figure 2B show the morphospace depicted from the first and third PCs derived from cranial analysis. The first PC account for shape changes associated from the crania of the red panda (*A. fulgens*) and the kinkajou (*P. flavus*), taking negative scores, to the crania of the rest of carnivores included in the sample, scoring positively (Fig. 2B; *x*-axis). The third PC mainly separates the crania of the giant panda (*A. melanoleuca*), taking positive scores, to the crania of other carnivores, taking now from less positive to negative scores in this axis (Fig. 2B, *y-axis*).

After visual inspection of these results, we can conclude that the combination of both PCs defines the mandibles of *Ailurus* and *Ailuropoda* into a delimited region of the morphospace (Fig. 2C.). In contrast, both pandas do not converge in the same place of the cranium shape space, but they are grouped in the upper left corner of this graph (Fig. 2B).This fact strongly suggests that both species of pandas have a combination of mandible and cranial traits in a unique manner among the rest of carnivores included in the analysis. These results are also confirmed by DA computed from mandible (Fig. 2E) and cranial (Fig. 2F) shapes to discriminate between *Ailurus* plus *Ailuropoda* and the other carnivores analyzed. The highly significant discrimination among both groups (see Table 1) allows us to recognize a set of shared traits between the pandas which distinguish them from other carnivores. The mandible of *Ailurus* and *Ailuropoda* is comparatively deep and concave, with a tall and narrow coronoid process, a robust articular condyle, an angular process more distantly positioned from the jaw condyle, and well developed cheek teeth (Fig. 2E). The cranium of the red and giant panda is brachycephalic with short snout, high-vaulted calvarium, short braincase, broad zygomatic arches, and well developed postglenoid process, large molars, and comparatively small canines (Fig. 2F).

Skull shape differences

The PCA of the aligned coordinates for the 13 landmarks describing mandible and cranium shape of *Ailurus* and *Ailuropoda* yielded 22 PCs (Supporting information Table S2; Figure S3).

Figure 3A shows the morphospace depicted from the first two PCs derived from mandible analysis which jointly explains more than 75% of variance explained (Supporting information Table S2). The first PC explains shape changes accounted from the mandibles of the red panda (*A. fulgens*), with negative scores, to the mandibles of the giant panda (*A. melanoleuca*) scoring positively (Fig. 3A, *x*-axis) according with a set of morphological traits (Fig. 3C). The second PC (Fig. 3A, *y*-axis) explains intraspecific changes in each species (Fig. 3C).

Figure 3B show the morphospace depicted form the first two PCs derived from cranial analysis. As in the case of the mandible analysis, the first PC is mainly related with the shape changes associated from the crania of the red panda (*A. fulgens*), taking negative scores, to the crania of the giant panda (*A. melanoleuca*) scoring positively (Fig. 3B; *x*-axis) according with a set of morphological traits (Fig. 3D). Again, the second PC (Fig. 3B, *y*-axis) explains changes related with intraspecific variation in each species. However, the fact that we are only analyzing two species and given that *Ailurus* and *Ailuropoda* posses high different body masses (~5 Kg and ~100 Kg, respectively; Roberts and Gittleman 1984; Chorn and Hoffmann 1978) preclude us to evaluate if this shape changes are associated with size (i.e., interspecific allometry).

The results obtained in DA from both mandible (Fig. 3E) and cranial (Fig. 3F) anatomy also shows a significant discrimination (Table 1) between both species of pandas, according with a set of morphological traits. It is worth noting that given the small sample size of both species of pandas and the high dimensionality of the data, the power of test could be low. However, all the specimens were correctly classified under cross-validation procedure and also, the morphological traits that allow to discriminate between both groups are the same of those obtained in the first PC obtained from mandible and cranial analyses. For example, the red panda posses deeper mandibular bodies, shorter molar tooth rows (for the absence of the third lower molar), a more vertically oriented coronoid process, more developed angular process and larger distances between the condylar and angular processes relative to the mandible of the giant panda (Fig. 3E). The cranium of the red panda is deeper, it has a less developed zygomatic and glenoid fossa, has a larger neurocranium and a shorter molar tooth row relative to the one of the giant panda (Fig. 3F).

DISCUSSION

Our results demonstrate that both species of pandas (i.e., *A. melanoleuca* and *A. fulgens*) share a number of craniodental traits that we interpret as common adaptations toward bamboo feeding. It is worth noting that in the case of the mandible morphology this convergence is particularly striking (Fig. 4). This opens the possibility of hypothesizing on the evolutionary causes below the shared morphological traits in both species of pandas.

There are two possible evolutionary scenarios that could explain the common morphological patterns related to the dietary preferences of pandas. The first is that these traits were present in their most immediate common ancestor (homology). Obviously, this evolutionary scenario would involve that both pandas must be grouped under the same clade. This could be the case if the red panda were closely related to Ursidae (Segall 1943; Hunt 1974; Ginsburg 1982; Wozencraft 1989). However, the most recent molecular and morphological phylogenies indicate that this species is more related to Procyonidae (e.g., Goldman *et al*. 1989; Wayne *et al*. 1989; Bininda-Edmonds *et al*. 1999) or even to Mephitidae (e.g., Flynn *et al*. 2000; Delisle & Strobeck 2005; Flynn *et al*. 2005). In addition, the scarce fossil record of both *Ailurus* and *Ailuropoda* points in the same direction. There is consistent evidence that the common ancestor of Ailuridae was a generalized carnivore, not a bamboo feeder, which indicates that the highly specialized morphology of *Ailurus* is secondary (Salesa *et al*. 2006b). The craniodental adaptations of *Ailuropoda* for durophagous feeding behavior centered on bamboo were already evident in the late Pliocene species *A. microta,* although dental and basicranial anatomy indicate a less specialized morphology in the early history of the lineage during Miocene times (McLellan & Reiner 1994; Jin *et al.* 2007).

