



Three-dimensional cranial ontogeny in pantherines (*Panthera leo*, *P. onca*, *P. pardus*, *P. tigris*; Carnivora: Felidae)

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The *Panthera* lineage is a monophyletic clade of felids, supported by both morphological and molecular evidence. The lineage includes large species with cranial similarity such as *Panthera leo* and *P. tigris*, and other with very different cranium such as *P. pardus*. The aim of our work was to study the cranial ontogeny of Pantherines, elucidating whether their cranial shape is a product of size or phylogeny, and to compare these findings with available information about other carnivores. We studied 370 specimens using geometric morphometrics technique in three dimensions. *Panthera leo* and *P. tigris* show similar ontogenetic trajectories, sharing adult crania with wider rostrum, shorter basicranium and vertical occipital plate. The cranial configuration of *P. leo* is a scaling version of *P. tigris*. *P. pardus* shows the most different cranial pattern, with adults having a rounded braincase and zygomatic arches less expanded than the rest, whereas *P. onca* occupies an intermediate place between these patterns. *P. pardus* is the species with the smallest birth weight and the lowest growth rate, reaching a final size and shape later than the remaining species. Adult shape morphology reflects no relation to phylogenetic placement of the species and it is probably related to Pantherine body size. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, 00, 000–000.

KEYWORDS: anatomy – development – geometric morphometrics – growth – morphology – skull.

INTRODUCTION

The Felidae includes hyper-carnivores, anatomically specialized for catching, subduing and consuming vertebrate prey (Ewer, 1973; Macdonald, Loveridge & Nowell, 2010), and exhibit an enormous variation in size, from 2 kg in *Leopardus guigna* (Molina, 1782) to 300 kg in *Panthera tigris* (Linnaeus, 1758) (Sunquist & Sunquist, 2009). Their skulls have a conservative shape (i.e. low morphological diversity) (Ewer, 1973); this fact could be related to their

restricted hypercarnivorous diet that could have acted as an evolutionary constraint (Holliday & Stepan, 2004). Even though the Felidae are considered to be homogeneous in skull morphology (Goswami, 2006; Sunquist & Sunquist, 2009; Sicuro, 2011), the Puma lineage shows noteworthy differences (Segura, Prevosti & Cassini, 2013); thus, the conservative shape of skull is directly related to the taxonomic level used in the work.

The Felidae include 37 extant species (Wozencraft, Wilson & Reeder, 2005), occurring in all continents except in Antarctica (Redford & Eisenberg, 1992; Sunquist & Sunquist, 2009). The species are

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separated into eight lineages (Johnson *et al.*, 2006; O'Brien & Johnson, 2007), of which the monophyletic *Panthera* lineage, supported by both morphological and molecular evidence (Hemmer, 1978; Herrington, 1986; Salles, 1992; Janczewski *et al.*, 1995; Johnson *et al.*, 1996, 2006; Bininda-Emonds, Gittleman & Purvis, 1999; Mattern & McLennan, 2000; Bininda-Emonds, Decker-Flum & Gittleman, 2001; Jae-Heup *et al.*, 2001; Yu & Zhang, 2005; Christiansen, 2008a; Davis, Li & Murphy, 2010), comprises six large felid species widely distributed in the world (Wozencraft *et al.*, 2005). Pantherines had an ancestor whose lineage branched off the rest of the felids at about 6.4 Mya (Fig. 1, Johnson *et al.*, 2006; O'Brien & Johnson, 2007). The split of this lineage was followed by climatically controlled divergence events during the Pliocene and Pleistocene periods (Johnson *et al.*, 2006; Werdelin & Peigné, 2010).

Here, we analyzed four species of Pantherines that were available to us: *Panthera leo* (Linnaeus, 1758) (150–225 kg, Haas, Hayssen & Krausman, 2005), *P. onca* (Linnaeus, 1758) (78–95 kg, Seymour, 1989), *P. pardus* (Linnaeus, 1758) (28–65 kg, Estes, 2012), and *P. tigris* (130–300 kg, Mazák, 1981). *P. leo* was present in Africa from the Late Pliocene, in the Middle Pleistocene it migrated to Europe and by the Late Pleistocene had a wide intercontinental distribution (Antunes *et al.*, 2008). The *P. onca* fossil record is limited to the Pleistocene of North and South America. [Correction added on 2nd September 2016, after first online publication: The fossil record of *P. onca* has been amended to reflect that the record is limited to

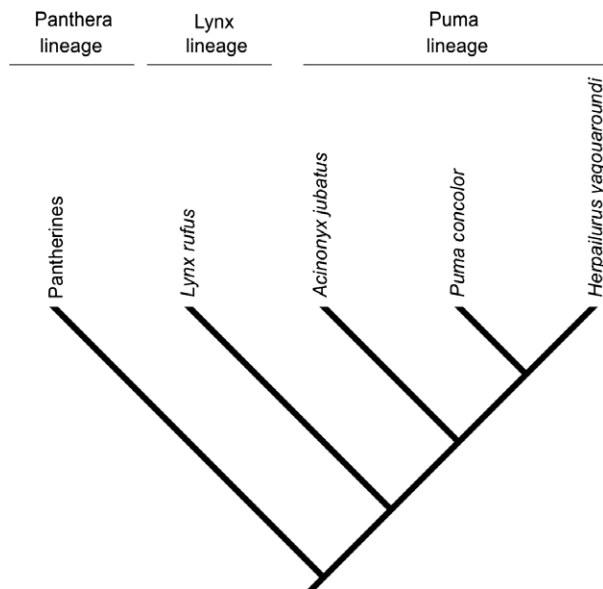


Figure 1. Cladogram of felid relationship based on Johnson *et al.*, 2006. This tree is pruned to indicate only species considered in this work.

the Pleistocene of North and South America]. Species of this clade could have migrated via Beringia to America during the early Pleistocene (Turner & Antón, 1997; Eizirik *et al.*, 2001). The earliest fossils for *P. pardus* were from Laetoli in Tanzania at about 3.5 Mya; in the Middle Pleistocene, the species was present in Africa and, earlier than the lion, in Europe (Uphyrkina *et al.*, 2001). The oldest fossils of *P. tigris*, of about 2 Mya, are from northern China and Java (Hemmer, 1987) and by the Late Pliocene and Early Pleistocene, tigers were widely distributed in eastern Asia (Luo *et al.*, 2004).

The phylogenetic relationships between Pantherines are contradictory, with *P. leo* and *P. pardus* being more frequently found as a sister group to the remaining species (e.g. Herrington, 1986; Janczewski *et al.*, 1995; Johnson *et al.*, 1996; Bininda-Emonds *et al.*, 1999, 2001; Jae-Heup *et al.*, 2001; Christiansen, 2008a; Davis *et al.*, 2010), although in some studies *P. leo* and *P. onca* (e.g. Johnson *et al.*, 2006) or *P. onca* and *P. tigris* (Mattern & McLennan, 2000) are sister species.

Previous studies have revealed morphological similarities and differences in crania among Pantherines (e.g. Pocock, 1917; Hemmer, 1978; Herrington, 1986; Salles, 1992; Christiansen, 2008b,c). Some studies refer to the osteological similarity between skulls of *P. leo* and *P. tigris* (e.g. Sunquist & Sunquist, 2002; Christiansen, 2008d; Sicuro & Oliveira, 2010; Sakamoto & Ruta, 2012) and others state that the *P. pardus* possess the most widely differing cranium of the Pantherines (Christiansen, 2008b,c). Previous studies on ontogeny in felids have been primarily descriptive, and focused on tooth eruption and age estimation (*Acinonyx jubatus*: Broom, 1949; Caro, 1994; *Caracal caracal*: Stuart & Stuart, 1985; *Felis silvestris*: García-Perea & Baquero, 1999; *Leopardus* spp.: Fagen & Wiley, 1978; *Leopardus wiedii*: Petersen & Petersen, 1978; *Lynx* spp.: García-Perea, 1996; *Lynx rufus*: Crowe, 1975; Tumilson & McDaniel, 1984; Jackson, Gluesing & Jacobson, 1988; Johnson, Brown & Bosomworth, 1981; *Prionailurus bengalensis*: Nakanishi *et al.*, 2009; *Puma concolor*: Biknevicius, 1996; Biknevicius & Leigh, 1997; Gay & Best, 1996; Laundré *et al.*, 2000; Shaw *et al.*, 2007). In addition some groups (Segura & Flores, 2009; Gianini *et al.*, 2010; Segura *et al.*, 2013; Segura, 2015) studied the ontogenetic changes and their functional consequences in *Acinonyx jubatus*, *Herpailurus yagouaroundi*, *Lynx rufus* and *Puma concolor*. The patterns of cranial ontogeny have been investigated in Pantherines using a descriptive approach that focused on general information about breeding in *P. tigris* (Sankhala, 1967), growth of body mass (in Pantherines: Carvalho, 1968; *P. leo*: Weilenmann, 1963; Smuts, Robinson & Whyte, 1980; *P. tigris*:

Weilenmann, 1963; Husain, 1966; Veselovsky, 1967; *P. onca*: Stehlik, 1971; Hunt, 1967; *P. pardus*: Weilenmann, 1963; Shukla *et al.*, 2003), and tooth eruption and age determination (in *P. leo*: Broom, 1949; Smuts, Anderson & Austin, 1978; *P. onca*: Stehlik, 1971; *P. pardus*: Broom, 1949; Stander, 1997; *P. tigris*: Veselovsky, 1967). The cranial ontogeny in Pantherines, however, has never been studied using a quantitative approach or in an evolutionary context.

