VARIATION IN CRANIO-FACIAL MORPHOLOGY SUPPORTS SPECIES-LEVEL DISTINCTIONS OF THE WHITE-MOUTHED TAMARINS (*LEONTOCEBUS*) IN PERU

Leila M. Porter
Tyler D. Kotowski

Introduction

The tamarins are a diverse radiation of callitrichines with a wide geographic distribution which extends from Panama south through the Amazon basin. Hershkovitz (1977) divided the tamarins into ten species and 33 subspecies and placed them into six groups (Hershkovitz, 1977). One of these groups, the "*nigricollis* group", is comprised of white-mouthed tamarins found throughout the western Amazon basin (Hershkovitz 1977). Historically, this "*nigricollis* group" was categorized into two species and 15 subspecies based on their geographic distributions, pelage patterns (Hershkovitz 1977). Recent genetic and morphological evidence however, inicates that Hershkovitz' taxonomic arrangement of tamarins requires revision as it does not properly represent their phylogenetic history.

Genetic comparisons across the tamarins indicate that these monkeys are more diverse than previously recognized. For example, Cropp *et al.* (1999) and Buckner *et al.* (2015) divide the tamarins into large-bodied and small-bodied clades on the basis of differences in mtDNA and nuclear DNA. Matauschek et al. (2011) estimate that the tamarins diverged from other callitrichines at 14.23 mya with a further split at 10.07 mya between the small-bodied "*nigricollis* group" and the larger-bodied tamarins. These results were supported by analyses of Buckner *et al.* (2015) who estimate these divergence times at 15-13 mya and 8-11mya respectively. As a result of this evidence, Rylands and colleagues (2016) reclassified members of the "*nigricollis* group" as their own genus, *Leontocebus*. In addition to this generic change, genetic comparisons of different populations of *Leontocebus* in Peru, led Matauschek *et al.* (2011) to elevate five of the Peruvian subspecies recognized by Hershkovitz (1977) to the species level. The phylogenetic species concept defines species as "an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent" (Cracraft 1989, page 35). Thus, if these newly recognized species represent "diagnosably distinct" species, it is important to determine if these taxa are also diagnosable based on morphological differences as Marroig *et al.* (2004) did for the "*jacchus* group" of marmosets.

The main objective of this study therefore, was to assess whether five of the *Leontocebus* subspecies elevated to the species-level based on molecular data (Matauscheck *et al.* 2011), have different craniofacial morphology. If so, these data will lend support to the recent taxonomic revisions proposed by Matauschek *et al.* (2011).

Methods

We measured *Leontocebus* specimens in the Mammals Collection at the Field Museum of Natural History (FMNH), Chicago. The species of tamarin included: *Leontocebus illigeri*, *Leontocebus lagonotus*, *Leontocebus leucogenys*, *Leontocebus nigrifrons*, and *Leontocebus weddelli weddelli* (Table 1). We measured four individuals from each taxa, for a total of twenty adult crania (Table 1). We identified adult crania as those which had fully fused cranial sutures and fully descended upper canines, as well as sharply defined superior temporal ridges. We took a total of thirty linear measurements following the methods of Marroig *et al.* 2004 (Tables 2 and 3), three times in order to minimize measurement error on each specimen; the mean of each repeated measurement as used in further analyses. We took measurements from the right side of the skull. We measured all specimens to the nearest 0.01 mm with Neiko Tools digital calipers, model 01407A.

We took the species map of Matauschek *et al.* (2011) and superimposed the collection location of each museum specimen onto this map using ArcMap 10.2.2. We log-transformed and analyzed the data using the Discriminate Analysis module of IBM SPSS 23. As no sexual dimorphism in cranial traits of tamarins was found in previous studies (Ackermann, 2001), we analyzed males and females together.

Results

In all cases the FMNH subspecies classifications and collection sites for each specimen matched the geographic distribution map for each taxa elevated to the species level by Matauschek *et al.* (2011) (Figure 1). Analyses of the craniofacial measures identified fifteen variables which could be used for the canonical discriminant functions: IS-PM, IN-NSL, IS-PNS, PM-ZI, NSL-NA, NSL-ZS, NA-BR, NA-FM, NA-PNS, BR-PT, PT-FM, PT-BA, PT-EAM, PT-ZYGO and PT-TSP. All other measures failed the tolerance test (set at 0.001) and were excluded from further anlayses. Eight measures, PM-ZI, NA-PNS, IN-NSL, NSL-ZS, NSL-NA, NA-FM, IS-PM and PT-TSP were used to classify the specimens, and the analysis sorted the twenty specimens into five well-defined clusters which correspond with their species-level categories (Table 4, Figure 2; functions 1-4 Wilk's lambda (^) = 0.000, X^2 = 103.40, df = 60, p = 0.0001; functions 2-4 ^ = 0.001, X^2 = 60.59, df = 42, p = 0.031; function 3-4 ^ = 0.034; X^2 = 30.48, df = 26, p = 0.248; function 4 ^ = 0.248; X^2 =12.54, df =12, p = 0.403).