Given that homology is an improbable cause for explaining the evolution of the pandas' common traits, how can we explain their extreme morphological and ecological resemblance? We hypothesize that similar selection pressures posed by similar ecological requirements (e.g., bamboo feeding) drove morphological evolution and feeding specialization in the unrelated *Ailuropoda* and *Ailurus*. Thus, the processes that more likely gave rise to the common morphology of pandas would be either convergent or parallel evolution (homoplasy).

The independent evolution of similar skull morphologies could result from a common developmental basis or genetic channeling, which would indicate that parallel evolution played a major role in the evolution of pandas. In contrast, if the morphological traits shared among these lineages evolved from different antecedent features (and thus through different developmental pathways), convergent evolution would be the main process for shaping these traits (Brakefield 2006). It is well documented that closely related taxa often use different developmental pathways to reach the same phenotype and vice versa (Arendt & Reznick 2008), at least within vertebrates (Leander 2008). For these reasons, distinguishing parallel from convergent evolution is a difficult task (Arendt & Reznick 2008). In addition, many authors cast doubts on if there is a clear-cut theoretical distinction between these two evolutionary processes, which are frequently envisaged as extremes of a continuum (e.g., Meyer 1999; Gould 2002; Desutter-Grandcolas *et al*. 2005; Arendt & Reznick 2008; Hall 2007; Abouheif 2008).

Could it be possible that natural selection may have favored other morphological designs highly specialized for bamboo feeding different to those shown by pandas? Or, on the contrary, are the pandas' morphologies the only possible given the underlying developmental channels and/or their shared genetic basis? The answers to these questions demand combined morphometric studies based on phylogenetic reconstructions, which are key points for providing a better understanding of the evolutionary history of pandas.

ACKNOWLEDGEMENTS

This work was supported by the Spanish Ministry of Science and Innovation (MICINN) Research Projects (CGL2006-13808-C02-02 and CGL2008-04896) and FPU PhD Research Fellowship to B. Figueirido.

LITERATURE CITED

Abouheif, E. 2008. Parallelism as the pattern and process of mesoevolution. Evol. Dev. 10:3-5.

Adams, D.C., F.J. Rohlf, and D. Slice. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. Ital. J. Zool. 71:5-16.

Alberch, P. 1982. Developmental constraints in evolutionary process. Pp. 313-332 in J. T. Bonner ed. *Evolution and Development*. New York: Springer.

Alves-Costa, C. P., G. A. Da Fonseca, and C. Christofaro. 2004. Variation in the diet of the brown-nosed coati (*Nasua nasua*) in southeastern Brazil. J. Mammal. 85:478-482.

Antón, M., M. J. Salesa, J. F. Pastor, S. Peigné, and J. Morales. 2006. Implications of the Functional Anatomy of the Hand and Forearm of *Ailurus fulgens* (Carnivora, Ailuridae) for the Evolution of the "False-Thumb" in Pandas. J. Anat. 209:757-764.

Arendt, J., and D. Reznick. 2008. Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends Ecol. Evol. 23:26-32.

Bardenfleth, K.S. 1913. On the systematic position of *Aeluropus melanoleucus*. Mindeskr. Japetus Steenstrup. 17:1-15.

Barone, R. 1986. Anatomie Compare des Mammifères Domestiques. Vol. 1: Ostéologie. Paris: Vigot.

Beddard, F.E. 1902. Mammalia. Cambridge Nat. Hist., London Macmillian.

Bininda-Emonds, O. R., J. L. Gittleman, and A. Purvis. 1999. Building large trees by combining phylogenetic information: A complete phylogeny of extant Carnivora (Mammalia). Biol. Rev. 74:143-175.

Bleijenberg, M. C. K., and J. Nijboer. 1989. Feeding herbivorous carnivores. Pp. 41-50 In Glatston, A. R. ed. Red Panda Biology. Netherlands: SPB Academic Publishing.

Bookstein, F. L. 1991. *Morphometric tools for landmarks data. Geometry and biology,* 1st ed. London: Cambridge University Press.

Brakefield, P.M. 2006. Evo-devo and constraints on selection. Trends Ecol. Evol. 21:362-368.

Chorn, J., and R. S. Hoffmann. 1978. *Ailuropoda melanoleuca*. Mamm. Spec. 110:1-6.

Choudhury, A. 2001. An overview of the status and conservation of the red panda *Ailurus fulgens* in India, with reference to its global status. Oryx 35:250-259

DeMaster, D. P., and I. Stirling. 1981. *Ursus maritimus*. Mamm. Spec. 145:1–7.

Davis, D.D. 1964. The giant Panda. A morphological study on evolutionary mechanisms. Fieldiana Zool. 3:1-339.