The aim of our work was to study the cranial ontogeny of Pantherines, which share the lineage and evolutionary history, in order to elucidate whether their cranial shape is a product of size or phylogeny, and to compare these findings with available information about other members of the Order Carnivora.

MATERIALS AND METHODS

BACKGROUND INFORMATION

The four study species (*Panthera leo*, *P. onca*, *P. pardus*, and *P. tigris*) have similar gestation periods (88–112 days: Seymour, 1989; Haas *et al.*, 2005; Sunquist & Sunquist, 2009; Stein & Hayssen, 2013) and litter size (1–4 cubs: Mazák, 1981; Seymour, 1989; Sunquist & Sunquist, 2002; Haas *et al.*, 2005). The smallest weight at birth is that of *P. pardus* (300–700 g Hemmer, 1979), followed by *P. onca* (700–900 g, Seymour, 1989), *P. leo* (1000 g, Hemmer, 1979), and *P. tigris* (780–1600 g, Mazák, 1981). Weaning is precocious in *P. pardus* (at 4 months, Stein & Hayssen, 2013), followed by *P. onca* at 5–6 months (Seymour, 1989), *P. tigris* at 6 months (Mazák, 1981) and *P. leo* is the most delayed (at 7–9 months, Estes, 2012). The deciduous teeth start to emerge between 14 and 21 days in *P. tigris* (Mazák, 1981), 21 days in *P. leo* (Haas *et al.*, 2005), 21–29 days in *P. pardus* (Stein & Hayssen, 2013), and 26–30 days in *P. onca* (Sunquist & Sunquist, 2009). Sexual maturity is reached at 2–3 years of age in both sexes in *P. pardus* (Hunter & Hinde, 2005), 2–3 years in females and 3–4 years in males of *P. onca* (Seymour, 1989), 3–4 years in females and 4–5 years old in males of *P. tigris* (Mazák, 1981), and 3–4 years in females and 2 years in males of *P. leo* (Hunter & Hinde, 2005). Lifespan is between 10 and 12 years in *P. pardus* (Hunter & Hinde, 2005), 11 years in *P. onca* (Sunquist & Sunquist, 2002), 12 years in *P. leo* (Haas *et al.*, 2005) and 17 years in *P. tigris* (Gittleman, 1986).

SAMPLE

The sample consisted of 370 specimens of four species of the *Panthera* lineage, and comprised juveniles

Table 1. Sample proportions

Age classes/ Species	<i>Panthera</i> <i>leo</i>	<i>Panthera</i> <i>onca</i>	<i>Panthera</i> <i>pardus</i>	<i>Panthera</i> <i>tigris</i>
N	5/8/4	0/0/1	0/1/0	0/0/2
B	0/3/5	2/0/0	1/3/3	5/4/5
J1	0/12/11	2/4/2	7/3/7	0/0/1
J2	0/1/3	1/2/0	3/3/0	0/1/0
J3	4/3/2	1/3/2	2/0/2	0/1/0
J4	1/6/1	0/1/0	3/0/3	0/0/1
A1	5/6/0	4/7/20	2/2/7	5/0/4
A2	6/5/4	6/16/37	6/10/9	13/7/8
A3	1/2/2	0/4/7	1/1/0	4/8/0

N, newborns; B, babies; J, juveniles; A, adults; estimated by dental eruption and tooth wear, and described by Segura (2015).

The numbers indicate females, males, and unsexed specimens, respectively.

and adults of different age classes (N: newborns, B: babies, J: juveniles, A: adults), estimated by dental eruption and tooth wear (Segura, 2015; Table 1). The material belongs to the mammal collections of the American Museum of Natural History (AMNH, New York, USA), Colección Félix de Azara (CFA, Buenos Aires, Argentina), Colección Mamíferos Lillo (CML, Tucumán, Argentina), Field Museum of Natural History (FMNH, Chicago, USA), Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN, Buenos Aires, Argentina), Museo de La Plata (MLP, La Plata, Argentina), National Museum of Natural History, Smithsonian Institution (NMNH, Washington, DC, USA) (Appendix 1).

LANDMARKS

Thirty-eight landmarks were digitized in three dimensions to describe the postnatal cranial ontogeny, using a Microscribe MX6DOF System (GoMeasured3D, Amherst, VA, USA). These landmarks were types 1 and 2, *sensu* Bookstein (1991) (Fig. 2). Only one-half of the cranium was digitized. The configuration was reflected in the plane of symmetry defined by sagittal landmarks, improving visualization and avoiding putative Procrustes alignment artifacts by using R-function AMP.r written by Annat Haber, University of Chicago (available online at http://www.tau.ac.il/~annat22/Annat_Haabr/R_scripts.html, also see Cassini & Vizcaíno, 2012).

DATA ANALYSIS

Both males and females were pooled in the same sample. A generalized Procrustes analysis (GPA: Goodall, 1991; Rohlf, 1999) was performed to

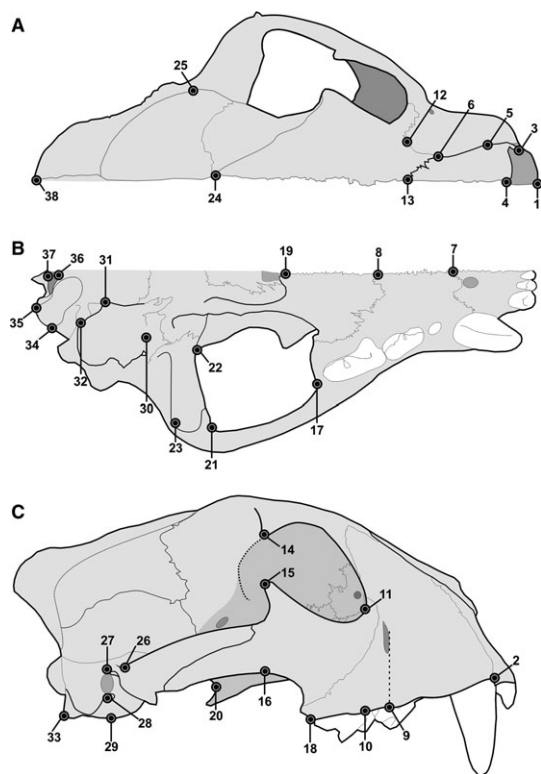


Figure 2. Placements of landmarks for dorsal and ventral (A), and lateral (B) views. Tip of premaxilla in the *sutura interincisiva* (1); frontal point in the *sutura incisivomaxillaris* at level of dentary row (2); tip of nasal process (3); anterior point of the nasals in the *sutura internasalis* (4); anterior contact of *sutura nasomaxillaris* (5); posterior contact of *sutura nasomaxillaris* (6); midline in *sutura incisivomaxillaris* (7); midline in *sutura palatomaxillaris* (8); point below the infraorbital foramen at level of dentary row (9); point below the lacrimal foramen at level of dentary row (10); lacrimal foramen (11); apex of *sutura frontomaxillaris* (12); midline of *sutura frontonasalis* (13); tip of the supraorbital process (14); tip of the infraorbital process (15); point below the infraorbital process in the zygomatic process of jugal (16); anterior point of temporal fossa (17); posterior point of dentary row (18); posterior point of palatine torus (19); point in the *sutura pterygopalatina* (20); internal edge of temporal fossa (21); external edge of temporal fossa (22); tip of postglenoid process (23); Intersection between *sutura coronalis*, *sutura sagittalis*, and *sutura interfrontalis* (24); apex of the braincase (25); tip of mastoid process (26); superior point of external auditory meatus (27); inferior point of external auditory meatus (28); apex of tympanic bulla (29); anterior point of tympanic bulla (30); internal point of tympanic bulla (31); posterior point of tympanic bulla (32); tip of paracondylar process (33); external apex of occipital condyle (34); internal apex of occipital condyle (35); inferior point in the foramen magnum (36); superior point in the foramen magnum (37); point in the inion (38).

superimpose the landmark configurations and remove the spatial variation that does not correspond to form (Dryden & Mardia, 1998). This analysis minimizes the sum of squared distances between homologous landmarks by translating, rotating, and scaling them to best fit. A principal component analysis (PCA) was performed for the four species along of all age classes to identify the major components of variation. This analysis allows the visualization of cranial shape changes, along the components of interest. A multivariate regression of the Procrustes coordinates against the log-transformed centroid size was performed to investigate how allometric variation in shape is associated with size (Monteiro, 1999; Drake & Klingenberg, 2008). The significance of regressions was tested using a permutation test with 10 000 resamples (Bookstein, 1991; Mitteroecker *et al.*, 2004). The angles between the corresponding regression vectors were computed as the arccosines of the inner products between the regression vectors in order to compare the regressions of the four species (Drake & Klingenberg, 2008; Klingenberg & Marugán-Lobón, 2013). The angle comparison allows us to compare the regression vectors which resume the shape change along the ontogeny. When these angles are close to zero then both regressions are similar and consequently this species shares a similar shape change along the ontogeny.

