

1 **Cranial shape variation in adult howler monkeys (*Alouatta seniculus*)**

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3 **Luca Fiorenza<sup>1,2</sup>, Emiliano Bruner<sup>3</sup>**

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5 <sup>1</sup>Department of Anatomy and Developmental Biology, Monash University, Melbourne, 3800

6 VIC [Australia]

7 <sup>2</sup>Earth Sciences, University of New England, Armidale, NSW 2351 [Australia]

8 <sup>3</sup>Centro Nacional de Investigación sobre la Evolución Humana, Burgos [Spain]

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16 Corresponding author:

Dr. Luca Fiorenza

17 Department of Anatomy and Developmental Biology

18 Monash University

19 Melbourne VIC 3800 Australia

20 Phone: +61 3 990 59809

21 Email: [luca.fiorenza@monash.edu](mailto:luca.fiorenza@monash.edu)

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1 **RESEARCH HIGHLIGHTS**

2 1. Cranial architecture in red howler monkeys (*Alouatta seniculus*) is influenced by  
3 allometric factors.

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5 2. This cranial morphology may be associated with the development of the  
6 masticatory apparatus and the enlargement of the vocal organs.

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8 3. Variation in the pteric area seems to be influenced by braincase morphogenesis.

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10 **ABSTRACT**

11 Howler monkeys (genus *Alouatta*) display a distinctive cranial architecture characterized by  
12 airorhynchy (or retroflexion of the facial skeleton on the cranial base), a small braincase, and  
13 a posteriorly oriented foramen magnum. This configuration has been associated with distinct  
14 factors including a high folivory diet, locomotion, and the presence of a specialized vocal  
15 tract characterized by large hyoid bone. However, the morphological relationships between  
16 the facial and neurocranial blocks in *Alouatta* have been scarcely investigated. In this study  
17 we quantitatively analyzed the cranial shape variation in *A. seniculus*, to evaluate possible  
18 influences and constraints in face and braincase associated with airorhynchy. We also  
19 considered the structural role of the pteric area within the cranial functional matrix. We  
20 applied landmark-based analysis and multivariate statistics to 31 adult crania, computing  
21 shape analyses based on 3D coordinates registration as well as the analysis of the Euclidean  
22 distance matrix to investigate patterns of intraspecific morphological variability. Our results  
23 suggest that allometry is the main source of variation involved in shaping cranial morphology  
24 in howlers, influencing the degree of facial proportions and braincase flattening, and  
25 generating the main sexual differences. Larger individuals are characterized by a higher

1 degree of airorhynchy, neurocranial flattening, and expansion of the zygomatic arch.  
2 Allometric variations influence the skull as a whole, without distinct patterns for face and  
3 braincase, which behave as an integrated morphological unit. A preliminary survey on the  
4 pteric pattern suggests that the morphology of this area may be the result of variations in the  
5 vertical growth rates between face and braincase. Future studies should be dedicated to the  
6 ontogenetic series and focus on airorhynchy in terms of differential growth among distinct  
7 cranial districts.

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9 **Key words: Atelidae; airorhynchy; geometric morphometrics; pterion sutural pattern;**  
10 **allometry**

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## 1 INTRODUCTION

2 Howler monkeys (*Alouatta* Lacépède, 1799) are the largest, most sexually dimorphic and  
3 most widely distributed neotropical primates. They occupy various habitat types ranging from  
4 southern Mexico to northern Argentina and can be found from sea level to high altitudes  
5 [Schultz, 1960; Gaulin and Gaulin, 1982; Bonvicino et al., 2001; Cortés-Ortiz et al., 2003;  
6 Bruner and Cucina, 2005]. They are considered the most folivorous of the New World  
7 monkeys although their diets can be quite flexible with different degree of frugivory [Milton,  
8 1980; Neville et al., 1988; Crockett, 1998; Silver et al., 1998; Rosenberger et al., 2001;  
9 Pavelka and Houston Knopff, 2004; Ludwig et al., 2008].