Table 1. Field Museum of Natural History (FMNH) specimens measured in this study.

FMNH Catalog Number	Species classficiation in FMNH catalog, following Hershkovitz (1977)	Species classifications according to Matauschek *et al.* (2011)	Coordinates of collection site in Peru		Sex
			Lat	Lon	
87147	*Saguinus fuscicollis illigeri*	*S. illigeri*	-4.83	-74.22	F
122754	*Saguinus fuscicollis illigeri*	*S. illigeri*	-4.83	-74.22	M
87146	*Saguinus fuscicollis illigeri*	*S. illigeri*	-4.83	-74.22	F
87145	*Saguinus fuscicollis illigeri*	*S. illigeri*	-4.83	-74.22	M
122757	*Saguinus fuscicollis lagonotus*	*S. lagonatus*	-4.28	-74.32	M
86963	*Saguinus fuscicollis lagonotus*	*S. lagonatus*	-3.77	-73.52	F
122756	*Saguinus fuscicollis lagonotus*	*S. lagonatus*	-4.28	-74.32	M
122753	*Saguinus fuscicollis lagonotus*	*S. lagonatus*	-3.83	-73.27	F
55410	*Saguinus fuscicollis leucogenys*	*S. leucogenys*	-8.85	-74.73	F
24191	*Saguinus fuscicollis leucogenys*	*S. leucogenys*	-9.30	-75.98	F
62071	*Saguinus fuscicollis leucogenys*	*S. leucogenys*	-8.30	-74.60	M
62072	*Saguinus fuscicollis leucogenys*	*S. leucogenys*	-8.30	-74.60	M
88874	*Saguinus fuscicollis nigrifrons*	*S. nigrifrons*	-4.45	-71.78	F
86965	*Saguinus fuscicollis nigrifrons*	*S. nigrifrons*	-3.43	-72.77	F
88873	*Saguinus fuscicollis nigrifrons*	*S. nigrifrons*	-4.22	-70.28	M
86958	*Saguinus fuscicollis nigrifrons*	*S. nigrifrons*	-3.43	-72.77	F
65669	*Saguinus fuscicollis weddelli*	*S. weddelli*	-13.40	-70.72	F
84231	*Saguinus fuscicollis weddelli*	*S. weddelli*	-12.78	-71.22	M
79880	*Saguinus fuscicollis weddelli*	*S. weddelli*	-14.00	-69.00	F
84230	*Saguinus fuscicollis weddelli*	*S. weddelli*	-12.78	-71.22	M

Table 2. Craniofacial landmarks recorded from tamarin crania

Landmark	Description	Position(s)
IS	Intradentale superior, A	Midline
PM	Premaxillary suture at the alveolus, A	Right, Left
NSL	Nasale, A	Midline
NA	Nasion, A	Midline
BR	Bregma, AP	Midline
PT	Pterion, AP	Right, Left
FM	Fronto-malare, A	Right, Left
ZS	Zygomaxillare superior, A	Right, Left
ZI	Zygomaxillare inferior, A	Right, Left
MT	Maxillary tuberosity, A	Right, Left
PNS	Posterior nasal spine, A	Midline
APET	Anterior petrous temporal, A	Midline
BA	Basion, AP	Midline
OPI	Opisthion, AP	Midline
EAM	Anterior external auditory meatus, A	Right, Left
PEAM	Posterior external auditory meatus, A	Right, Left
ZYGO	Inferior zygo-temporal suture, A	Right, Left
TSP	Temporo-spheno-parietal junction, A	Right, Left
TS	Temporo-sphenodial junction at petrous, AP	Right, Left
JP	Juglar process, AP	Right, Left
LD	Lambda, P	Midline
AS	Asterion, P	Right, Left
Designation A (anterior) or P (posterior) after landmark indicates which position(s) the landmark was recorded (from from Marroig *et al*., 2004).		