Delisle, I., and C. Strobeck. 2005. A phylogeny of the Caniformia (order Carnivora) based on 12 complete protein-coding mitochondrial genes. Mol. Phylogenet. Evol. 37:192-201.

Desutter-Grandcolas, L., F. Lengendre, P. Grandcolas, T. Robillard, and J. Murienne. 2005. Convergence and parallelism: Is a new life ahead of old concepts? Cladistics 21:51-61.

Endo, H., N. Sasaki, D. Yamagiwa, Y. Uetake, M. Kurohmaru, and Y. Hayashi. 1996. Functional anatomy of the radial sesamoid bone in the giant panda (*Ailuropoda melanoleuca*). J. Anat 189:587-592.

Endo, H., T. Makita, M. Sasaki, K. Arishima, M. Yamamoto, and Y. Hayashi. 1999. Comparative anatomy of the radial sesamoid bone in the polar bear (*Ursus maritirnus*), the brown bear (*Ursus arctos*) and the giant panda (*Ailuropoda melanoleuca*). J. Vet. Med. Sci. 61:903-907.

Endo, H., M. Sasaki, Y. Hayashi, H. Koie, Y. Yamaya, and J. Kimura. 2001a. Carpal bone movements in gripping action of the giant panda (*Ailuropoda melanoleuca*). J. Anat. 198:243-246.

Endo, H., M. Sasaki, H. Kogiku, M. Yamamoto, and K. Arishima. 2001b. Radial sesamoid bone as a part of the manipulation system in the lesser panda (*Ailurus fulgens*). Ann. Anat. 183:181-184.

Endo, H., N. Hama, N. Niizawa, J. Kimura, T. Itou, H. Koie, and T. Sakai. 2008. Three-dimensional Imaging of the Manipulating Apparatus in the Lesser Panda and the Giant Panda. Pp. 61-66 in Endo H. and R. Frey eds. Anatomical Imaging: Towards a New Morphology, Springer.

Ewer, R. F. 1973. *The Carnivores,* 2nd ed. New York: Cornell University Press.

Figueirido, B., P. Palmqvist, and J.A. Pérez-Claros. 2009. Ecomorphological correlates of craniodental variation in bears and paleobiological implications for extinct taxa: an approach based on geometric morphometrics. J. Zool. (London) 277:70-80.

Figueirido, B., J.A. Pérez-Claros, V. Torregrosa, A. Martín-Serra, and P. Palmqvist. 2010. Demythologizing *Arctodus simus,* the 'short-faced', long-legged and predaceous bear that never was. JVP 30:in press.

Flower, W.H., and R. Lydekker. 1891. An introduction to the study of mammals living and extinct. F.Z.S. London: Adam & Charles Black. New-York: Macmillan & Co.

Flynn, J. J., N. A. Neff, and R. H. Tedford. 1988. Phylogeny of the Carnivora. Pp. 73-116 in M. Benton ed. The Phylogeny and Classification of the Tetrapods Vol. 2, Mammals. Clarendon, Oxford.

Flynn, J. J., and M. Nedbal. 1998. Phylogeny of the Carnivora (Mammalia): Congruence vs Incompatibility among Multiple Data Sets. Mol. Phylogenet. Evol. 9:414-426.

Flynn, J. J., M. Nedbal, J. Dragoo, and L. Honeycutt. 2000. Whence the red panda? Mol. Phylogenet. Evol. 17:190-199.

Flynn, J. J., J. A. Finarelli, S. Zehr, J. Hsu and M. Nedbal. 2005. Molecular Phylogeny of the Carnivora (Mammalia): Assessing the Impact of Increased Sampling on Resolving Enigmatic Relationships. Syst. Biol. 54:317-337.

Fulton, T. L., and C. Strobeck. 2007. Novel phylogeny of the raccoon family (Procyonidae: Carnivora) based on nuclear and mitochondrial DNA evidence. Mol. Phylogenet. Evol. 43:1171-1177.

Gatti, A., R. Bianchi, C. Regina, X. Rosa, and S. Lucena. 2006. Diet of two sympatric carnivores, *Cerdocyon thous* and *Procyon cancrivorus*, in a restinga area of Espirito Santo State, Brazil. J. Trop. Ecol. 22:227–230.

Ginsburg, L. 1982. Sur la position systématique du petit panda, *Ailurus fulgens* (Carnivora, Mammalia). Geobios (Mem. Spec.). 6:247-258.

Ginsburg, L. 1999. Order Carnivora. Pp 109-148 in G. Rössner and K. Heissig eds. The Miocene Land Mammals of Europe. Munich: Pfeil.

Gittleman, J. L. 1994. Are the Pandas Successful Specialists or Evolutionary Failures? BioScience 44:456-464.

Goldman, D., P. Rathna Giri, and S. J. O'Brien. 1989. Molecular Genetic-Distance Estimates Among the Ursidae as Indicated by One- and Two-Dimensional Protein Electrophoresis. Evolution 43:282-295.

Gompper, M. E. 1995. *Nasua narica.* Mamm. Spec. 487:1-10.

Gompper, M.E. 1996. Sociality and asociality in white-nosed coatis (*Nasua narica*): foraging costs and benefits. Behav. Ecol. **7**:254–263.

