Morphological Comparisons of Five Species of Tamarins in Peru

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Abstract Tamarins of the genus *Leontocebus* (formerly *Saguinus*), subfamily Callitrichinae, represent one of the most diverse primate taxa but detailed information about their phylogeny is still lacking. Recent molecular phylogenetic analyses have led to the reclassification of many taxa. In Peru, ten subspecies of tamarins were reclassified as eight new species and four new subspecies based on genetic differences among these taxa. However, no one has attempted to determine whether these new "genetic species" have distinguishable morphological traits. To do this, we examined twenty crania and skins representing five Peruvian "genetic species" housed in the Mammals Collection at the Field Museum of Natural History in Chicago, Illinois. We measured thirty linear craniofacial characters using digital calipers and photographed the pelage of all specimens. We log-transformed and analyzed the data using the Discriminant Analysis module of SPSS 23. We qualitatively compared the pelage color and pattern of all furs. Our study demonstrates that the "genetic species" can be distinguished by their cranial anatomy. Thus, the "genetic species" represent morphologically distinct populations, as is expected if they represent evolutionarily distinct taxa.

Introduction

Defining a species is important not just for taxonomy and systematics, but also for a wide variety of other fields such as biogeography, ecology, and conservation biology (Groves, 2012). Historically, animals were divided into different taxa based on their physical appearance,

beginning with *Systema Naturae* by Carl Linnaeus in 1735. In the 20th century taxonomists expanded on the Linnaean system using comparative anatomy as a means for subjectively distinguishing taxa from each other. In the 21st century, taxonomists have increasingly used comparisons of DNA to help determine the taxonomy of different groups. Some of these molecular phylogenies have led to surprising results, as animals which appear similar may actually have quite different classifications. For example, the mouse lemurs, *Microcebus*, of Madagascar were historically considered to be one species based on morphological similarities; however, genetic studies have led to the identification of more than ten distinct species since 2000 (Rylands, 2007). Molecular analyses may therefore be useful in identifying populations which superficially appear similar, but which actually represent genetically distinct populations. However, morphological comparisons of these species identified by their DNA are important because they can help scientists to determine if these populations represent distinct species or local variants within a species.

There is considerable debate about how species should be defined. One of the most widely cited species concepts is the Biological Species Concepts proposed by Mayr (1942). He defines species as groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups (Mayr, 1942). However, this species concept is problematic with allopatric speciation because it does not offer criteria with which to decide if different groups warrant species status or subspecies status. Another problem with this species concept is with hybridization. Hybridization is a problem because some species which appear distinct, have been found to interbreed with one another (which is in violation with reproductive isolation). Lastly, there is a problem with asexual reproducing species because by nature all these organisms would be reproductively isolated. These cases proved serious drawbacks to this

definition of species, because it makes the species concept unrepeatable and unfalsifiable, and thus not strictly scientific (Groves, 2012). As a result, several other species concepts have been proposed.

The Morphological Species concept proposed by Linnaeus and later expanded upon by Darwin defines species as: "Given for the sake of convenience to a set of individuals closely resembling each other, and that it does not essentially differ from the term variety, which is given to less distinct and more fluctuating forms (Darwin, 1859, page 52)." The problem with this method of identifying species, is that it is subjective, as how much species must resemble one another may be disagreed upon, and what features should be used to distinguish a species is not always clear. For example, Hershkovitz (1977) used the Morphological Species concept to give species names to the callitrichines, and as a result, this is one of the primate lineages with the most controversial taxonomies (Digby, 2007).

More recently, geneticists have embraced the Phylogenetic Species Concept proposed by Cracraft. He defines a species as follows: "A phylogenetic species is 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, pages 34-35)." So species have fixed heritable differences, and they are genetically isolated, though not necessarily reproductively isolated.

For living taxa, it should be possible to assess whether they represent "good" species by determining whether they meet the criteria of several of these species concepts. For example, two lineages which have been separated by many generations should have both distinctly different DNA and distinctly different anatomical, physical and/or behavioural traits.