10 The taxonomy of howler monkeys remains ambiguous with disagreements on how many  
11 subspecies exist and how many of them should be elevated to species rank [Crockett, 1998;  
12 Rylands et al., 2000; de Oliveira et al., 2002]. Hershkovitz [1949] initially recognized five  
13 distinct species grouped into three different morphotypes based on the morphology of the  
14 hyoid bone: the South American *Alouatta seniculus* group, the Central American *A. palliata*  
15 group and *A. caraya* group. Dermatoglyphic, molecular and genetic evidence suggest a higher  
16 number of species, ranging from seven to twelve [Froehlich and Froehlich, 1987; Gregorin,  
17 2006; Groves, 2001; de Oliveira et al., 2002; Cortés-Ortiz et al., 2003; Rylands and  
18 Mittermeier, 2009; Cortés-Ortiz et al., 2015]. There is, however, a general consensus in  
19 considering the genus *Alouatta* to be a monophyletic group within the Atelidae family, which  
20 separated from the atelines around 15 million years ago [Hershkovitz, 1949, Meireles et al.,  
21 1999; Schneider, 2000; Bonvicino et al., 2001; de Oliveira et al., 2002].

22 The cranial morphology of howler monkeys is characterized by a set of features that is  
23 unique among living platyrrhine primates: the braincase is relatively small and with a  
24 pyramidal shape; the face is projecting and flexed onto the neurocranial axis; and the foramen  
25 magnum is positioned and oriented posteriorly [Hill, 1962]. This dorsal rotation of the face

1 relative to the neurobasicranium is called *airorhynchy* [Hofer, 1952; Shea, 1985; Lieberman,  
2 2000; Bruner et al., 2004; Singleton, 2013], and in primates it has been associated with the  
3 presence of enlarged laryngeal sacs [Biegert, 1963]. Howler monkeys are indeed  
4 characterized by an extremely large hyoid apparatus, forming a specialized vocal tract that  
5 plays a crucial social function [e.g. Baldwin & Baldwin, 1976; Whitehead, 1987]. The large  
6 hyoid apparatus is housed between large ascending mandibular rami with pronounced  
7 bigonial breadth [Herskovitz, 1949; Hill, 1962; Schultz, 1960; Ravosa and Ross, 1994]. The  
8 association between airorhynchy and enlarged vocal sacs seems supported by the fact that  
9 other primates, like oranges, display a similar combination of features although the  
10 morphogenetic mechanisms may be distinct [Bruner et al., 2004; Senck and Coquerelle,  
11 2015]. This spatial relationship of the face in relation to the neurocranium could be also  
12 responsible for the howlers' bone arrangement at the infratemporal fossa and interorbital area,  
13 which is different from common platyrrhine schemes [Gegenbaur, 1884; Forsyth-Major,  
14 1901; Collins, 1925; Schultz, 1926; Ashley-Montagu, 1933; Hill, 1962; Bruner et al., 2004;  
15 Koppe et al., 2005]. In fact, in most neotropical monkeys the sutural pattern of the pterion  
16 (the region of the cranium where frontal, parietal, temporal and sphenoid bones meet  
17 [Williams et al., 1998]) is characterized by the direct contact between the zygomatic and  
18 parietal bones while howler monkeys frequently display a sutural pattern typical of Old World  
19 monkeys (where frontal and sphenoid bones articulate between each other). Collins [1925]  
20 and Ashley-Montagu [1933] were the first to observe that in *Alouatta* more than 50% of the  
21 specimens exhibit a pterion type where the greater wing of the sphenoid bone articulates with  
22 the frontal bone, separating thus the zygomatic from the parietal bone. Concerning the  
23 interorbital area, early works reported a significant variability in the nasal bones and lacrimal  
24 fossa of *Alouatta* [Gegenbaur, 1884; Forsyth-Major, 1901; Schultz, 1926; Hill, 1962], and  
25 different bony arrangements among atelids dealing with the contact between the nasal and

1 lacrimal bones [Schultz, 1960; Bruner et al., 2004]. Finally, in howlers the basisphenoid and  
2 the frontal bone are also highly pneumatized due to a significant enlargement of the maxillary  
3 sinus [Koppe et al., 2005]. It remains to be evaluated whether all these specific traits in  
4 *Alouatta* are consequences of a global cranial arrangement, or else independent characters  
5 influenced by local factors and distinct morphogenetic mechanisms.