Gould, S. J. 2002. The structure of the evolutionary theory. 6th ed. Harvard University Press.

Gould, S.J. 1978. The panda's peculiar thumb. Nat. Hist. 87:20-30.

Gregory, W.K. 1936 .On the phylogenetic relationships of the giant panda (*Ailuropoda*) to other arctoid carnivora. Amer. Mus. Nov. 878:1-29.

Hall, B. K. 2007. Homoplasy and homology: Dichotomy or continuum? J. Hum. Evol. 52:473-479.

Hunt, R. M., 1974. The auditory bulla in Carnívora: an anatomical basis for reappraisal of carnivore evolution. J. Morphol. 143:21-76.

Jin, C., R. L. Ciochon, W. Dong, R. M. Hunt Jr., J. Liu, M. Jaeger and Q. Zhu. 2007. The first skull of the earliest giant panda. Proc. Natl. Acad. Sci. USA 104:10932-10937.

Johnson, A.S. 1970. Biology of the racoon (*Procyon lotor varius* Nelson and Goldman) in Alabama. Agric. Exp. Stat. Auburn Univ. Bull. 402:1-148.

Johnson, K. G., G. B. Schaller, and J. Hu. 1988. Comparative behavior of red and giant pandas in the Wolong Reserve, China. J. Mammal. 69:552-564.

Joshi, R., D. L. Garshelis, and J.L. Smith. 1997. Seasonal and habitat-related diets of sloth bears in Nepal. J. Mammal. 78:584–597.

Julien-Laferrière, D. 2002. Frugivory and seed dispersal by kinkajous. Monogr. Biol. 80:217-225.

Kays R. W. 2000. The behavior and ecology of olingos (*Bassaricyon gabbii*) and their competition with kinkajous (*Potos flavus*) in central Panama. Mammalia 64:1-10.

Koepfli, K., M. E. Gompper, E. Eizirik, C. Ho, L. Linden, J. Maldonado, R. K. Wayne. 2007. Phylogeny of the Procyonidae (Mammalia: Carnivora): Molecules, Morphology and the Great American Interchange. Mol. Phylogenet. Evol. 43:1076-1095.

Krause, J., T. Unger, A. Noçon, A. Malaspinas, S. Kolokotronis, M. Stiller, L. Soibelzon, P. Spriggs, A. Dear, W. Briggs, S. Bray, S. J. O'Brien, G. Rabeder, P. Matheus, A. Cooper, M. Slatkin, S. Pääbo, and M .Hofreiter. 2008. Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. BMC Evol. Biol. 8:220.

Lankester, E.R., and R. Lydeker. 1901. On the affinities of *Aeluropus melanoleucus*. Trans. Soc. London 8:163-171.

Larivière, S. 2001. *Ursus americanus*. Mamm. Spec. 647:1-11.

Leander, B. S. 2008. Different modes of convergent evolution reflects phylogenetic distances: a reply to Arendt and Reznick. *Trends Ecol. Evol.* 23:481-482.

Ledje, C. and U. Arnason. 1996. Phylogenetic analyses of complete cytochrome*b* genes of the order Carnivora with particular emphasis on the Caniformia. J. Mol. Evol. 42:135-144.

Lotze, J. and S. Anderson. 1979. *Procyon lotor*. Mamm. Spec. 119:1-8.

Liu, J., Z. Ouyang, W. Taylor, R. Groop, Y. Tan, and H. Zhang. 1999. A Framework for Evaluating the Effects of Human Factors on Wildlife Habitat: the Case of Giant Pandas. Conserv. Biol. 13:1360-1370.

Marugán-Lobón, J., and A. D. Buscalioni. 2004. Geometric morphometrics in macroevolution: morphological diversity of the skull in modern avian form in contrast to some theropods dinosaurs. Pp. 157-171 in A.M.T. Elewa ed. *Morphometrics: applications in biology and paleontology*. New York: Springer.

Marugán-Lobón, J., and A. D. Buscalioni. 2006. Avian skull morphological evolution: exploring exo- and endocranial covariation with two-block partial least squares. Zoology 109:217-230.

McLellan, B., and D. C. Reiner. 1994. A review of bear evolution. Int. Conf. Bear Res. Mgmt. 9:85-96.

Meyer, A. 1999. Homology and homoplasy: the retention of genetic programmes. Pp. 141-157 in G. Bock ed. *Homology*. Chichester: Wiley & Son.

Mivart, G. 1885. On the anatomy, classification, and distribution of the Arctoidea. Proc. Zool. Soc. London 1885:340-404.

Nowak, R. M., and E.P. Walker. 1999. *Walker's mammals of the world*. 6th ed. JHU Press.

Pasitschniak-Arts, M. 1993. *Ursus arctos.* Mamm. Spec. 439:1-10.

Pelton, M. R. 1993. The American black bear. In *Bears: majestic creatures of the* wild. Pp.108-115 in I. Stirling ed. Pennsylvania: Rodale Press.

Peng J., Z. Jiang, and J. Hu. 2001. Status and conservation of giant panda. Folia Zoologica 50:81-88.

Peyton, B. 1980. Ecology, distribution and food habits of spectacled bears, *Tremarctos ornatus*, in Peru. J. Mammal. 61:639-652.