Tamarins provide an interesting case study for examining species concepts. Tamarins represent one of the most diverse primate taxa, but the taxonomy and phylogeny of this group is still debated (Digby et al., 2007; Rylands et al., 2016). Tamarins are squirrel sized primates (their body lengths range from 13 to 30 cm, their tails are 25 to 44 cm long, and they weigh from 220 to 900 grams) that are diurnal, arboreal, frugivore-insectivores of the forests of South and Central America (Digby et al., 2007; Rylands et al., 2016). They are members of the Platyrrhines, the New World monkeys, in the family Callitrichinae. Like the other callitrichines, tamarins have small body sizes, claw-like nails, twin births, postpartum ovulation, cooperative care of young, one less molar than other Platyrrhines, and dramatic variation in coloration (including ear tufts and "mustaches")(Digby et al., 2007).

Studies have shown members of the *nigricollis* group are ecologically and behaviourally similar (Ryland et al., 2016). A notable feature to this group is the prevailing use of the lower forest strata and the higher proportion of leaping between vertical trunks (Ryland et al., 2016). They are also distinct in their prey foraging behavior: they search for prey concealed in knotholes, crevices, bromeliad tanks, leaf litter, and other substances (Ryland et al., 2016). Even though their prey foraging behavior is distinct their social structure is similar to other tamarins. Groups contain between two and eleven individuals and polyandry as their prevailing social mating system (Ryland et al., 2016). So, members of the *nigricollis* group differ from other tamarins with regard to forest strata use, locomotion, and prey foraging techniques.

Tamarins historically were classified as ten species and 33 subspecies which were divided into six groups (Hershkovitz, 1977). One of these tamarin groups Hershkovitz (1977) identified was the aforementioned *nigricollis* group, which is found in the Amazon basin (Figure 1). Hershkovitz (1977) divided the smaller bodied *nigricollis* group into two species and ten

subspecies based on their geographical distributions, body sizes, craniofacial morphology, pelage patterns, and pelage coloration. Hershkovitz' taxonomy has been used by researchers studying the behavior and ecology of the tamarins in the Amazon region for the last 4 decades (e.g. Garber, 1991, 1992, 1993, 2016; Peres, 1993b, 1996; Goldizen,1988, 1996; Heymann, 1997, 2000, 2000b, 2001).

However, genetic analysis indicates that taxonomy of Hershkovitz requires revision. Genetic studies show that the tamarins are sister to all other callitrichids, diverging 15-13 Ma (Rylands et al., 2016). The small-bodied *nigricollis* group diverged from the remaining, larger tamarins 11-8 Ma (Rylands et al., 2016). As a result, Rylands and colleagues (2016) have reclassified members of the *nigricollis* group as a new genus *Leontocebus* (previously *Saguinus*). Furthermore, comparisons of the tamarins' DNA have led to their reclassification as eight species and four subspecies under the framework of the Phylogenetic Species Concept (Table 1) (Matauschek et al., 2011).

Given that the new molecular data suggests that six of the Peruvian subspecies within the *nigricollis* tamarin group should be elevated to the species level, in this research project we asked, are these "genetic species" Matauschek identified based on DNA identifiable as distinct groups based on their morphology? That is do these "genetic species" have identifiable morphological differences? This question is important because many primate taxonomies have been modified based on genetic differences in the recent years (like the mouse lemurs from Madagascar); however, in many cases no one has tested to see whether these genetic differences correspond with differences in behavior and morphology.

Materials and Methods

Leontocebus specimens were obtained from the Mammals Collection at the Field Museum of Natural History in Chicago, Illinois. The species of tamarin include: *Leontocebus illigeri*, *L. lagonotus*, *L. leucogenys*, *L. nigrifrons*, and *L. weddelli weddeli* (Figure 2 and Table 2). We measured a total of twenty adult crania consisting of four specimens from each taxon. Our criteria for adult crania were that the crania had fully fused cranial sutures, the upper canines were fully descended, and the crania had sharply defined superior temporal ridges. We did this to avoid ontogenetic size changes which occur over the course of development from infancy to adulthood. We took a total of thirty linear measurements (Figure 3, Tables 3 and 4) three times on each skull in order to minimize measurement error on each specimen; the mean of the three measurements was used in further analysis. We did not take measurements of any skulls which were broken, and we took measurements from one side of the skull. We measured all specimens to the nearest 0.01 mm with Neiko Tools digital calipers, model 01407A. We log-transformed and analyzed the data using the Discriminate Analysis module of IBM SPSS 23. There is no sexual dimorphism in cranial traits of tamarins, so males and females were analyzed together (Ackermann, 2001). Photos of the pelage were taken with a Samsung Galaxy S5 for qualitative comparison (Figure 4). All photos were color normalized using a Camera Trax 24 Color Card.