To evaluate whether the adult cranial shape can be explained by allometric scaling (i.e., a shift in their ontogenetic trajectory), a group-centered multivariate regression of the whole sample (all ontogenetic series) was performed. Then, the vector of regression shape change was applied to the adults subset (which has its own Procrustes space) to compute the shape residuals, as a way to correct for ontogenetic scaling. These shape residuals were analyzed via a canonical variate analysis (CVA) using species as grouping category. We use CVA instead of PCA because it finds the shape features that best distinguish among multiple groups of specimens. Then, those species whose adult cranial shape could be explained by a shift in their ontogenetic trajectory from a second species are expected to be indistinguishable from each other.

All the analyses described above were performed with MorphoJ 1.05a software (Klingenberg, 2011).

Procrustes coordinates data, obtained in the Procrustes superposition, were used as an index of shape change (e.g. Tanner *et al.*, 2010; Segura & Prevosti, 2012), by calculating Procrustes distance (PD) as the square root of the sum of the squared distances between each landmark of one specimen and the mean configuration of the smallest age class. In addition, Centroid size (CS) was used as an estimate of cranial size (Bookstein, 1991; Zelditch *et al.*,

2004). These estimators were calculated with R 2.9.2 software (R Development Core Team 2004) and were used to determine the age class at which the final adult cranial size (CS) and shape (PD) were reached.

Previous studies have suggested the existence of sexual size dimorphism for Pantherines (Seymour, 1989; Sunquist & Sunquist, 2002; Mazák, 2004). For this reason, we tested for dimorphism in the sample; in the case of allometric variation, both males and females clearly exhibited the same ontogenetic trajectory, with non-significant slope or intercept differences in the regression analyses, indicating that the observed allometric pattern was not biased by sexual dimorphism. We also tested for the presence of sexual size dimorphism in the skull (CS) and sexual shape dimorphism (PD) (Table S1), using the Mann–Whitney *U*-test (Zar, 1999) with the software PAST version 1.98 (Hammer, Harper & Ryan, 2001).

RESULTS

PC1 explained 39.88% of the total variation (Fig. 3) and showed information related to ontogeny, with N and B age classes followed by the remaining

juveniles progressively disposed from the negative to the positive side. The three classes of adults (A1, A2, and A3) were located at positive values and exhibited overlap between them. The configuration of the juvenile cranium was relatively rounded, with broad and short rostrum, palate and nasal bones, wide frontal bones, no postorbital constriction and coronal suture in a more forward position. Moreover, the cranium had unexpanded and weak zygomatic arches, broad bullas, large orbits, occipital plate with low height and foramen magnum at the same level as the inion. The adult cranium was relatively elongated and narrow, with long and thin rostrum, and palate, wide nasal process of nasal bones, narrow and long frontal bones, with postorbital constriction and coronal suture in a more backward position. In addition, the adult cranium had broad and strong zygomatic arches, small bullas and orbits, tall inion that forms a straight line with landmark 24 (intersection between *sutura coronalis*, *sagittalis*, and *interfrontalis*), and foramen magnum in anterior position in relation to inion (Fig. 2).

PC2 explained 8.93% of the total variation (Fig. 3) and showed different species of Pantherines sequentially organized in the morphospace. With the

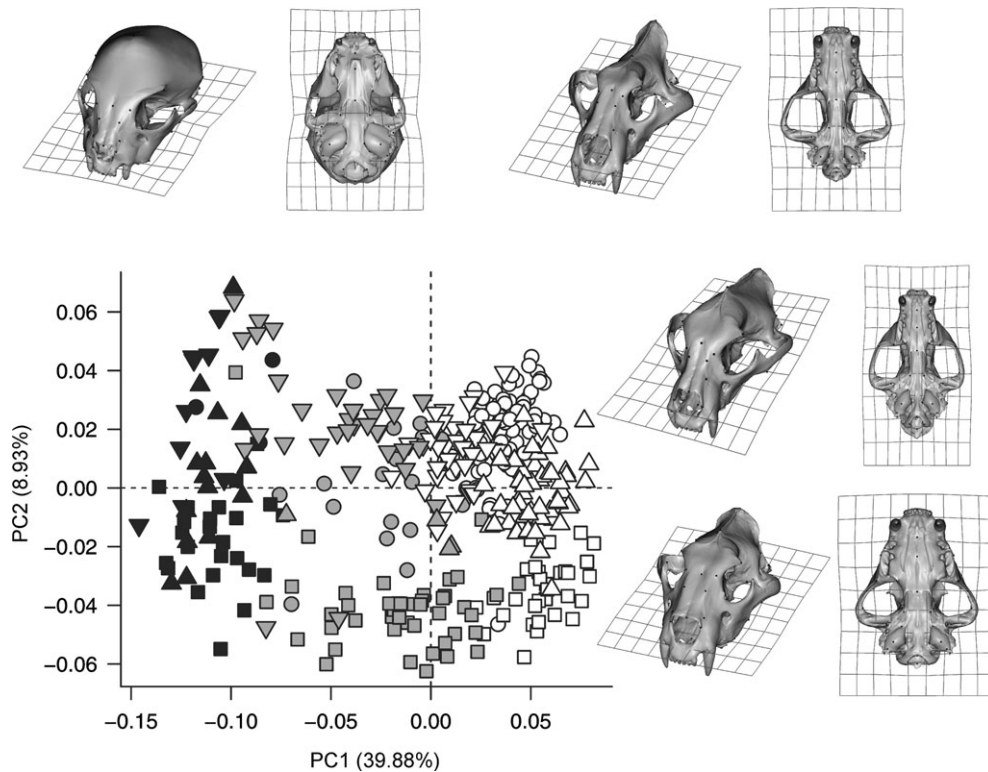


Figure 3. Plot of the results of principal components 1 and 2 for the four species. Squares represent *Panthera leo* specimens, triangles represent *Panthera tigris* specimens, circles represent *Panthera onca* specimens, and inverted triangles represent *Panthera pardus* specimens. Colour key for symbols: black, Newborn and Baby classes; grey, juvenile classes; white, adult classes.

exception of some new born and juveniles, most *Panthera leo* specimens were placed at negative values, whereas *P. tigris*, *P. onca* and *P. pardus* were placed in a more overlapped position, at positive values. At negative values (where *P. leo* was placed), the cranium was flattened, short and wide, with broad and long rostrum and nasal bones, and coronal suture in a more posterior position; the posterior part of the cranium (parietal) seems to be shorter than the anterior part (frontal). In addition, this configuration had well developed zygomatic arches, orbits in a more posterior position, with supraorbital and infraorbital processes close each other, and tall inion at the same level as foramen magnum. At positive values, the cranium was narrow and long, with short and narrow rostrum and nasal bones, and coronal suture in a more forward position. This configuration had also less expanded zygomatic arches, orbits in a more anterior position, with supraorbital and infraorbital processes more distant to each other, and an inion that protrudes into the foramen magnum. In addition, in the morphospace depicted by both components (PC1 and 2) the ontogenetic trajectories seems to follow a semicircular shape.

Size in *Panthera leo* explained 46.88%, in *P. tigris* 52.52%, in *P. onca* 21.32%, and in *P. pardus* 38.05% of shape variation ($P < 0.00001$). For the four species, overall smaller forms (younger specimens) showed a rounded cranium, tall braincase, a short rostrum, and a short inion at the same level as the foramen magnum, and larger forms showed a narrow and flat braincase and a long rostrum (Figs 4A and B, 5A and B). In addition, particularly in *P. onca* (Fig. 5A), smaller forms showed less developed zygomatic arches, whereas larger forms showed more developed (stronger, higher) zygomatic arches. In *P. pardus* (Fig. 5B) the coronal suture was in a more forward position in smaller forms and in a more backward position in larger forms. In *P. tigris* (Fig. 4B) the braincase was wide and the inion was more posterior than foramen magnum. The similarity of shape change associated with size was observed in the angles between the regression vectors (Table 2), with *P. pardus* having greater differences than the remaining species ($\sim 150^\circ$).