6 A preliminary inter-specific cranial shape comparison between howlers and the other  
7 atelids showed minor average difference in overall facial geometry, suggesting that most of  
8 the features associated with howlers' airorhynchy could be the result of braincase pattern of  
9 development constraining the spatial relationships between facial and neurocranial blocks  
10 [Bruner et al., 2004]. At intra-specific level, spatial factors and shape patterns associated with  
11 this peculiar cranial arrangement has still to be investigated. Cranial size is assumed to have a  
12 major role in this variation, being a relevant factor in shaping skull morphology through  
13 differences in ontogenetic scaling associated with sexual dimorphism, socio-ecological  
14 factors, diet, and masticatory function [Ravosa and Ross, 1994; Jones et al., 2000; Koppe et  
15 al., 2005; Blanco and Godfrey, 2006; Flores and Casinos, 2011; Youlatos et al., 2015].  
16 Allometric comparisons in cranial proportions of *Alouatta seniculus* have shown large  
17 differences between adults of each sex, which were explained as the results of ontogenetic  
18 scaling [Ravosa and Ross, 1994]. Similarly, Youlatos and colleagues [2015], who analyzed  
19 cranial variation in *Alouatta* at the species level, found that gender differences and overall  
20 variation was more pronounced in *A. seniculus* than for any other howler species.

21 In this study, we want to extend the initial work of Bruner and colleagues [2004] by  
22 analyzing cranial shape changes in the sexually dimorphic species of *A. seniculus*, and  
23 investigating shape factors associated with airorhynchy at intraspecific level. We evaluate to  
24 what extent the cranial geometry and the characters associated with airorhynchy in red  
25 howlers are influenced by size and allometric schemes, under the null hypothesis of no

1 correlation between size and shape changes. If the hypothesis is rejected, then we can state  
2 that size (and, consequently, growth) is likely to have a major effect on airorhynchy and its  
3 associated traits.

4 We also evaluate whether sexual dimorphism is based on size factors or on sex-specific  
5 characters. Moreover, because of the specific bone articulation at the pterion in this genus,  
6 *Alouatta* is a good case study to evaluate whether the morphological pattern associated with  
7 the junction between face and braincase is the result of spatial constraints associated with  
8 airorhynchy. Considering that bone articulations are sensitive to morphogenetic rates and  
9 spatial distances between bone centers [Enlow, 1990], it is expected that pteric sutural  
10 scheme, formed by the contact between four bony elements, can be influenced by the flexion  
11 between facial and neurocranial blocks. Accordingly, we have investigated here the  
12 association between cranial form and pteric patterns in *Alouatta seniculus*.

## 14 **METHODS**

15 This study was performed on dry skulls from museum collections. The research complies  
16 with the American Society of Primatologists Principles for the Ethical Treatment of Primates,  
17 protocols of the appropriate Institutional Animal Care Committee, and legal requirements of  
18 each country housing collections.

19 The sample used in this study consists of 31 adult specimens of *Alouatta seniculus* housed  
20 at the Senckenberg Natural History Museum (Frankfurt, Germany), whereof 14 are males, 11  
21 are females and six have unknown sex. *A. seniculus* was investigated in this intra-specific  
22 survey because it is the most common howler monkey species, it has a documented sexual  
23 dimorphism, and at the same time is the taxon that has been most examined in literature  
24 [Ravosa and Ross, 1994; Plavcan, 2002; Youlatos et al., 2015]. We considered only  
25 individuals with fully erupted dentition (third molar), and with a recognizable pterion sutural

1 pattern on both cranial sides.

