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Craniofacial morphometric analysis as a differentiation tool in *mystax* group of *Saguinus* Hoffmannsegg, 1807 (Cebidae, Callitrichinae): a preliminary test

Daysa A. Fernandes[1,2*] & Cayo A. R. Dias[1,2]

1- PG Zoologia, Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais – Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG; 2- Laboratório de Evolução de Mamíferos, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais – Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG. *Corresponding author: daysa.athaydes.primatology@gmail.com

Abstract

This study provides a quantitative assessment of the craniofacial variation among currently recognized species and subspecies in the *Saguinus mystax* species group and tests the reliability of the proposed method in detecting differences among taxa. Thirty measurements were taken of 66 tamarin specimens representing *Saguinus imperator*, *S. inustus*, S. labiatus and *S. mystax*. We used the non-parametric MANOVA to test for differentiation among species and among subspecies (*S. i. imperator* and *S. i. subgrisescens*; *S. m. mystax* e *S. m. pileatus*). Interspecific analyses showed significant variation among all species, except for *S. inustus* (which we attribute to the small sample size). We also detected significant differentiation between *S. mystax mystax* and *S. m. pileatus*, whereas no significant morphometric difference was found between *S. imperator* subspecies. In order to explore the variation of each individual measurement, we plotted diagrams comparing the mean variation among species. Measurements presenting some degree of differentiation among species were selected for subsequent analysis of variance. Univariate analyses detected significant differences among species only for three measurements. Our results suggest that the cranial morphometric variation has limited information to discriminate among the taxa in the *S. mystax* group. However, we cannot disregard the lack of statistical power due to small sampling in some taxa, absence of some subspecies in the analyses, or even lack of informativeness of the chosen measurements. Morphometric analyses were useful to detect some degree of differentiation among species, but it was also insufficient to demonstrate complete differentiation in *Saguinus*. Therefore, the use of morphometric analyses should not be considered as a definitive method for taxa differentiation and delimitation in *Saguinus*.

Keywords: Tamarins, quantitative characters, morphometry, multivariate analysis, skull.

Introduction

Morphometric analyses are considered morphological quantification tools, which may help in assessing organism shape disparity and, consequently, providing more information about diversity (Klingenberg 2010). This method have been useful in species differentiation in some groups of mammals, such as marsupials (e.g. Cerqueira & Lemos 2000), rodents (e.g. Percequillo et al. 2008) and primates (e.g. Hanihara & Natori 1987).

Most of alpha taxonomy of the genus *Saguinus* (Primates, Platyrrhini, Callitrichidae) has been based on pelage coloration associated to geographic distribution patterns (e.g. Hershkovitz 1977), but some studies also employed morphometric analyses as a complementary approach to address taxa delimitation issues in this genus, or in closely related groups.

Gregorin & Vivo (2013) re-validated *S. ursulus* Hoffmannsegg, 1807 based on morphological differences, corroborating previous molecular studies. However no significant morphometric difference was found between *S. ursulus* and related species. Similar results have been found by Röhe et al. (2009), which reported that most measurements used for comparing *S. fuscicollis mura* to related subspecies showed too much overlap to be useful to differentiate them. In other studies, however, morphometric methods have proved to be an efficient tool for separating closely related species of *Saguinus* (Natori & Hanihara 1992; Ackermann & Cheverud 2002).

In the absence of a consensus on morphometric analyses usefulness for the differentiation of closely related taxa, we provide a quantitative analysis of craniofacial variation existing among currently

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recognized species (and some subspecies) included in the *Saguinus mystax* group, in order to test the reliability of morphometric analyses for taxonomic

Materials and Methods

Table 1: Number of specimens analyzed per species in this study. For details, see supporting information S1.

Taxon	Number of specimens
S. i. imperator (Goeldi, 1907)	3
S. i. subgrisescens (Lönnberg, 1940)	17
S. l. labiatus (É. Geoffroy in Humboldt, 1812)	11
S. m. mystax (I. Geoffroy & Deville, 1848)	24
S. m. pileatus (Spix, 1823)	9
S. inustus (Schwarz, 1951)	2
Total:	66

We examined 66 skulls of Saguinus belonging to five recognized species and subspecies of the *S. mystax* group (Tab. 1), including *S. inustus* Schwarz, 1951. The specimens examined are deposited in the following Brazilian institutions (see supporting information S1): Museu Paraense Emílio Goeldi, Belém (MPEG), Museu de Zoologia da Universidade de São Paulo (MZUSP) and Museu Nacional da Universidade Federal do Rio de Janeiro (MN).