The CVA performed on residuals of a subset composed of adults, corrected by ontogenetic scaling, showed three canonical axes ($n - 1$ groups), with the first two explaining $\sim 82\%$ of the group variation. All pairs of group's mean of the PDs were significantly different from zero after 10 000 rounds of the permutation test. The lowest value was obtained for the pair composed of *P. tigris* and *P. leo* (0.0488), whereas the largest value was obtained for *P. leo* and *P. pardus* (0.1132). The shape change in the CVA ordination space associate the CV1 (45.21%)

with a low and flat cranium with elongated rostrum, small orbits, laterally expanded zygomatic arches and narrow braincase at negative values. The positive values associate the CV1 with a rounded and high cranium, with a relative shorter and narrower rostrum, anteriorly oriented orbits and a wider braincase. Similarly, the ordination in CV2 (36.52%) relates negative values with a similar cranium of CV1 negative values with narrower frontal bones. The positive values are related to a rounded cranium, a short and narrower rostrum, less expanded zygomatic arches, larger orbits and wide frontal bones (Fig. 6). This ordination morphospace allows us to visualize the variation among groups. *P. tigris* and *P. leo* showed the lowest variation, as they are clustered together in two adjacent point clouds in the double-negative quadrant. In contrast, *P. onca* and *P. pardus* occupied their own morphospace, with *P. pardus* being the most distant of all the Pantherines (Fig. 6).

Centroid size values in *P. leo* (Fig. 7A) and *P. tigris* (Fig. 7B) gradually increased during ontogeny, showing an asymptote from J2 to J4 classes. Then, the increase remained constant up to the A3 class, in which the final size of cranium was obtained. CS values in *P. onca* (Fig. 7C) rapidly increased from N to J3 classes, and then showed an asymptote from J3 to J4. Finally, the increase was constant up to the A3 class, in which the final size of cranium was obtained. CS values in *P. pardus* (Fig. 7D) rapidly increased up to the A1 class, in which the final size was obtained.

Procrustes distance values in *P. leo* (Fig. 8A) increased constantly from N to A3 classes, where the final shape of cranium was reached. PD values in *P. tigris* (Fig. 8B) increased rapidly from the N to B classes. Then, the increase was constant up to the A2 class, in which the final shape was obtained. PD values in *P. onca* (Fig. 8C) and *P. pardus* (Fig. 8D) increased rapidly from N to B classes. Then, the increase was constant from B to A3 classes (in *P. onca*, Fig. 8C) up to A1 classes (in *P. pardus*, Fig. 8D), where the final shape of cranium was reached.

DISCUSSION

CRANIAL ONTOGENY IN *PANTHERA* LINEAGE

Our results suggest differences in the ontogenetic trajectories in *Panthera*; juvenile cranium (N and B classes) of the four species are similar, but juveniles (J classes) and adult cranium are not uniform, because the four species have some differences in shape. *Panthera leo* and *P. tigris* showed more similar ontogenetic trajectories between each other than

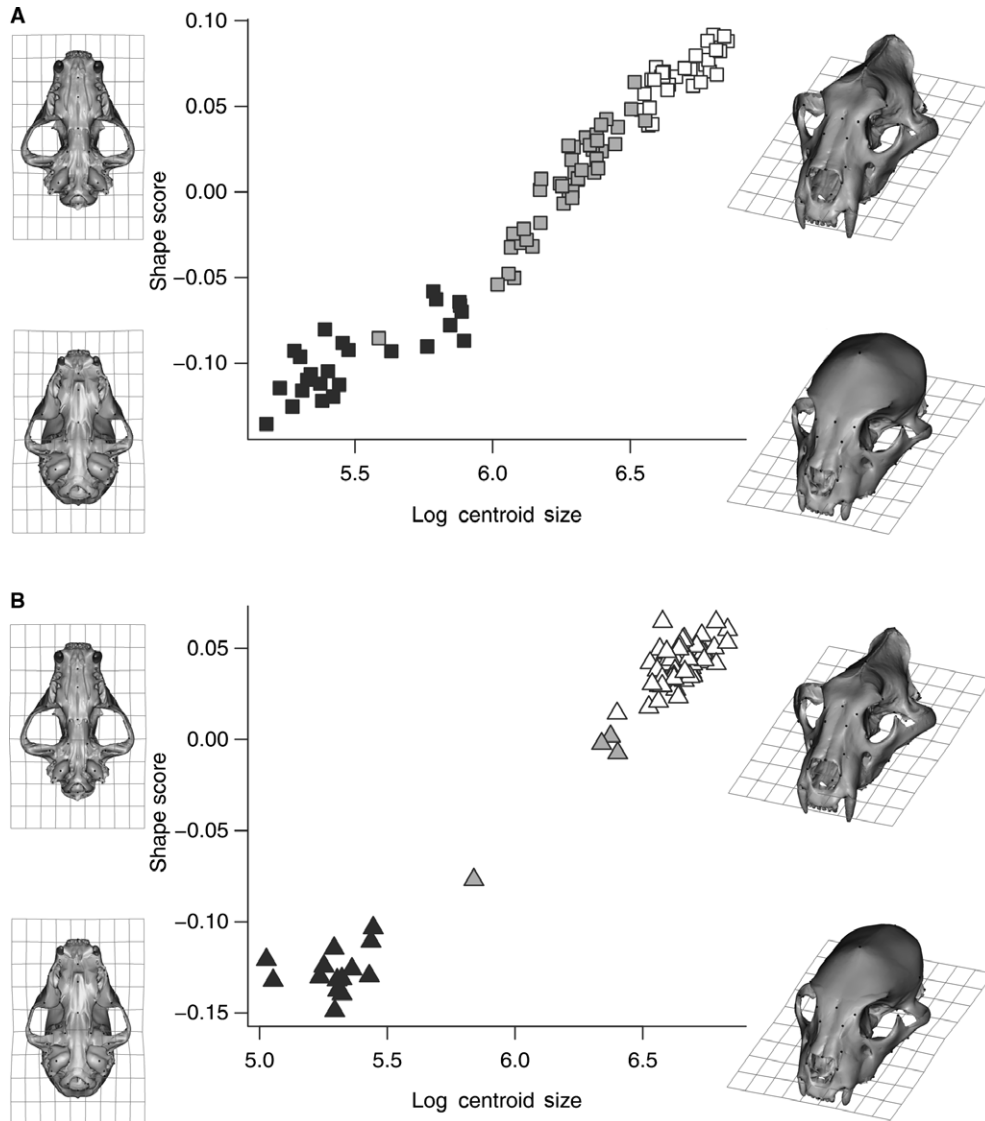


Figure 4. Individual analysis of multivariate regression of the Procrustes coordinates against the log-transformed centroid size for all age classes for *Panthera leo* (A) and *Panthera tigris* (B). Colour key for symbols: black, Newborn and Baby classes; grey, juvenile classes; white, adult classes. Thin plate spline gridlines exaggerated three times.

between other species (Fig. 3, Table 2), sharing adult crania with wider rostrum, shorter basicranium and a more vertical occipital plate. Christiansen (2008d) found differences between *P. leo* and *P. tigris*, *P. tigris* was progressively longer with increase in skull size, and with longer nasal bones than *P. leo*. We did not find these differences, however. Our CVA results show that the cranial configuration of *P. leo* is a scaled version of that of *P. tigris* (Fig. 6). Conversely, skulls of adults of *P. pardus* show the most widely differing patterns, with a rounded braincase and less expanded zygomatic arches, whereas *P. onca* occupies an intermediate position between these patterns.

The similarity in shape between *P. leo* and *P. tigris* was not a reflection of the relationship within the phylogeny reported to date, which placed *P. leo* as sister group of *P. onca* (Johnson *et al.*, 2006) or *P. pardus* (Herrington, 1986; Janczewski *et al.*, 1995; Johnson *et al.*, 1996; Bininda-Emonds *et al.*, 1999, 2001; Jae-Heup *et al.*, 2001; Christiansen, 2008a; Davis *et al.*, 2010). The similarity is probably related to their body size, which is sensibly larger than in the rest of the Pantherines (*P. leo*: 150–225 kg and *P. tigris*: 130–300 kg, Haas *et al.*, 2005; Mazák, 1981). Likewise, *P. pardus* is the smallest of Pantherines (28–65 kg, Estes, 2012), whereas *P. onca* is 78–95 kg (Seymour, 1989). Previous studies on