2 We used a geometrical model with 42 three-dimensional landmarks covering most of the  
3 facial, neurocranial and basicranial regions following White and Folkens [2000] and Bruner  
4 and colleagues [2004] (Fig. 1). Pterion (pt) was defined as the midpoint along the sphenoparietal suture. Landmark's coordinates were sampled on dry skulls using a 3D device  
5 (MicroScribe® G2X 3D, Solution Technologies, Inc., Oella, Maryland, USA). Landmark data  
6 were registered through Procrustes superimposition, minimizing residuals among  
7 corresponding landmarks by translation to a common centroid, scaling to unitary centroid  
8 size, and rotation following a criterion of least square differences [Bookstein, 1991].  
9 Superimposed coordinates were analyzed with multivariate statistics according to the  
10 principles of geometric morphometrics [Zelditch et al., 2004]. Data were symmetrized by  
11 averaging the bilateral landmarks according to the midline landmarks. This is a standard  
12 procedure when asymmetries are not the aim of the study [Klingenberg et al., 2002].

13 Registered coordinates were analyzed through Principal Component Analysis [Jolliffe,  
14 2002] to localize and quantify the main patterns of covariation generating the phenotypic  
15 variability. Allometry was tested by correlation between centroid size (the square root of the  
16 sum of the squared distances from each landmark to the centroid [Hammer et al., 2001]) and  
17 shape coordinates, and between centroid size and principal components. Sex differences were  
18 assessed by Discriminant Function Analysis on shape variables. Integration between facial  
19 and braincase morphological variation was further evaluated through Partial Least-Square  
20 (PLS) analysis, analyzing the covariation between the two multivariate blocks, computed on  
21 both single and independent registrations [Rohlf & Corti, 2000].

22 Pteric area displays two main sutural patterns (Fig. 1A): Zygoparietal (ZP) and  
23 Sphenofrontal (SF). In the ZP pterion (frequent in platyrrhines but not in howlers), the  
24 zygomatic bone contacts the parietal bone, separating thus the frontal from the sphenoid bone.  
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1 Conversely, in the SF configuration (frequent in howlers and chatarrhines), the zygomatic and  
2 parietal bones are disjointed by the contact between the frontal and the sphenoid bones. We  
3 also evaluate an asymmetric morphotype (asym), where distinct patterns characterize the two  
4 cranial sides of the same individual. The sample includes only three individuals with  
5 symmetric SF pattern and three individuals with asymmetric pteric patterns. Taking into  
6 consideration a small number of specimens in these two groups, the current analysis of the  
7 pteric patterns must be therefore considered as preliminary. Pterion groups were analyzed  
8 according to overall shape analysis (PCA) and by comparing their respective average  
9 coordinates (mean shapes). Geometric morphometrics was computed with MorphoJ 1.06a  
10 ([http://www.flywings.org.uk/MorphoJ\\_page.htm](http://www.flywings.org.uk/MorphoJ_page.htm)) [Klingenberg, 2011] and PAST 2.17c  
11 (Hammer and Harper<sup>®</sup>, Oslo, Norway; <https://folk.uio.no/ohammer/past/>) [Hammer et al.,  
12 2001]. Group differences were tested by permutation test. Statistical significance was set at P  
13 < 0.05.

14 Sexes and pterion groups were also compared by Euclidean Distance Matrix Analysis  
15 (EDMA) to quantify differences in terms of absolute dimensions (form) and not only  
16 according to shape proportions [Richtsmeier et al., 2002]. In this case, the distribution of all  
17 the corresponding interlandmark distances was analyzed in terms of their ratio to quantify the  
18 average size differentiation between groups, and to localize distances showing larger  
19 departures from this mean value. This approach is useful to localize local areas involved in  
20 morphological changes beyond raw size increase. EDMA was computed with WinEdma  
21 (<sup>®</sup>2001 T. M. Cole III, Kansas City, Missouri, USA;  
22 <http://www.getahead.psu.edu/comment/edma.asp>).