Poglayen-Neuwall, I. 1989. Procyonids. Pp. 450-468 in S. Parker ed. *Grzimek's Encyclopedia of Mammals*. New York: McGraw-Hill.

Pradhan, S., G. K. Saha, and J. A. Khan. 2001. Ecology of the red panda *Ailurus fulgens* in the Singhalila National Park, Darjeeling, India. Biol. Conserv. 98:11-18.

Radinsky, L. A. 1981. Evolution of skull shape in carnivores 2. Additional modern carnivores. Biol. J. Linn. Soc. 16:337-355.

Raven, H.C. 1936. Notes on the anatomy on the viscera of the giant panda (*Ailuropoda melanoleuca*). Amer. Mus. Nov. 877:1-223.

Reid, D. G., J. Hu, and Y. Huang. 1991. Ecology of the red panda in the Wolong Reserve, China. J. Zool. (Lond.) 225:347-364.

Roberts, M. S., and J. L. Gittleman. 1984. *Ailurus fulgens*. Mamm. Spec. 222:1-8.

Rodríguez-Bolaños, A., and P. Sánchez. 2000. Trophic characteristics in social groups of the Mountain coati, *Nasuella olivacea* (Carnivora: Procyonidae). Small Carnivore Conservation 23:1-6.

Rodríguez-Estrella, R., A. Rodríguez, and K. Grajales. 2000. Spring diet of the endemic ring-tailed cat (*Bassariscus astutus insulicola*) population on an island in the Gulf of California, Mexico. J. Arid Environ. 44:241-246.

Rohlf, F.J. 1993. Relative warp analysis and an example of its application to mosquito wings. Pp. 131-159 in L.F. Marcus, E. Bello, and A. García-Valdecasas ed. *Contributions to morphometrics*. Madrid: Museo Nacional de Ciencias Naturales.

Rohlf, F.J. 2007. TpsRelw ver. 1.45. Department of Ecology and Evolution, State University of New York at Stony Brook [computer program and documentation].

Rohlf, F.J. 2008. TpsDig, ver. 2.11. Digitize landmarks and outlines. Department of Ecology and Evolution, State University of New York at Stony Brook [computer program and documentation].

Salesa, M. J., G. Siliceo, M. Antón, J. Abella, P. Montoya, and J. Morales, J. 2006a. Anatomy of the "false thumb" of *Tremarctos ornatus* (Carnivora, Ursidae, Tremarctinae): phylogenetic and functional implications. Estudios Geol. 62**:**389-394.

Salesa, M. J., M. Antón, S. Peigné, and J. Morales. 2006b. Evidence of a false thumb in a fossil carnivore clarifies the evolution of pandas. Proc. Natl. Acad. Sci. USA 109:379-382.

Santos, M., and S. Hartz. 1999. The foods habits of *Procyon cancrivorus* (Carnivora, Procyonidae) in the Lami Biological Reserve, Porto Alegre, Southern Brazil. Mammalia 63:525-530.

Schaller, G. B., J. Hu, W. Pan, and J. Zhu. 1985. The giant panda of Wolong. Chicago: University of Chicago Press.

Schaller, G. B., T. Qitao, K. G. Johnson, X. Wang, H. Shen, and J. Hu. 1989. The feeding ecology of giant pandas and Asiatic black bears in the Tangjiahe Reserve, China. Pp. 212-241 in J.L.Gittleman ed. *Carnivore behaviour, ecology, and evolution*. Ithaca, New York: Cornell University Press.

Senshu, T., A. Ohya, K. Ide, J. Mikogai, M. Morita, T. Nakao, K. Imazu, L. Jingcao, L. Xuanzhen, L. Wenqi, and N. Lili. 2007. Studies on the digestion in the giant panda, *Ailuropoda melanoleuca*, fed feedstuffs including bamboo. Mamm. Study 32:139-149.

Segall, W. 1943. The auditory region of the arctoid carnivores. Zool. Ser. Field Mus. Nat. Hist. 29:33-59.

Simpson, G.G. 1945. The principles of classification and classification of mammals. Bull. Amer. Mus. Nat. Hist. 85:1-350.

Tagle, D. A., M. M. Miyamoto, M. Goodman, O. Hofmann, G. Braunitzer, R. Göltenboth, and H. Jalanka. 1986. Hemoglobin of pandas: Phylogenetic relationships of carnivores as ascertained with protein sequence data. Naturwissenschaften 73:512-514.

Wade-Smith, J. and B. J. Verts. 1982. *Mephitis mephitis*. Mammalian species 173: 1-7.

Walker, T.R., I. L. Boyd, D. J. McCafferty, N. Huin, R. I. Taylor and K. Reid. 1998. Seasonal occurrence and diet of leopard seals (*Hydrurga leptonyx*) at Bird Island, South Georgia. Antarctic Science 10 : 75-81.

Wayne, R. K., R. E. Benveniste, D. N. Janczewski, and S. J. O'Brien. 1989. Molecular and biochemical evolution of the Carnivora. Pp. 465-494 in J. Gittleman ed. *Carnivore Behavior, Ecology, and Evolution*. Ithaca, New York: Cornell University Press

Wei, F., Z. Feng, Z. Wang, and J. Hu, J. 1999a Current distribution, status and conservation of wild red pandas *Ailurus fulgens* in China. Biol. Conservation 89:285-291.