Twenty-nine morphometric cranial and dental characters were selected based on literature (Ackerman & Cheverud 2002; Vivo 1991). An additional character was defined in the present study (height of the coronoid process of the mandible, HCPM), totaling thirty variables (for details, see Tab. 2). In order to avoid interference of age in our analyses, only adult specimens were measured. Specimens that presented all teeth erupted and a closed basisphenoid-basioccipital suture were considered adults (Vivo 1991).

Our dataset containing 30 measurements from 66 specimens was submitted to statistical analyses using the software PAST 3 (Hammer et al. 2001). We employed the Doornik-Hansen test in order to test for multivariate normality.

Prior to comparisons among taxa, and in order to avoid the confounding effects of sexual variation, we tested for sexual dimorphism employing a non-parametric multivariate analysis of variance (NPMANOVA). Since no significant difference between sexes was detected, males and females were

Table 2: List of the measurements used in skull morphometric analyses. Measurements are based on Ackerman et al. (2002) and Vivo (1991).

Abbreviation	Description
IS-PM	Distance between intradentale superior point and premaxillary superior at the alveolus
IS-PNS	Distance between intradentale superior point and posterior nasal spine
PM-ZS	Distance between premaxillary superior at the alveolus and zygomaxillare superior
PM-MT	Distance between premaxillary superior at the alveolus and maxillary tuberosity
NA-BR	Distance between nasion and bregma
NA-FM	Distance between nasion and fronto-malare
BR-PT	Distance between bregma and pterion
PT-FM	Distance between pterion and fronto-malare
PT-BA	Distance between pterion and basion
PT-ZIGO	Distance between pterion and inferior zygo-temporal suture
PT-TSP	Distance between pterion and temporo-spheno-parietal junction
FM-ZS	Distance between fronto-malare and zygomaxillare superior
ZS-ZI	Distance between zygomaxillare superior and zygomaxillare inferior
ZI-ZIGO	Distance between zygomaxillare inferior and inferior zygo-temporal suture
LD-AS	Distance between lambda and asterion
BR-LD	Distance between bregma and lambda
OPI-LD	Distance between opisthion and lambda
PT-AS	Distance between pterion and asterion
ML	Mandible length
HMC	Height of the mandibular condyle
IMDS	Inferior molar dental series length plus inferior canine
HCPM	Height of the coronoid process of the mandible
SL	Skull length
WS	Width of skull
SLBC	Skull length at the basal condyle
OW	Orbital width
SWPO	Skull width at post orbital constriction
LUMS	Length of upper molar series
WUM	Width across upper molars
WUC	Width across upper canines

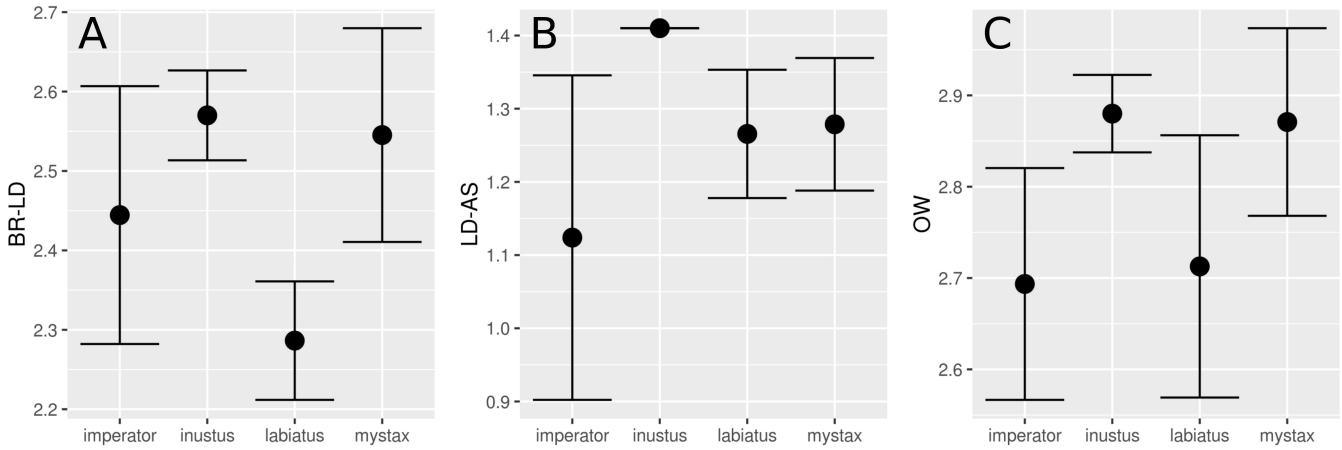

Figure 1: Diagrams showing the mean variation among species (*S. labiatus*, *S. mystax*, *S. inustus* e *S. imperator*) for the three measurements that showed significant differences among species in the ANOVA. A) Diagram of BR-LD measure (ANOVA F = 10.82, p <0.01); B)Diagram of LD-AS measure (ANOVA F = 5.84, p <0.01); C)Diagram of BR-LD measure (ANOVA F = 12.4, p <0.01). Vertical bars indicate standard deviation.

pooled together in a single dataset.