23

## 24 **RESULTS**

### 25 *Cranial shape analysis*

1 The variance distribution after Principal Component Analysis shows a multivariate space  
2 that is characterized by two main components (Fig. 2). Each of the following components  
3 explains less than 7% of the total variance and they are below a threshold of random noise  
4 variation according to a broken stick model [Hammer et al., 2001], and hence they will be not  
5 discussed here. The first component (33.5% of the variance) is associated with flattening and  
6 widening of the cranium, and it separates males from females. Flattening is particularly  
7 localized at the vault and upper face, and associated with widening of the zygomatic arch and  
8 muzzle, and backward rotation of the foramen magnum. Vault flattening and muzzle  
9 expansion (males) lead to increased neuro-facial angle and airo-rhynch. The opposite pattern  
10 (females) involves taller vault, smaller muzzle and zygomas, and inclination of the foramen  
11 magnum. The second component (14.4% of the variance) is associated with enlargement of  
12 the orbito-malar region, taller vault, shorter braincase, and larger premaxilla. Females display  
13 larger values, although their range largely overlaps with males distribution. A larger orbito-  
14 malar region is related to an apparent displacement of the pterion. It is worth noting that three  
15 specimens with bilateral zygoparietal pteric pattern and two specimens up to three with  
16 unilateral zygoparietal pteric pattern do not show similar values for PC1, but they are all  
17 positioned in the upper part of the distribution of PC2. Thus, five up to six specimens with  
18 zygo-parietal contact at the infratemporal fossa display an enlarged orbito-malar area (Fig.  
19 2A).

20 Size explains 28% of the whole morphological variation ( $P < 0.0001$ ). Shape pattern  
21 associated with size variation is the same as described for the first principal component, and  
22 centroid size is strongly correlated to PC1 ( $R^2 = 0.83$ ;  $P < 0.0001$ ). Size is not correlated to  
23 PC2 ( $P = 0.47$ ). Males and females show significant differences in PC1 and size ( $P < 0.0001$ )  
24 but not in PC2 ( $P = 0.15$ ). Discrimination between males and females is significant ( $P <$   
25  $0.0001$ ) and the shape pattern was the same as described for PC1, with males displaying

1 larger crania, flatter vault, wider zygoma, and larger muzzle. A Partial Least Square (PLS)  
2 analysis between the facial and neuro-basiscranial blocks confirms an integrated pattern of  
3 variation (PLS1;  $P < 0.0001$ ), in which a taller vault is associated with relatively shorter  
4 muzzle and larger orbito-malar areas (Fig. 3). Results are the same when considering blocks  
5 correlation according to a single shared registration or independent Procrustes  
6 superimposition. Shape variations are similar to the pattern associated with PC1, suggesting  
7 that flattening of the braincase, rotation of the foramen magnum, and facial flexion are part of  
8 an integrated morphological change.

9 When analyzing all the interlandmark distances as ratios between the male and female  
10 values, males are on average 20% larger than females (Fig. 4). Diameters increasing less than  
11 2 standard deviations ( $<5\%$  - that is, proportionally shorter in males) are associated with the  
12 vertical dimensions of upper face, vault, and nuchal area. Diameters increasing more than 2  
13 standard deviations ( $>34\%$  - that is, proportionally longer in males) are associated with the  
14 dimensions of the zygomas and with the length of the cranial base.