Wei, F., Z. Feng, Z. Wang, A. Zhou, and J. Hu. 1999b. Use of the nutrients in bamboo by the red panda (*Ailurus fulgens).* J. Zool. (London) 248:535-541.

Wei, G., H. Lu, Z. Zhou, H. Xie, A. Wang, K. Nelson, and Zhao, L. 2007. The microbial community in the feces of the giant panda (*Ailuropoda melanoleuca*) as determined by PCR–TGGE profiling and clone library analysis. Microb. Ecol. 54:194-202.

Wozencraft, W. C. 1989. The phylogeny of the Recent Carnivora. Pp. 495-535 in J. Gittleman ed. *Carnivore Behavior, Ecology, and Evolution*. Ithaca New York: Cornell University Press.

Wyss, A. R., and J. J. Flynn. 1993. A phylogenetic analysis and definition of the Carnivora. Pp. 32-52 in F. Szalay, M. Novacek, and M. McKenna, eds. Mammal Phylogeny: Placentals. Springer-Verlag, New York.

Young, K.A., J. Snoeks, and O. Seehausen. 2009. Morphological diversity and the roles of contingency, chance and determinism in African cichlid radiations. PLoS ONE 4:e4740.

Zelditch, M.L., D.L. Swinderski, H.D. Sheets, and W.L. Fink. 2004. Geometric morphometrics for biologist: a primer. 1st ed. New York/London: Elsevier Academic Press.

Zhang, S., R. Pan, M. Li, C. Oxnard, and F. Wei. 2007. Mandible of the giant panda (*Ailuropoda melanoleuca*) compared with other Chinese carnivores: functional adaptation. Biol. J. Linn. Soc. 92:449-456.

Table 1. Sample sizes (*N*, crania/mandibles) of the species included in the morphometric analyses.

Figure 1. Landmarks used in the morphometric analysis of crania and mandibles. *Mandible*, 1- most antero-dorsal border of the canine alveolus, 2- most postero-dorsal border of the canine alveolus, 3- point between the fourth premolar and lower carnassial, 4- point between the first and second lower molar, 5- posterior edge of the lower tooth row, 6- postero-ventral edge of the coronoid process, 7- intersection between the coronoid and condylar processes, 8- most posterior edge of the articular surface condyle, 9- intersection between the most antero-dorsal point of the angular process and the ascending ramus, 10- tip of angular process, 11- ventral outline below the mesial end of the tooth row, 12- ventral outline below the intersection of the fourth premolar/first molar, 13- ventral outline below the most posterior edge of the canine; *Cranium*, 1- most anterior edge of the nasal bones, 2- dorsal outline directly superior to post-orbital process, 3- dorsal outline directly superior to the end of the zygomatic arch, 4- intersection between sagittal and nuchal crests, 5- postero-dorsal border of the zygomatic arch, 6- ventral tip of postglenoid process, 7- lacrimal duct, 8- ventral tip of the post-orbital process, 9- most antero-dorsal border of the canine alveolus, 10- most postero-dorsal border of the canine alveolus, 11- point between the third and fourth upper premolars, 12- ventral intersection between the zygomatic arch and the maxilla, 13- posterior edge of the upper tooth row.

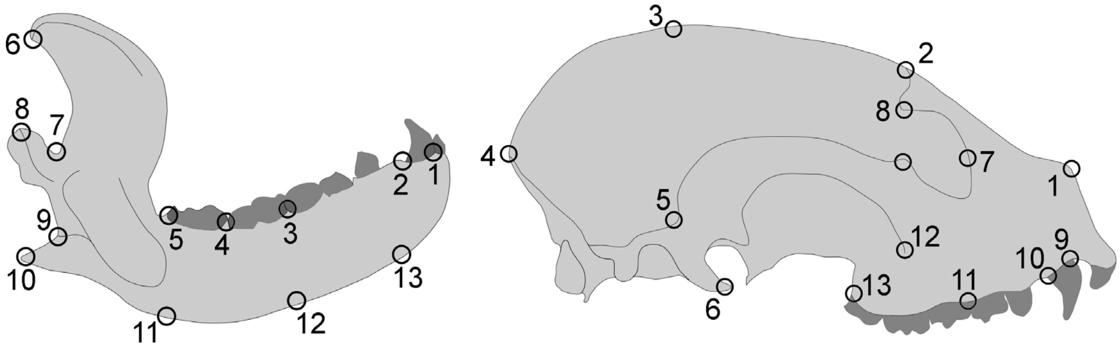

6
8
7
9
10
5
4
3
2
1
13
11
12
3
2
8
4
7
1
5
12
10
9
13
11
6

Figure 2. Multivariate analyses to reveal skull shape similarities between *Ailurus* and *Ailuropoda*. A, Bivariate plot of the scores derived from PCA of jaw anatomy on the morphospace depicted by the first two PCs. B, Bivariate plot of the scores derived from PCA of cranial anatomy on the morphospace depicted by PCI and PCIII. In both cases, the 95% of confidence ellipses are also showed. C, Morphological variation accounted by the first two PCs derived from mandible morphology. D, Morphological variation accounted by PCI and PCIII derived from the morphology of the crania. In both cases, the transformation grids show the deformation from the consensus to each extreme of the axis. E, Histogram showing the frequency of the specimens within the scores of the discriminant function obtained from the analysis of mandible morphology. F, Histogram showing the frequency of the specimens within the scores of the discriminant function obtained from the analysis of mandible morphology. The morphological variation accounted by each discriminant function is also showed as a grid deformation from one group (*Ailurus* plus *Ailuropoda*) to the second group (other related carnivores).