We tested for morphometric differentiation at the intraspecific and interspecific levels using NPMANOVA. In order to test for intraspecific differentiation, we divided our dataset into groups defined by currently valid subspecies (Rylands et al. 2016). Interspecific variation was assessed after dividing our dataset into groups which consisted of currently recognized species.

In order to explore the variation of each measurement, we plotted diagrams comparing the mean variation among species (S. labiatus (E. Geoffroy em Humboldt, 1812), *S. mystax* (Spix, 1823), *S. inustus* and *S. imperator* (Goeldi, 1907)). Those displaying among species differentiation were selected for a subsequent Analysis of Variance

Results and Discussion

Non-parametric MANOVA indicated significant differences among different pairs of taxa (F = 4.314, p < 0.01), as observed in Tab. 3, which contains the corrected p values (after Bonferroni correction).

Our results indicate significant differentiation between the *S. mystax* subspecies analyzed, *S. m. mystax* and *S. m. pileatus*, which are parapatrically distributed. Conversely, Saguinus imperator showed no significant morphometric differentiation between its subspecies *(S. i. imperator* and *S. i. subgrisescens*), which also occur in parapatry. However, this result may be explained by the lack of statistical power due to the small sample size of *S. i. imperator* (n=3). The interspecific test resulted in significant variation among all species, except for *S. inustus* (Tab. 4), which we attribute to the small sample size (n = 2).

Considering the results of univariate statistical analyses (supporting information S2 and S3), we detected significant differences among species only in the following measurements: i) BR-LD (distance between bregma and lambda) showed significant difference (ANOVA F = 10.82, p <0.01) among *S. labiatus*, *S. mystax* and *S. inustus* (Fig. 1A) ii) LD-AS (distance between lambda and asterion) showed that, on average, *S. imperator* is smaller than *S. labiatus and S. mystax* (ANOVA F = 5.84, p <0.01) (Fig. 1B) iii) OW (orbital width) showed that, on average, *S. mystax* is larger than *S. labiatus* and *S. imperator* (ANOVA F = 12.4, p <0.01) (Fig. 1C).

In summary, this study showed that the multivariate analysis of quantitative morphological variation detected significant differences among currently recognized species of *S. mystax* group. The univariate analyses, on the other hand, showed too much overlap among species for most measurements, even for those three which showed significant differentiation among some pairs of species.

We conclude that the degree of differentiation found was not sufficient to discriminate among currently recognized taxa in the *S. mystax* group, especially in the subspecies level, unlike the works of Ackermann & Cheverud (2002) and Natori & Hanihara (1992). However, we cannot disregard problems related to the small sample size of some taxa (as *S. inustus* and *S. imperator imperator*), the lacking of available specimens of *S. mystax pluto* and *S. labiatus thomasi*, or even problems related to the selection of measurements.

Table 3: Pairwise test for differentiation between groups resulted from NPAMOVA analysis (F = 4.31) as indicated by corrected p Values (Bonferroni). Bold values indicate significant differences among groups.

	S. i. imperator	*S. i. subgrisescens*	*S. l. labiatus*	*S. m. pileatus*	*S. m. mystax*	*S. inustus*
S. i. imperator		0.0307	0.0773	**0.0046**	**0.0009**	0.0998
S. i. subgrisescens	0.0307		**0.0042**	**0.0017**	**0.0002**	0.0816
S. l. labiatus	0.773	**0.0042**		0.0107	**0.0001**	0.0389
S. m. pileatus	**0.0046**	**0.0017**	0.0107		**0.0036**	0.1255
S. m. mystax	**0.0009**	**0.0002**	**0.0001**	**0.0036**		0.276
S. inustus	0.0998	0.0816	0.0389	0.1255	0.2676	

Morphometric analyses may be useful to detect some degree of differentiation among species, as observed in this study. But taking into account that, in other studies, quantitative morphometrics was also insufficient to demonstrate complete differentiation in *Saguinus* (e.g. Gregorin & Vivo 2013), we believe that the use of morphometric analyses should not be considered as a definitive method for taxa differentiation and delimitation in *Saguinus*.

Table 4: Corrected p Values (Bonferroni correction) resulted from NPAMOVA analysis with species group.

	S. imperator	*S. labiatus*	*S. mystax*	*S. inustus*
S. imperator		**0.0139**	**0.0001**	1
S. labiatus	**0.0139**		**0.0001**	1
S. mystax	**0.0001**	**0.0001**		1
S. inustus	1	1	1	

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