#### 16 *Morphological comparisons of pterion sutural patterns*

17 Comparison of the mean shape of all the specimens with symmetric sphenofrontal junction  
18 with the mean shape of the three specimens with zygoparietal junction shows that in the latter  
19 group the vault is relatively taller and the muzzle is relatively narrower (Fig. 5). The analysis  
20 of the Euclidean Distance Matrix of the two groups evidences that the sphenofrontal group  
21 has a larger size (5%), and that morphological differences are actually due to (in this group)  
22 reduced vault and orbit distances ( $-3\%$ ) and larger basicranial and midface distances ( $> 13\%$ ).

## 24 **DISCUSSION**

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1      *Cranial shape variation*

2      A preliminary inter-specific analysis among Atelids suggested that the peculiar cranial  
3 shape in howlers might be due to braincase constraints influencing the spatial relationships  
4 with the facial block [Bruner et al., 2004]. In the present study we investigate cranial shape  
5 variation within the most common species of howlers, *A. seniculus*. The null hypothesis of no  
6 correlation between size and shape is rejected by this dataset, suggesting that in red howler  
7 monkeys airorhynchy is influenced by size variation. Size increase involves an allometric  
8 pattern characterized by flattening of the cranial vault, backward rotation of the foramen  
9 magnum, expansion of the zygomatic arch, and relative enlargement and rotation of the facial  
10 block. All these variations are expressed proportionally to size, and larger skulls have  
11 relatively flatter braincase, more posterior foramen magnum, wider zygomatic arches, and  
12 larger and more rotated face. Our survey indicates that sexual dimorphism in *A. seniculus* is  
13 generally only based on size differences and allometric changes, and that facial and braincase  
14 variations are integrated.

15 In large primates such as howlers, cranial size changes are functionally correlated with the  
16 typical masticatory apparatus of this genus, which scales positively with body size [Biegert,  
17 1963], and is ultimately associated with a folivorous diet and with enlarged vocal organ. The  
18 flat cranial vault seen in larger specimens of *A. seniculus* suggests that brain growth in this  
19 species might not expand in a vertical direction as in other primates. Chopra [1957] noted that  
20 howler monkeys are characterized by an early suture closure when compared to other  
21 primates, and Shultz [1960] suggested that the vault growth tends to be directed posteriorly  
22 rather than superiorly or inferiorly. Accordingly, the flattened braincase that characterizes *A.*  
23 *seniculus* could be related to the early cessation of cranial growth and the obliteration of the  
24 cranial sutures [Höfer, 1969; Youlatos et al., 2015]. During ontogeny, elements maturing  
25 earlier channel and constrain the spatial arrangements of the elements maturing later [Bastir et

1 al., 2006]. Thus, it seems that limited vertical expansion of the braincase is likely to be the  
2 factor constraining the following morphogenetic changes and leading to flexure of the angle  
3 between the splanchnocranial and neurocranial axis. According to Hartwig and colleagues  
4 [2011], the limited brain size of howler monkeys was also associated with basicranial spatial  
5 re-arrangement for accommodating the large hyoid complex. The forward and upward  
6 shifting of the face, and the simultaneous repositioning of the foramen magnum may have  
7 constrained brain size growth patterns [Hartwig et al., 2011]. Our results show that the facial  
8 skeleton and the neurocranium are not independent modules, displaying patterns of  
9 morphological integration that suggests a shared morphogenetic process. Thus, it is possible  
10 to hypothesize that airorhynchy and the rotation of the foramen magnum are probably the  
11 simultaneous by-product of the limited vertical expansion of the neurocranium and the  
12 considerable enlargement of the hyolaryngeal system. It remains to be evaluated to what  
13 extent such spatial rearrangement of the cranial elements can have a role in functional and  
14 behavioral aspects associated with orbit orientation and vision [Bruner et al., 2003; Martin  
15 and Ross, 2005; Fleagle et al., 2010], and posture and locomotion [Schön Ybarra, 1984;  
16 Bruner et al., 2004].