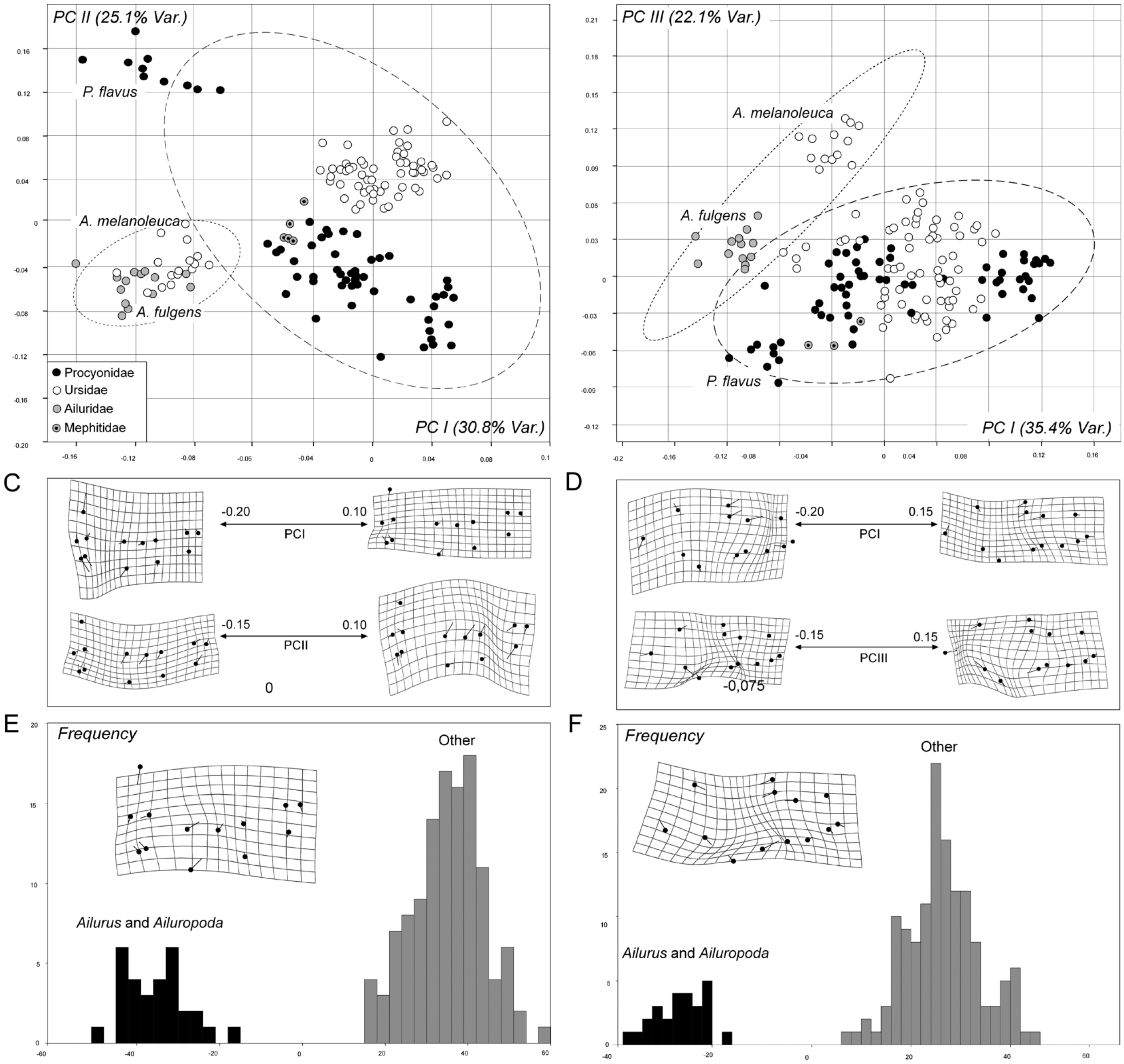
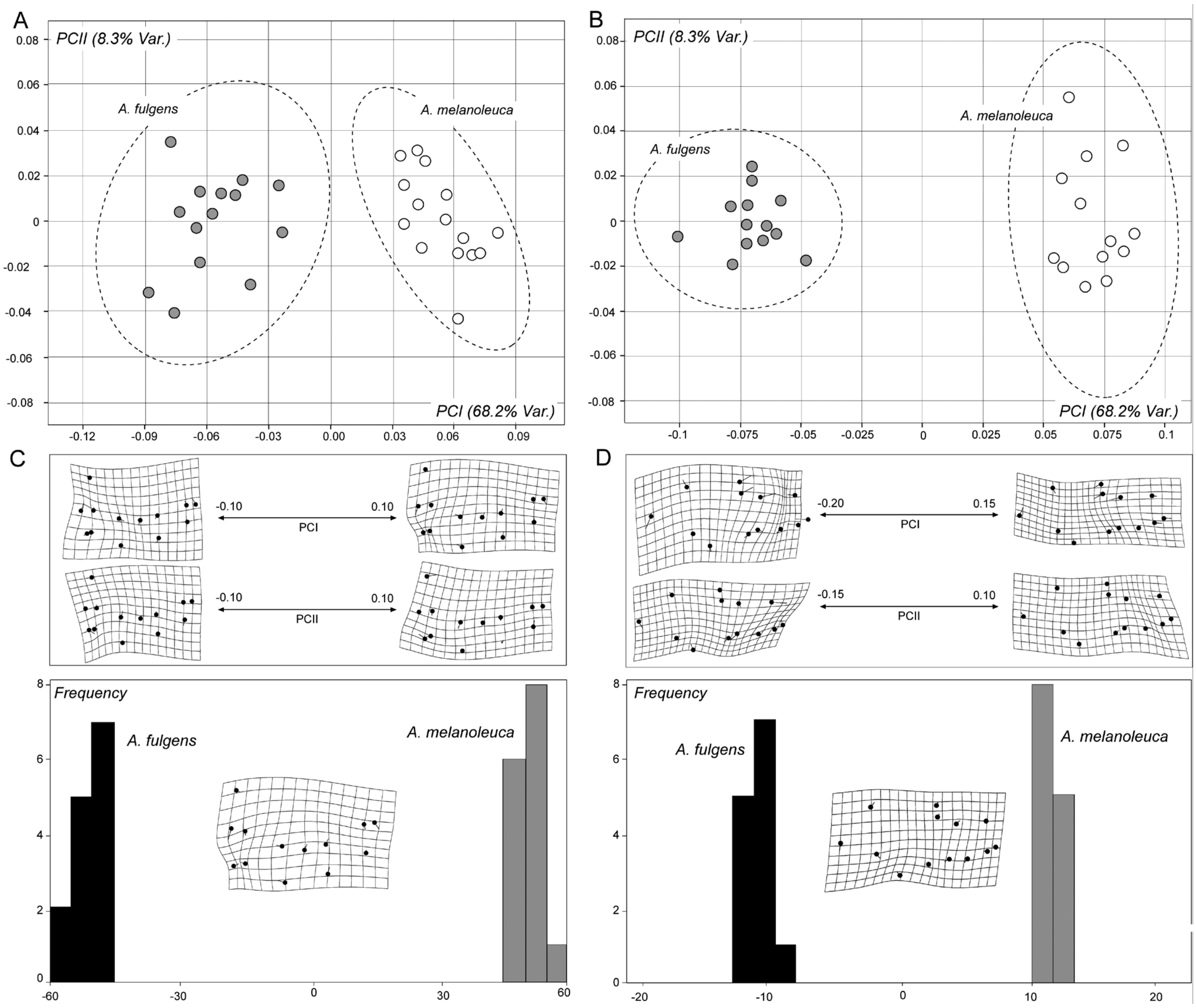