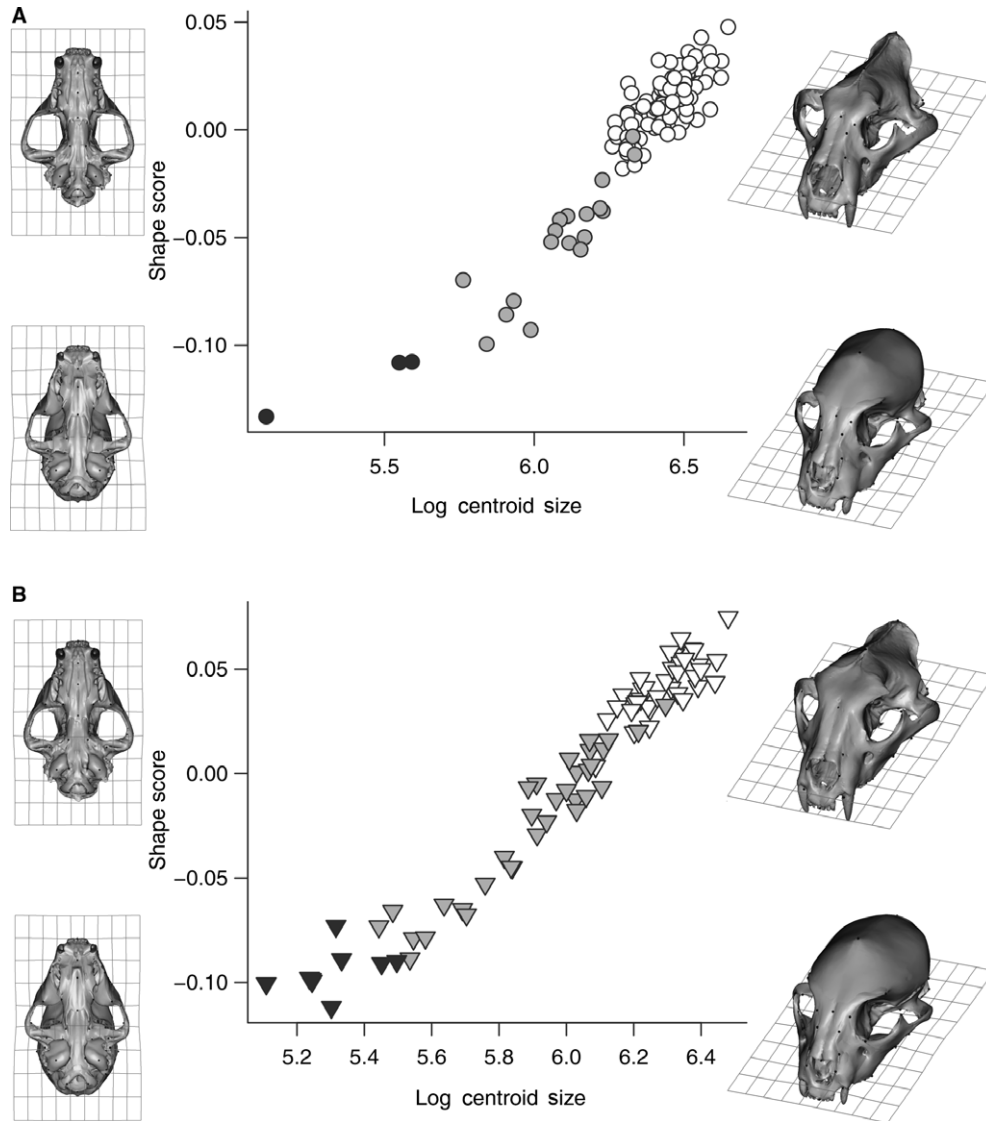


Figure 5. Individual analysis of multivariate regression of the Procrustes coordinates against the log-transformed centroid size for all age classes for *Panthera onca* (A) and *Panthera pardus* (B). Colour key for symbols: black, Newborn and Baby classes; grey, juvenile classes; white, adult classes. Thin plate spline gridlines exaggerated three times.

Table 2. Angular comparison of regression vectors

	<i>Panthera leo</i>	<i>Panthera onca</i>	<i>Panthera pardus</i>	<i>Panthera tigris</i>
<i>Panthera leo</i>	–	31.98° $P < 0.00001$	157.49° $P = 1$	17.40° $P < 0.00001$
<i>Panthera onca</i>	31.98° $P < 0.00001$	–	151.12° $P = 1$	30.72° $P < 0.00001$
<i>Panthera pardus</i>	157.49° $P = 1$	151.12° $P = 1$	–	159.05° $P = 1$
<i>Panthera tigris</i>	17.40° $P < 0.00001$	30.72° $P < 0.00001$	159.05° $P = 1$	–

cranial morphology of felids (Werdelin, 1983; Morales & Giannini, 2010, 2014; Sicuro, 2011; Segura *et al.*, 2013) also reported a stronger relationship between shape and size than between shape and phylogeny.

Previous works have shown the morphological similarity of adult skulls of *P. leo* and *P. tigris* (Sunquist & Sunquist, 2002; Sicuro & Oliveira, 2010; Sakamoto & Ruta, 2012) and that *P. pardus* is more different

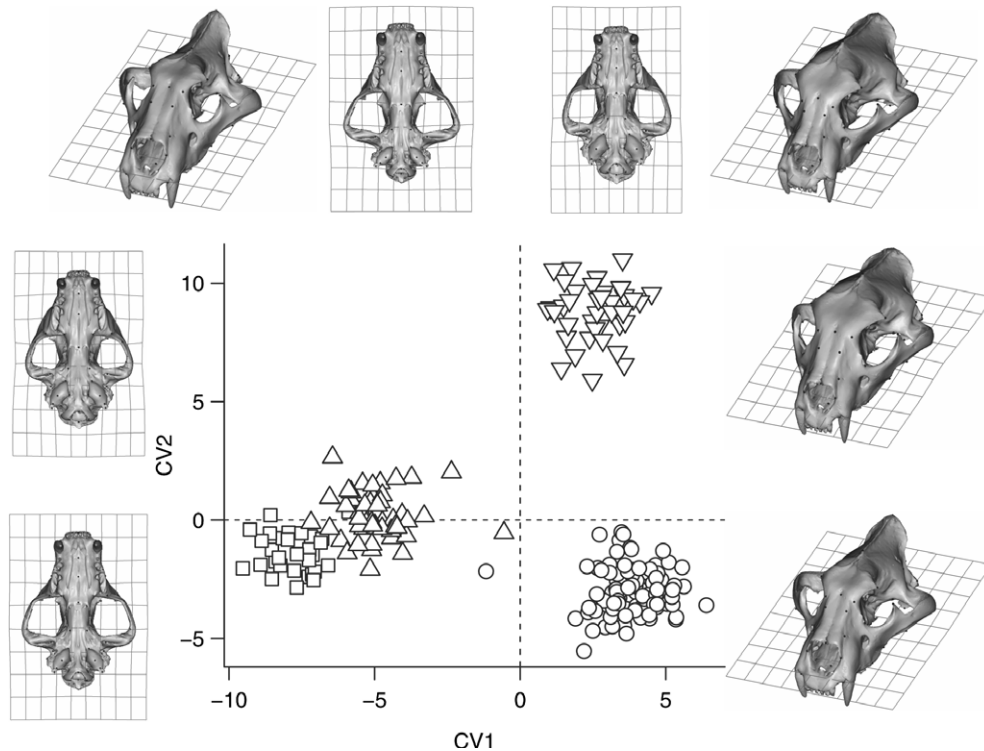


Figure 6. Plot of the results of canonical variate analysis, axes 1 and 2 for all the adult residuals of four species. Circles represent *Panthera leo* specimens, triangles represent *Panthera tigris* specimens, squares represent *Panthera onca* specimens, and inverted triangles represent *Panthera pardus* specimens.

(Christiansen, 2008b,c). Our results agree with these findings and show that these similarities and differences derive from juvenile age classes (J). Taking into account that the gestation period is similar among Pantherines, *P. pardus* is the species with the smallest birth weight and lowest growth rate (Fig. 9). This finding is consistent with the pattern observed in *P. pardus*, reaching final size and shape before than the remaining Pantherines (in A1 and A2 classes respectively, Figs. 7D, 8D, 10), and shows that they need less time to grow because they grow less than the other Pantherines. The opposite situation was observed in *P. leo* and *P. tigris*, which are born larger, show a higher growth rate and reach final size and shape later (Figs. 7A and B, 8A and B, 10). The difference in size between *P. pardus* and the remaining Pantherines start to increase at about 50 days (Fig. 9), before weaning in the Pantherines (Husain, 1966; Stehlik, 1971; Smuts *et al.*, 1980; Shukla *et al.*, 2003). Even when size begins to differentiate early in the lineage (Fig. 9), the final size and shape was obtained after weaning, when felids have their definitive hypercarnivore diet (Figs. 7, 8 and 10). Moreover, they reached final shape and size after sexual maturity (Figs. 7, 8 and 10).

Size also explains a high percentage of shape variation (allometric scaling) during ontogeny in *P. leo* and *P. tigris* (regression results, Fig. 4). *P. onca* and *P. pardus* showed a low percentage of shape variation during ontogeny, meaning that there is lower difference in shape (associated with size) between young and adult skulls than that observed in the remaining Pantherines. The small change observed in *P. pardus* is more similar to the change that occurred in *P. onca*, and was previously reported by Christiansen (2008b). When Pantherines were analyzed with other small- and medium-sized felids, they appear integrated and show particular characteristics such as large skull, long rostrum, large temporalis and high bite force (Morales & Giannini, 2014). It is clear that this morphotype, called snouted/massive headed cats by Sicuro (2011) co-evolved with the body size differentiation. Thus, although they have different ontogenetic trajectories, the adults of the Pantherines converge in shape, in relation to size.