17 Our results suggest that sexual dimorphism in *A. seniculus* is generally based only on size  
18 variation. In this species, males reach larger sizes by growing faster than females, a process  
19 known as rate hypermorphosis [Ravosa and Ross, 1994]. As in other primates, braincase  
20 shows fewer differences, due to the early cessation of the brain growth [Shea, 1983]. Also, in  
21 primates, strongest sexual differences are associated with areas of muscle attachments  
22 [Plavcan, 2002], and in this case, they deal mostly with the masseters (zygomatic bone),  
23 which is particularly developed in folivorous primates [Anapol and Lee, 1994].

24

25 *Airorhynchy and pteric patterns*

1 Although the sample size is limited, some preliminary comments can be provided on the  
2 characteristic pteric junction in howlers. Airorhynchy is associated with an increase of the  
3 angle between facial and neurocranial axes [Shea, 1985], and the infratemporal fossa  
4 represents a structural hinge sensitive to facial rotation [Enlow, 1990]. It can be hypothesized  
5 that the high frequency of sphenofrontal joint at the pteric area in howler monkeys could be  
6 related to the morphogenetic cranial pattern associated with facial rotation. There are at least  
7 two possible mechanisms behind such bone arrangement. The peculiar (Catarrhine-like)  
8 sutural scheme in howlers can be a secondary consequence of airorhynchy. In this case,  
9 growing bone centers will contact each other depending upon the timing of bone filling of the  
10 pteric space, which in turn depends upon the distance between facial and neurocranial block  
11 (influenced by airorhynchy) but also by the general skull proportions (relative height of the  
12 skull). Alternatively, the pteric pattern can have an active role in channeling skull proportions,  
13 constraining the distance between face and braincase, and, because of airorhynchy,  
14 influencing the vertical proportions of the skull. This second hypothesis is less likely, but we  
15 still do not have sufficient ontogenetic information to support or exclude one of the two  
16 alternatives. Unfortunately, individuals with zygomatic-parietal junctions are rare, and in our  
17 sample we found only three specimens with this feature present bilaterally. In these three  
18 cases, a zygomatic-parietal junction is associated with taller braincase and narrower muzzle,  
19 that is, a less pronounced platycephaly and a reduced splanchnocranial size. It can be  
20 hypothesized that in *Alouatta seniculus*, the flat braincase induces sphenofrontal contact  
21 simply by approaching spatially the two bones. In specimens with taller braincase the  
22 zygomatic and parietal bones can come in contact before the frontal and sphenoid bones  
23 contact, closing the pteric space. According to our survey, there is no evidence that such  
24 structural adjustment can be due to airorhynchy and rotation of the neurocranial axis onto the  
25 facial axis, and platycephaly alone can explain such peculiar morphological arrangements.

1 Nonetheless, as mentioned previously, this comparison is performed only on three individuals  
2 and should be improved by increasing the sample size. Furthermore, this same approach  
3 should be extended also to other primates, including those that do not display airorhynchy.  
4

#### 5 *Final remarks*

6 Red howlers are large and highly sexually dimorphic neotropical primates with a diet mostly  
7 consisting of leaves, powerful masticatory systems, and large vocal sacs characterizing their  
8 behaviors and social structure. Their skulls show many atypical features, like flat braincase,  
9 posterior foramen magnum, and upward rotation of the facial axis, a condition named  
10 airorhynchy. Our results suggest that in the most common howler, *A. seniculus*, all these  
11 features are proportionally influenced by size, integrating the facial and braincase morphology  
12 and generating differences between males and females. Because of the maturation sequence,  
13 an early interruption of the brain vertical growth can be the primary factor inducing the  
14 following spatial changes. Larger sample and more specific landmark configurations can add  
15 more details to these conclusions, particularly when dealing with the role of the sutures within  
16 the cranial functional matrix. Also, a comparison with other species is necessary considering  
17 that at least sexual dimorphism is not uniform among howler monkeys [Ravosa and Ross,  
18 1994]. Finally, studies on ontogenetic series are mandatory, not only during growth and  
19 development but also among adults, taking into consideration that cranial modeling in  
20 *Alouatta* continues beyond dental maturity [Jones et al., 2000].