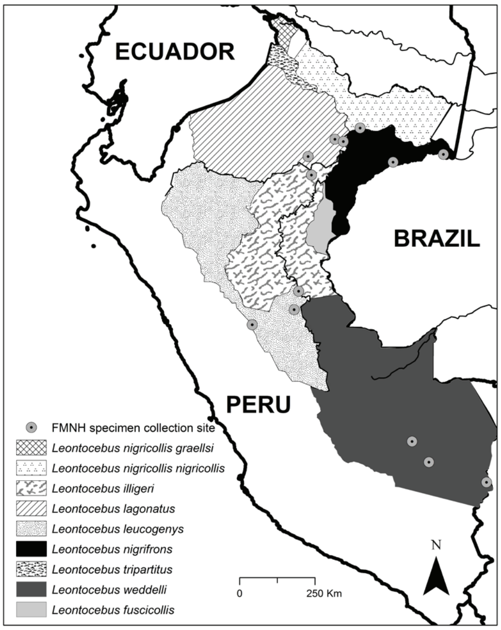

Figure 1. Locations where the FMNH specimens were collected. Coordinates were obtained from the FMNH Mammals Collection digital data base.

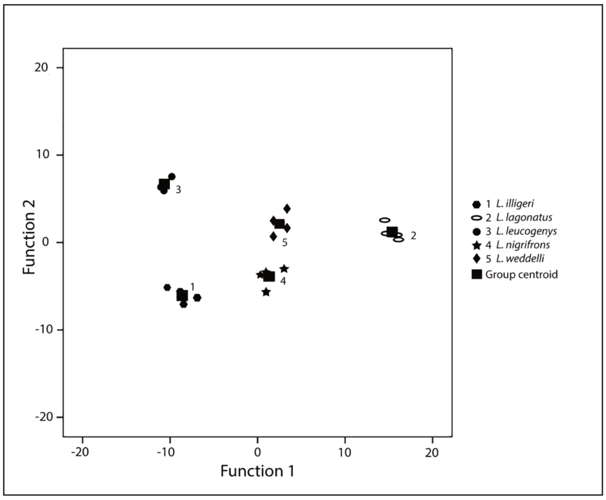

Figure 2. Discriminant analysis plot of the specimens based on eight craniofacial measurements. The black squares represent the centroid for each group.

Table 3. Thirty linear craniofacial measurements calculated from the landmarks in Table 2.

IS-PM	PT-FM	PNS-BA
IS-NSL	PT-BA	BA-EAM
IS-PNS	PT-EAM	EAM-ZYGO
PM-ZI	PT-ZYGO	ZYGO-TSP
NSL-NA	PT-TSP	LD-AS
NSL-ZS	FM-ZS	BR-LD
NA-BR	FM-MT	OPI-LD
NA-FM	ZS-ZI	PT-AS
NA-PNS	ZI-ZYGO	JP-AS
BR-PT	MT-PNS	BA-OPI
Landmark acronyms are defined in Table 2		

Table 4. Structure matrix developed from the distances between craniofacial landmarks. The values represent the pooled within-group correlations between discriminating variables and standardized canonical discriminant functions.

	Function			
Linear Craniofacial Measurements	**1**	**2**	**3**	**4**
PM-ZI	0.660	-0.074	0.155	-0.048
NA-PNS	0.059	-0.042	0.085	-0.024
IS-NSL	0.053	-0.015	0.197	-0.295
NSL-ZS	0.063	-0.002	0.184	0.263
NSL-NA	0.017	0.022	0.062	0.188
NA-FM	0.081	-0.034	-0.128	-0.164
IS-PM	0.086	-0.075	-0.103	0.154
PT-TSP	0.096	0.003	0.093	-0.151

Discussion

Our results demonstrate that the specimens we measured at the FMNH can be divided into five morphologically distinct groups which correspond with the species classifications proposed by Matauschek *et al.* (2011). Therefore, our data support the elevation of the subspecies within the "*nigricollis* group" to the species level.

Morphological comparisons have also proven useful for developing hypotheses about the evolutionary processes which led to tamarin diversification and speciation. For example, Ackermann and Cheverud (2002) investigated craniofacial variation among tamarins and found support for Garber's (1992) hypothesis that there was selection for smaller size in the "*nigricollis* group" as they became increasingly specialized to forage on large vertical supports. Furthermore, Marroig *et al.* (2004) compared craniofacial traits across the "*jacchus* group" of marmosets and hypothesized that speciation among these monkeys was driven by allopatric speciation resulting from rapidly changing climatic conditions in the last 0.5-1.5 million years. We suggest that additional morphological comparisons among the tamarin species in the "*nigricollis* group" could allow for a better understanding of their evolutionary history.

Given the genetic and morphological support for the species level classifications within the white-mouthed tamarins, additional studies should be undertaken to determine if similar craniofacial differences are present among other species and subspecies of *Leontocebus*. To date few major behavioral and ecological differences have been documented among the "*nigricollis* group" (Rylands *et al.* 2016), thus, different hypotheses should be tested to better understand if their differences are due to genetic drift or other evolutionary processes (Cropp *et al.*, 1999; Ackerman and Cheverud, 2002). Furthermore, additional studies are needed to assess the population sizes of these taxa and the threats they face, as these data are crucial for assessing their conservation status.