Different body size involves different size of prey and different hunting methods: *Panthera leo* is a generalist hunter that consumes a great variety of vertebrates, with preference for larger ungulates, and a modal prey size of about 150 kg (Packer, 1986;

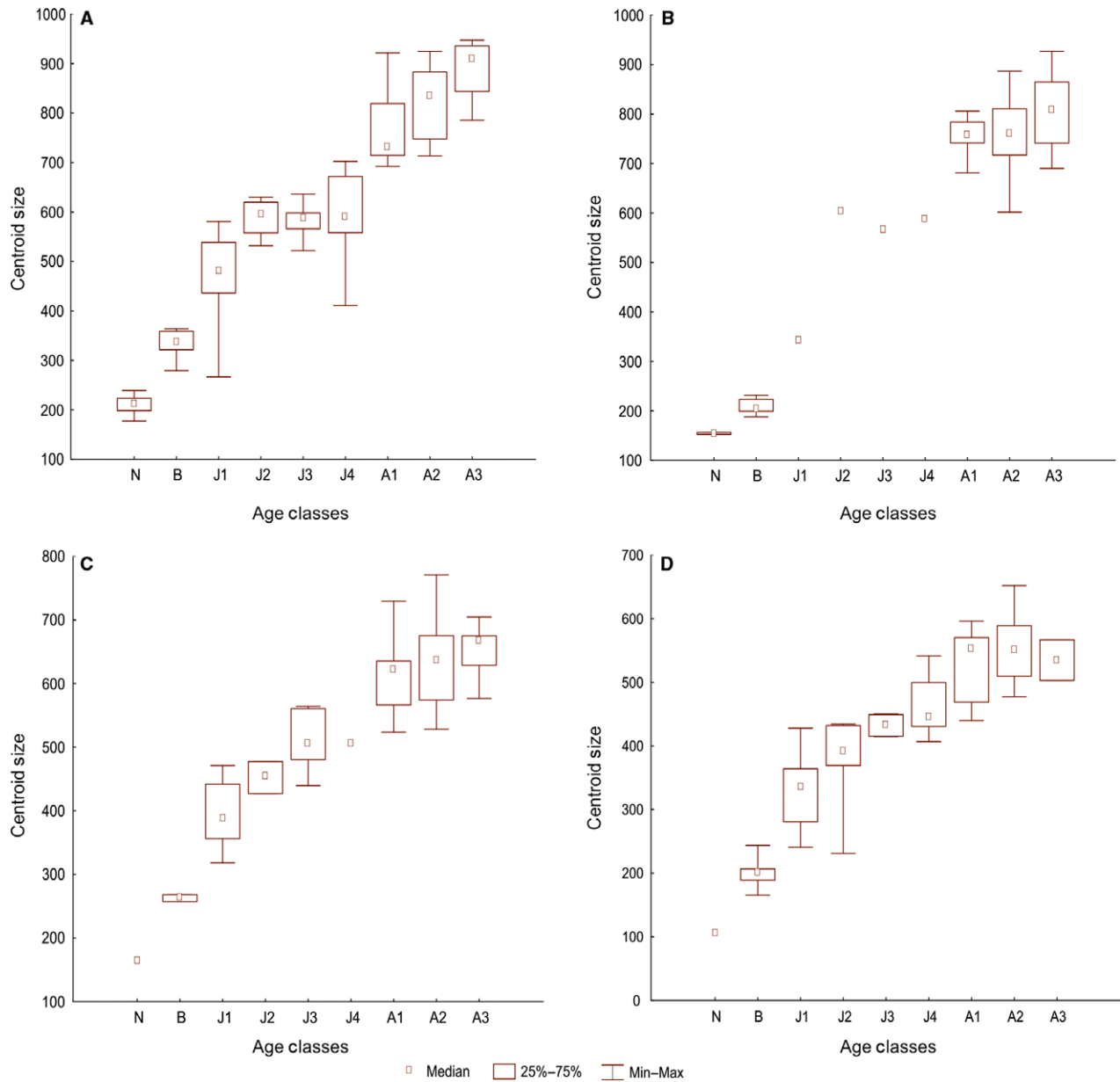


Figure 7. Box plots of centroid size versus age classes for *Panthera leo* (A) *Panthera tigris* (B), *Panthera onca* (C), and *Panthera pardus* (D). N is newborn, B is baby, J is juvenile, and A is adult.

Estes, 2012). They usually hunt in groups and cooperation allows them to subdue prey of about four times their weight (Estes, 2012). In *P. tigris* the main prey is also a large ungulate, but this species is a solitary hunter (Ewer, 1973). *P. onca* is an opportunistic solitary predator, capable of killing almost any prey encountered, which weigh three to four times its own weight; this species catches the prey with a bite to the back of the skull, rather than the more common neck or throat bite employed by other Pantherines (Sunquist & Sunquist, 2009).

P. pardus is a solitary hunter that has a wider range of different prey species (of about 10–40 kg) (Kitchener, 1991). The diet is generally dominated by medium-sized ungulates (Bailey, 1993; Hayward *et al.*, 2006; Owen & Mills, 2008). However, when ungulate prey is scarce, *P. pardus* switches to smaller bodied prey (Nowell & Jackson, 1996; Hayward *et al.*, 2006), a similar behavior reported for *Puma concolor* (Linnaeus, 1771) (Iriarte *et al.*, 1990). Different prey size could involve different functional requirements; for example, smaller prey require less bite force, effort

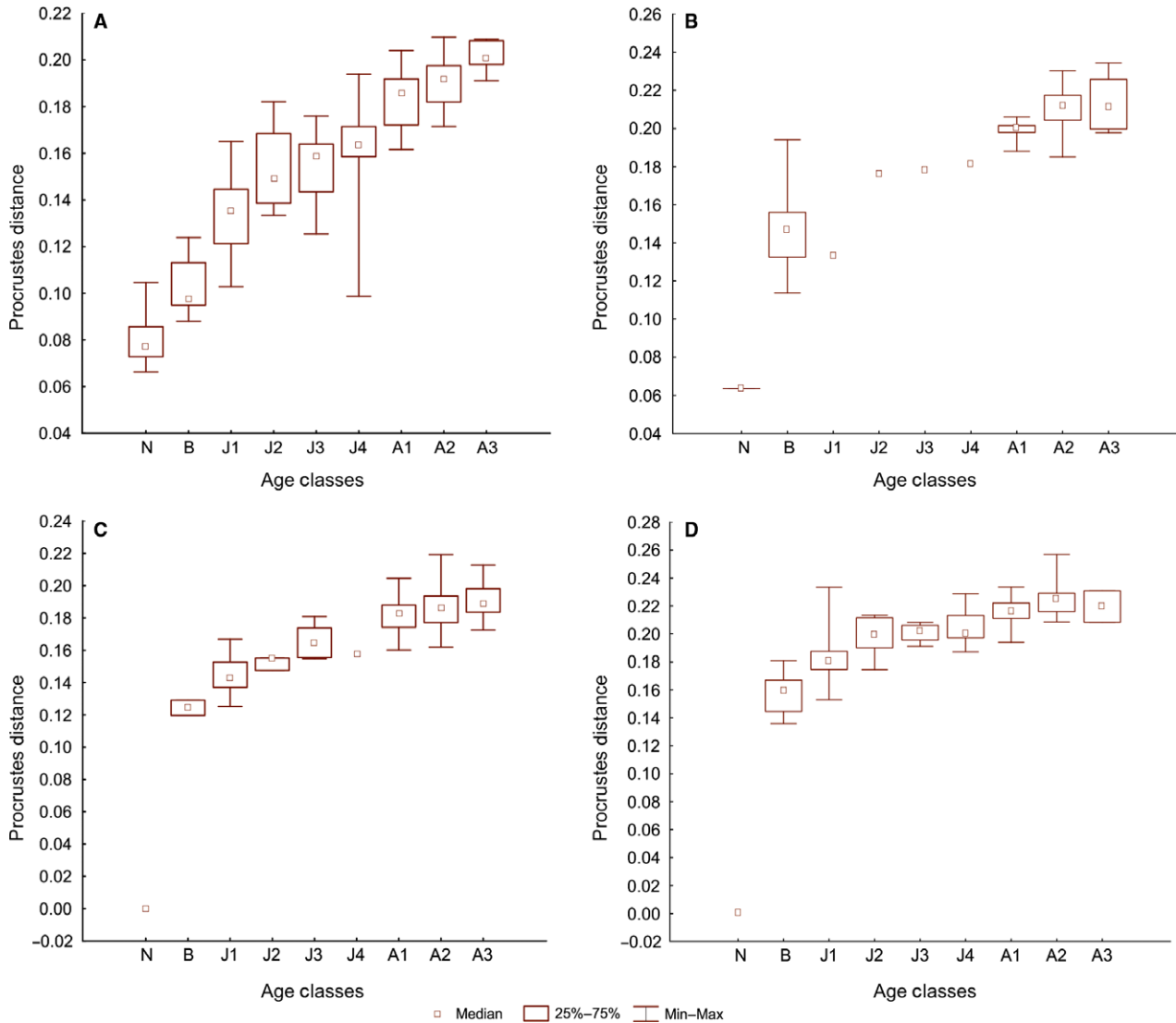


Figure 8. Box plots of Procrustes distance versus age classes for *Panthera leo* (A) *Panthera tigris* (B), *Panthera onca* (C), and *Panthera pardus* (D). N is newborn, B is baby, J is juvenile, and A is adult.

and muscular commitment to be captured, killed and processed than large prey (Wroe, McHenry & Thomason, 2005; Piras *et al.*, 2013). Variation in prey size increases the food resources available to the predator and the areas where it can survive (Estes, 2012). Moreover, shape changes along with size in a compensatory manner, allowing these predators to achieve the same functions or to improve the capacity, for example, to process larger prey (Emerson & Bramble, 1993).