21 It is worth noting that other primates, like orangs, display similar combinations of cranial  
22 features, with important changes in the spatial relationships between face and braincase [Moss  
23 and Young, 1960; Shea, 1985]. In addition, the cranial architecture is largely integrated  
24 although the mechanisms associated with airorhynchy are not clear and may be different from  
25 those of howlers [Bruner et al., 2004; Senck and Coquerelle, 2015]. Taking into account their

1 phylogenetic distance, further analysis of the differences and similarities between these two  
2 taxa would represent an excellent model for investigating principles of primate functional  
3 craniology.

4

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1 **FIGURE LEGENDS**

2

3 **Figure 1.** Landmark three-dimensional configuration shown in lateral, superior and inferior  
4 view (A). Schematic illustration of a generic *Alouatta* cranium displaying the two different  
5 morphotypes: Sphenofrontal and Zygoparietal (B). The pterion is a region where the  
6 zygomatic (Z), sphenoid (S), frontal (F) and parietal (P) bones join together. Midsagittal  
7 landmarks [12 landmarks: Basion, *ba*; Bregma, *br*; Glabella, *gl*; Hormion, *ho*; Inion, *in*;  
8 Lambda, *ld*; Nasion, *na*; Nasospinale, *nsp*; Opisthion, *op*; Posterior Nasal Spine, *pns*;  
9 Prosthion, *pr*; Rhinion, *rh*]. Bilateral landmarks [30 landmarks (15 points x 2 sides):  
10 Articulare, *art*; Asterion, *as*; Frontomolare Orbitale, *fmo*; Foramen Ovale (anterior most  
11 point), *foa*; Nasomaxillare, *nsm*; Orbitale Superior, *os*; Petrosal (anterior most point), *pet*;  
12 Premolare, *pm*; Premaxillare, *pmx*; Porion, *po*; Pterion, *pt*; Zygomotemporale Inferior, *zti*;  
13 Zygomotemporale Superior, *zts*; Zygomaxillare, *zym*; Zygoorbitale, *zyo*].

14

15 **Figure 2.** Principal Component Analysis of cranial shape variation. PCA plot (A) of the first  
16 two principal components accounting for 47.9% of variation (PC1: 33.5%; PC2: 14.4%);  
17 males are colored in blue, females in red and indeterminate individuals in black stars.  
18 Screeplot and broken stick model (B) and wireframes (Black: positive values; Grey: negative  
19 values) of PC1 (C) and PC2 (D) scores. ZP = zygomatic-parietal contact; As = asymmetric  
20 morphotype.

21

22 **Figure 3.** Partial Least Square correlation between facial and neurocranial blocks, computed  
23 through independent (A) and shared (B) Procrustes superimposition. The patterns are similar  
24 to PC1, associating neurocranial flattening with facial flattening and rotation, and  
25 displacement of the foramen magnum. Arrows are just visual aids to highlight the general

1 pattern of shape differences. Wireframe in **A** has been simplified to improve visualization of  
2 the blocks.

3

4 **Figure 4.** Euclidean Distance Matrix Analysis (EDMA) comparing males (numerator) and  
5 females (denominator) matrices, showing diameters which, in males, are larger (red) and  
6 shorter (blue) than two standard deviations. Males are on average 20% larger than females,  
7 but their cranial base and malar bones are more than 34% larger, while the braincase is less  
8 than 5% taller. FDM = form difference matrix; FM = form matrix; FD = form difference.

9

10 **Figure 5.** Mean shape Procrustes comparisons between individuals with bilateral SF (black)  
11 and ZP (red) patterns at the pterion (A), and EDMA comparison between the two groups  
12 showing longer (red) and shorter (blue) distances associated with the SF cranial morphology  
13 (B). FDM = form difference matrix; FM = form matrix; FD = form difference.