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Leila M. Porter, Department of Anthropology, Northern Illinois University, DeKalb, IL USA, E-mail: <lmporter@niu.edu> and **Tyler D. Kotowski**, Department of Biology, Northern Illinois University, DeKalb, IL USA.

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COLOMBIAN BLACK-SPIDER MONKEY (*ATELES FUSCICEPS*) IN TATAMÁ NATIONAL NATURAL PARK, WESTERN COLOMBIA

María Elena Giraldo
Carolina Guzmán Valencia
Luis Enrique Gallego López
Luis Guillermo Gallego Patiño
Libaniel Osorio Parra
Jhon Eduar Rojas Osorio
Ricardo José Pérez Montalvo
Néstor Roncancio Duque

Introduction

The rarity of a taxon may be expressed in three ways: a restricted range (endemicity), low population densities, and demanding ecological requirements (habitat specialist) (Rabinowitz *et al.* 1986; Gaston 1994). Taxa with small geographic ranges are intrinsically vulnerable to habitat transformation even over relatively small areas, exacerbated if their population densities are low, and they are habitat specialists or otherwise, demanding in terms of specific ecological requirements. Specific habitat conditions may be high productivity and broad and functional connectivity to supply the needs of a viable population. If these conditions are not available, populations may be rapidly depleted and isolated (Rabinowitz and Zeller 2010).

The original range of *Ateles fusciceps* in Colombia extended approximately 142,000 km2 (Hernández-Camacho and Cooper 1976, Rodríguez-Bolaños *et al.* 2013), but has been reduced 35% with a total current rate of deforestation of 0,2% per year. Habitat lost has occurred mainly in the Caribbean region with an increasing rate of deforestation from 1.55% per year between 2002 to 2009, to 2.98% per year between 2009 – 2012 (IDEAM 2002, 2009, 2012). Of 92,300 km2 of remnant forest in the Colombian range of this species, only 850 km2 are in national natural parks, and in most there are no confirmed records of its presence. These parks partially overlap with indigenous and afrocolombian communities that hunt them for food. Their current distribution is unknown, we have no information where they occur and, no data on the sizes of remaining populations and their growth rates are available.

Spider monkeys have features like its body mass (7-9kg), diet (up to 85% of their diet is composed of ripe fruit), reproduction (1 birth every 3-4 years), activity patterns (1.5-3.5 km traveled per day) and home range (60-350ha) that make them extremely vulnerable to the loss, reduction and fragmentation of habitat (Chapman and Onderdonk 1998; Stevenson *et al.* 2002; Link and Di Fiore 2006; Takahashi, 2008; Urbani *et al.* 2008; Defler, 2010). Thus, the current rate of habitat loss, the likely high hunting pressure, plus its intrinsically vulnerability, *A. fusciceps* is categorized as Critically Endangered (CR); it is estimated that more than an 80% population decline has occurred over the past 45 years (based on a generation time of 15 years) (Cuarón, et al 2008).

To plan effective strategies for the conservation of wildlife species, it is necessary to do a quantitative diagnosis of their conservation status, measuring some indicators that compared over time can lead to evaluate the effect of the implemented interventions. In order to evaluate the conservation status of the Colombian Black-spider monkey, it is necessary to know the current distribution and the current available habitat (size and spatial configuration) as well as its population density in different zones of the landscapes. Therefore, the location of remnants population must be documented.

Records of *Ateles fusciceps*

Tatamá National Natural Park is one out of the 10 national protected areas that probably has populations of this taxon; the park is in the eastern border of its distribution, where the Andean (left margin of the Cauca River) and the Pacific regions get in contact. While carrying out sampling activities of the monitoring plan of Tatamá National Natural Park, we obtained two records of *Ateles fusciceps*. Eight individuals of *A. fusciceps* were seen moving and foraging at 1,780 m a. s. l. (5°13′48′′N, -76°05′57′′W, Fig. 1) and three individuals were seen at 700 m a.s.l. in the Área de Manejo Especial de Comunidades Negras Alto Amurrapá, an afrocolombian community adjacent to the park (05°18′56.8′′N - W 076°09′06.5′′W). Based on these observations, Tatamá National Natural Park is the only confirmed National Park that protects this taxon at the eastern border of its distribution.

Discussion

The borders of the distribution of any species are usually marginal habitat with low population density, compared with the core areas (Hengeveld and Haeck, 1982, Soley-Guardia, et al 2014); thus, the fact that our records are in a limit of the distribution of the species, makes this eastern population of the Tatamá Natural Park more vulnerable. Another important aspect lies on the fact that this park