It has been previously reported that *P. pardus* competes for food with *P. leo*, *P. tigris*, *Crocuta crocuta* (Erxleben, 1777), and *Lycaon pictus* (Temminck, 1820) (Hayward & Kerley, 2008; Stein & Hayssen, 2013). To avoid attacks from potential

predators, *P. pardus* tends to hunt at different times of the day and to avoid areas where potential predators are most abundant (Hayward & Kerley, 2008; Stein & Hayssen, 2013). Under high competition for large prey items, *P. pardus* tends to select smaller prey, which reduces interspecific competition (Karanth & Sunquist, 1995, 2000). The broad diet variety of *P. pardus* seems to be an indicator of the presence of these competitors (Stein & Hayssen, 2013). Accordingly, any trait that allows species to exploit different, novel or otherwise not exploitable resources will be under strong selection (Dayan & Simberloff, 1998; Pfennig & Pfennig, 2009), and probably the smaller body size and cranial shape of *P. pardus* is related to this pressure. Moreover, the

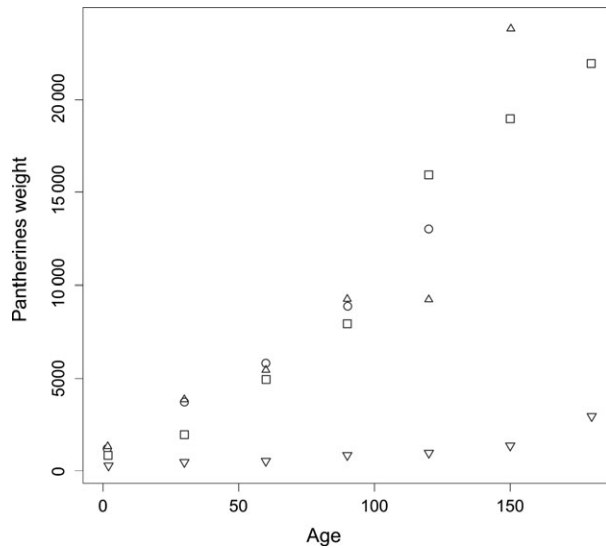


Figure 9. Growth of body size in Pantherines taken from literature: Veselovsky, 1967; Stehlik, 1971; Smuts *et al.*, 1980; Shukla *et al.*, 2003. Plot age in days vs. weight in grams. Inverted triangles represent *Panthera leo* specimens, triangles represent *Panthera tigris* specimens, squares represent *Panthera onca* specimens, and circles represent *Panthera pardus* specimens.

variation in body size is a rapid mode of differentiating niches in a young group such as felids (Late Miocene) (Gittleman, 1985; Piras *et al.*, 2013), and it is important for larger carnivores, for which prey is difficult to partition except by size (Van Valkenburgh & Wayne, 1994).

In *P. pardus* the evolutionary tendency could be size reduction; therefore, this tendency could be considered paedomorphic in relation to individuals' size and cranial configuration. Accordingly, paedomorphosis could be produced by a post-displacement (delayed onset of growth) or by neoteny or deceleration (reduced rate of growth) (Reilly, Wiley & Meinhardt, 1997; Klingenberg, 1998; McNamara, 2012). However, it is also possible that the evolutionary tendency in *P. leo* and *P. tigris* was an increase in size and, in this case, they could be considered peramorphic. Peramorphosis could be produced by a pre-displacement (earlier onset of growth) or by acceleration (increased rate of growth) (Reilly *et al.*, 1997; Klingenberg, 1998; McNamara, 2012) (Fig. 10).

CORRELATION BETWEEN FORM AND FUNCTION

Following Mitteroecker *et al.* (2013) in the classic concept of allometry, a trait is considered as negatively (positively) allometric if it increases less (more) in size than other traits or overall size do. In

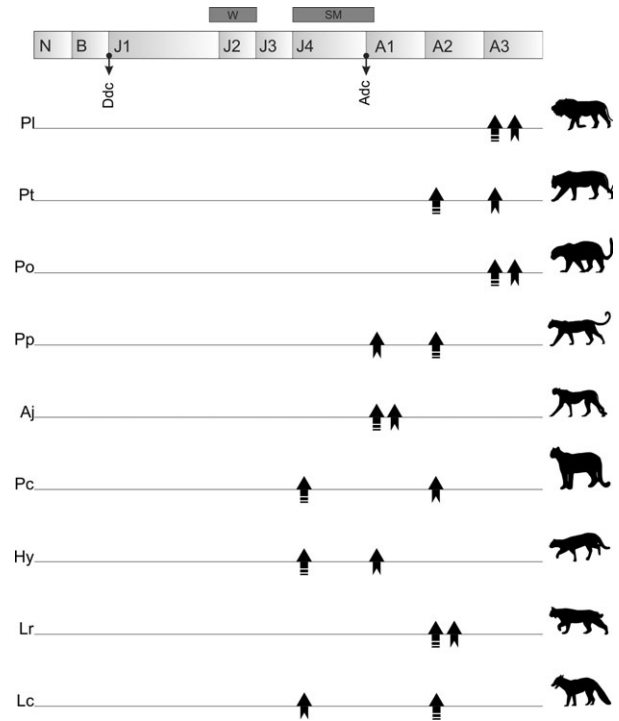


Figure 10. Timeline illustrating the age classes in relation to life-history events for *Panthera leo* (Pl), *Panthera tigris* (Pt), *Panthera onca* (Po), *Panthera pardus* (Pp), *Acinonyx jubatus* (Aj), *Puma concolor* (Pc), *Herpailurus yagouaroundi* (Hy), *Lynx rufus* (Lr), *Lyncalopex culpaeus* (Lc). W is weaning period, SM is sexual maturity period, Ddc is deciduous dentition complete, and Adc is adult dentition complete. Cranial size noted by solid arrow, cranial shape noted by broken arrows.

contrast, in geometric morphometrics positive or negative allometry cannot be inferred from single shape coordinates because they are shape variables not size variables. Instead all shape coordinates must be visualized together in order to characterize the relative decrease or increase in size of specific parts described by the shape change vector (i.e., negative and positive allometry respectively). Therefore, the changes observed in the crania of Pantherines imply the negative allometry of neurocranium (braincase, orbits and auditory bulla) and positive allometry of splanchnocranium (rostrum, palate). These patterns were previously recognized and reported in felids using different methodologies (Biknevicius & Leigh, 1997; Segura & Flores, 2009; Slater & Van Valkenburgh, 2009; Giannini *et al.*, 2010; Prevosti, Turazzini & Chemisquy, 2010; Segura *et al.*, 2013; Segura, 2015) and seem to be plesiomorphic to mammals (Emerson & Bramble, 1993).

Changes in size can result in a relative loss of function, unless there are compensatory adjustments

in shape (Emerson & Bramble, 1993). Accordingly, the accelerated morphogenesis of the central nervous system, which induces the formation of the membranous bone of the braincase, produces rounded crania of Pantherines juveniles, with taller, shorter and broader braincase, weaker and unexpanded zygomatic arches, smaller temporal fossa, and large sensory capsules (orbits and bulla) (Smith, 1997; Byron, 2006; Rice, 2008). Moreover, the palate is broader and shorter in juveniles than in adults. This condition is probably related to the function of the palate as a platform on the tongue during lactation (German & Crompton, 1996; Smith, 1997).

These juvenile crania undergo drastic modifications to reach the configuration of adult cranium, in which the predator characteristics are emphasized (Figs. 3, 4 and 5), such as the rostrum scales with positive allometry. Other examples are the expansion of zygomatic arches, along with the narrowness of the braincase, providing a broader temporal fossa, a larger space to be occupied by masticatory muscles, such as masseter and temporal muscles (Radinsky, 1981). The increase in the temporalis (the most important masticatory muscle in carnivores), and its origin in a most posterior placement in the cranium, imply a more powerful action when the adult Pantherines catch and kill the prey (García-Perea, 1996). The reorganization of the occipital plate, which includes posterior part of sagittal crest and dorsal part of nuchal crest, provides a greater surface for attachment of larger muscles, both masticatory and cervical (Wickland, Baker & Paterson, 1991; Richmond, Thomson & Loeb, 1992; Evans, 1993; Duckler, 1998; Antón *et al.*, 2004). In this sample, the reorganization was represented by changes in the position of the inion and foramen magnum (Fig. 3).