PC II (25.1% Var.)
P. flavus
A. melanoleuca
A. fulgens
Procyonidae
Ursidae
Ailuridae
Mephitidae
PC I (30.8% Var.)
PC III (22.1% Var.)
A. melanoleuca
A. fulgens
P. flavus
PC I (35.4% Var.)
C
-0.20
0.10
PCI
-0.15
0.10
PCII
0
D
-0.20
0.15
PCI
-0.15
0.15
PCIII
-0,075
E
Frequency
Other
Ailurus and Ailuropoda
F
Frequency
Other
Ailurus and Ailuropoda

Figure 3. Multivariate analyses to reveal skull shape differences between *Ailurus* and *Ailuropoda*. A, Bivariate plot of the scores derived from PCA of jaw anatomy on the morphospace depicted by the first two PCs. B, Bivariate plot of the scores derived from PCA of cranial anatomy on the morphospace depicted by PCI and PCII. In both cases, the 95% of confidence ellipses are also showed. C, Morphological variation accounted by the first two PCs derived from mandible morphology. D, Morphological variation accounted by PCI and PCII derived from the morphology of the crania. In both cases, the transformation grids show the deformation from the consensus to each extreme of the axis. E, Histogram showing the frequency of the specimens within the scores of the discriminant function obtained from the analysis of mandible morphology. F, Histogram showing the frequency of the specimens within the scores of the discriminant function obtained from the analysis of mandible morphology. The morphological variation accounted by each discriminant function is also showed as a grid deformation from one group (*Ailurus*) to the second group (*Ailuropoda*).


A
PCII (8.3% Var.)
PCI (68.2% Var.)
A. fulgens
A. melanoleuca
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
-0.12
-0.09
-0.06
-0.03
0.00
0.03
0.06
0.09
B
PCII (8.3% Var.)
PCI (68.2% Var.)
A. fulgens
A. melanoleuca
-0.1
-0.075
-0.05
-0.025
0
0.025
0.05
0.075
0.1
C
-0.10
0.10
PCI
-0.10
0.10
PCII
D
-0.20
0.15
PCI
-0.15
0.10
PCII
Frequency
A. fulgens
A. melanoleuca
8
6
4
2
0
-60
-30
0
30
60
Frequency
A. fulgens
A. melanoleuca
-20
-10
0
10
20