COMPARISON WITH OTHER CARNIVORES

Panthera leo and *P. onca* obtained their final cranial shape and size in a synchronous and delayed way, in A3 class, when weaning and sexual maturity has been reached (Figs. 7, 8 and 10). This pattern is more protracted and delayed than in the remaining Pantherines (*P. tigris*, *P. pardus*) and even in other felids studied to date, such as *Puma concolor* (A2, J4, see Segura *et al.*, 2013) (Fig. 10), which is also a large felid (53–72 kg, Currier, 1983). *P. leo* resembles the hyaenid *Crocuta crocuta* in that both are born large (1400 g in *P. leo*, 1000 g in *C. crocuta*), both are fed for a prolonged period (9 months, 12 months, respectively) by their mothers, and in the case of *P. leo*, also by other females of the pride (Estes, 2012), and both present a protracted growth and development of their crania during ontogeny. In both species, becoming an efficient predator is a gradual

process that requires more time to obtain an optimal cranium with the ability to support the stress imposed by an extreme diet (large-sized prey in *P. leo*; durophagous in *C. crocuta*). In addition, both species developed adult crania with massive braincase, strong zygomatic arches and well developed sagittal and nuchal crests (See Tanner *et al.*, 2010, Fig. 3). The extension of the ontogenetic period could be related to a behavioral characteristic of both species related to the group living, which allows them to have a prolonged development; activities such as capturing and killing the prey are in the charge of other members of the pride or clan while they grow. The longer growth and development may also be a result of the development of the secondary sexual characters (e.g. mane in males of *P. leo*, phallus in females of *C. crocuta*), which are important to mate, making these individuals more competitive in groups; however, this hypothesis cannot be tested with the little information about sexes in the sample.

Most felids (*Lynx rufus* (Schreber, 1777), *Acinonyx jubatus* (Schreber, 1775), *Panthera leo*, *P. tigris*, *P. onca* and *P. pardus*) obtained both definitive shape and size of cranium in adult classes, when weaning and sexual maturity have been reached (Fig. 10, see Segura *et al.*, 2013); this pattern is shared with the hyaenid *Crocuta crocuta* (See Tanner *et al.*, 2010). However, other felids (*Puma concolor* and *Herpailurus yagouaroundi*) grew or developed in juvenile classes, when weaning has been reached, but sexual maturity has not (Fig. 10, see Segura *et al.*, 2013); this pattern is shared with the canids *Lycalopex culpaeus* (Molina, 1782) (Fig. 10, see Segura & Prevosti, 2012) and *Canis latrans* Say, 1823 (La Croix *et al.*, 2011). *P. leo* and *P. onca* reached definitive cranial shape and size at the same age class, and *P. onca* reached the final cranial shape before to cranial size. This pattern was previously observed in felids, such as *Herpailurus yagouaroundi* and *Puma concolor* (Segura *et al.*, 2013), and is clearly opposite to the pattern of *P. pardus* and canids, such as *L. culpaeus*, which obtained definitive cranial size before obtaining final cranial shape (Segura & Prevosti, 2012) (Fig. 10). The latter pattern does not occur in other felid or canid previously studied as *Canis latrans* (La Croix *et al.*, 2011) and could be related to specific ecological events suffered by this species (e.g. the early end of parental care).

In summary, we observed that *Panthera pardus* are the most different between Pantherines, and present less changes between juvenile and adults. Meanwhile, *P. leo* and *P. tigris* present a large change between juvenile and adults and are very similar each other. In fact, *P. leo* seems to be a scaled version of *P. tigris*. Pantherines share specific characteristics that group them together (i.e. large skull, long rostrum, large temporalis

and high bite force) and, at the same time, these characteristics split them off from the rest of small and medium-sized felids. In this sense, the effect of size plays an important role, because although the four species analyzed in this work have different ontogenetic trajectories, the adults converge in shape because their sizes are more similar if compared with other felids, suggesting a shape constraint. We hypothesize that this pattern (and its allometry implication) could be structured in the cranial evolution in felids in general. It would be interesting to study this aspect taking into account a sample that includes a higher number of taxa, in order to obtain a more robust reconstruction of skull ontogenetic evolution in the Felidae.

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APPENDIX 1

LIST OF SPECIMENS USED IN THIS STUDY

Panthera leo ($N = 100$). AMNH: 6353; 7899; 7905; 7906; 7909; 7986; 7995; 8355; 8364; 13904; 13998; 14028; 16875; 21516; 22706; 35088; 35353; 52071; 54370; 54371; 54372; 54393; 54394; 54395; 83621; 83622; 83623; 83624; 83625; 85146; 130117; 135885; 140396. FMNH: 1443; 15530; 20756; 20757; 20758; 20760; 20762; 30778; 31121; 33479; 33480; 35131; 35132; 35133; 35134; 35164; 35739; 35740; 35741; 35743; 38134; 60314; 60438; 60788; 75608; 75609; 89926; 121667; 127836; 127838; 127839; 127841; 135278; 163109; 173259; 206425. MACN: 36.939. NMNH: 61753; 63110; 86924; 113238; 113239; 114659; 120581; 120582; 120583; 161915; 161916; 161917; 163881; 181584; 181588; 181591; 182143; 182143; 182300; 182301; 182302; 182303; 182304; 182305; 182306; 182312; 182325; 182330; 256096; 470174.

Panthera onca ($N = 122$). AMNH: 110; 331; 6293; 6294; 11084; 22915; 25009; 25010; 25011; 35897; 36949; 36950; 37503; 37504; 37549; 37550; 42405; 70075; 75462; 78520; 80234; 96127; 98671; 98679; 98683; 98684; 98841; 11083; 120998; 135929; 139959; 146602; 146987; 147510; 147513; 149326; 149327; 149328; 176373; 180275; 209135; 209136. CFA: 12823; 12824; 12825. CML: 6236. FMNH: 25352; 48175; 48723; 51475; 60477. MLP: 24.IX.01.6; 24.IX.01.7; 24.IX.01.8; 294; 456. NMNH: A04362; A06480; A08003; A09704; A12296; A13845; A13846; A25097; A49393; 9390; 12176; 61192; 100122; 100541; 122602; 122603; 123527; 125349; 125353;

127542; 130362; 131498; 131499; 131998; 137039; 155603; 167894; 179170; 179171; 225613; 231961; 239343; 244858; 247337; 249821; 249822; 249823; 249824; 249825; 249826; 251118; 251119; 256385; 256386; 259038; 268871; 269786; 270363; 270414; 281420; 281421; 289015; 338356; 338357; 338358; 339678; 361043; 362249; 362250; 374849; 388248; 395084; 395085; 456774; 464972; 583256.

Panthera pardus ($N = 79$). AMNH: 35408; 52001; 52021; 52028; 52029; 52034; 52036; 52037; 52038; 52045; 52048; 54942; 54943; 57009; 70553; 81302; 164151; 238075. FMNH: 1444; 1446; 22364; 27006; 27279; 27443; 30779; 31792; 31793; 32943; 33469; 33475; 34590; 34591; 34872; 35257; 54247; 57956; 58966; 60051; 60615; 60626; 60634; 60740; 83654; 85215; 89916; 89917; 91259; 91290; 99534; 99535; 99536; 99538; 123760; 127842; 129396; 134486; 135075; 135076; 153776; 153777; 199748; 199749. NMNH: A22643; 13067; 21654; 102583; 164544; 164545; 182318; 182328; 182331; 254537; 254637; 256125; 256668; 271289; 290875; 292310; 364963.

Panthera tigris ($N = 69$). AMNH: 62; 63; 7901; 7903; 7904; 10556; 14032; 14033; 17420; 35444; 35482; 35558; 35756; 35799; 70309; 80175; 90087; 119632; 119633; 135954; 139847; 143502; 143823. FMNH: 21325; 25706; 31152; 31153; 31797; 31798; 54220; 54221; 54222; 60760; 104937; 121357; 134497; 134607; 142009; 153778; 159999; 165401; 186834; 188486. NMNH: A03804; A16144; A49728; 111982; 152192; 152193; 152194; 174981; 188638; 218321; 239445; 239450; 253285; 253286; 253289; 253290; 253291; 253293; 254977; 269320; 278470; 396137; 396654; 399096; 399556; 536895.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Summary of results of the Mann–Whitney U -test for skull allometry, between age classes and sexes of *Panthera leo*, *Panthera tigris*, *Panthera onca* and *Panthera pardus*. Values in italics are statistically significant ($P < 0.05$). CS, centroid size; PD, Procrustes distance.

Table S2. Regression vectors of *Panthera tigris*, *Panthera leo*, *Panthera onca* and *Panthera pardus*.