Results

We compared the morphology of the five *Leontocebus* species with the primary purpose of determining whether their cranial measurements clustered within the five species designations given to these taxa by geneticists. This classification analysis was based upon the 30 craniofacial characters measured on each specimen. In an overall discriminant analysis, the five *Leontocebus* species were sorted into well-defined clusters differing markedly on both size and shape (Figure 5). All specimens were classified with 100% accuracy into their taxonomic groups. Figure 5 shows a clear gradient of size along the axis of Function 1 and a clear secondary gradient of shape on Function 2. Thus, these analyses provide evidence that the five *Leontocebus* species are distinctly different in their morphology.

Differences in tamarin morphology can also be seen in pelage coloration (Groves, 2001). However, qualitative comparison of the five *Leontocebus* species furs (Figure 4) to the artistic renditions (Figure 2) shows similar dorsal coloration. The only noticeable difference between the five species is the slight difference in coloration of the front legs and the hind legs (which is shown in the artistic renditions in Figure 2). This can be subjective and might not be shown in all the members of each taxon. The preservation of the furs by the Field Museum of Natural History limits the comparison of these animals pelage, as not all parts of the animals are preserved: for example, the facial pelage is not well preserved in any of the specimens. Since these five species have extremely similar pelage this could explain why they have previously been grouped together under one species making them a cryptic species.

Discussion

The taxonomic challenge posed by cryptic species (two or more distinct species classified as a single species) has been recognized for nearly 300 years, but the advent of relatively inexpensive and rapid DNA sequencing has given biologists a new tool for detecting and differentiating morphologically similar species. The frequency with which cryptic species are uncovered with DNA sequence data (and often subsequently confirmed with morphological and/or ecological data) suggests that molecular data should routinely be incorporated in the research of taxonomists and/or that genetic material should be preserved so that subsequent molecular analysis is possible (Bickford, 2006). Our results demonstrate that the individuals we measured came from five morphologically distinct populations which correspond exactly with their new species classifications as suggested by the DNA evidence from Matauschek (2011). These results are expected if they represent evolutionarily distinct taxa. This provides evidence that DNA sequence data is a valid research method for taxonomists, and vice versa.

Future studies could also compare vocalizations and behavior of the tamarin taxa. Rylands et al. (2016) sited that the *nigricollis* tamarin group are ecologically and behaviorally similar (Rylands et al., 2016). These tamarins generally have similar diets, social organization, forest strata use, locomotion, and prey foraging techniques (Rylands et al., 2016). However, long-term studies are restricted to only a few species within the *nigricollis* group, thus the variation among taxa may not yet be known (Rylands et al., 2016). Since these generalizations have been made based upon few long-term studies, the addition of more species specific studies could provide new information to determine if taxa differ in some aspects of their behavior and ecology.

A characteristic shared by all endangered callitrichine species is a relatively small geographic range combined with critical levels of anthropogenic habitat alteration (Digby, 2007). Accurate species identifications are often crucial for the diagnosis and prevention of disease, and the identification of invasive and pest species (Bickford, 2006). Also, the identification and description of cryptic species have important implications for conservation programs for each taxon and natural resource protection management (Bickford, 2006). Under the "old" taxonomic group, the *nigricollis* group has a large geographic range (Figure 1) and would likely be considered to be low risk of extinction; however, the "new" species have much smaller geographic ranges, with smaller population sizes, and thus they may be under much greater risks of extinction if there are local threats to the areas (Figure 2). For example, the mouse lemurs of Madagascar, when they were classified as one species, had a large geographic range across the island but, when their taxonomy was changed, the geographic range of each species was actually determined to be very small, as well as the population size for each species. As a result, according to the IUCN Red List, the mouse lemurs went from being one species of least concern in 2000, to three species being critically endangered (*Microcebus gerpi*, *M. marohita,* and *M. mamiratra*), eleven species being endangered (*M. arnholdi*, *M. berthae, M. bongolavensis*, *M. mittermeieri*, *M. danfossi*, *M. margotmarshae*, *M. jollyae*, *M. macarthurii*, *M. ravelobensis*, *M. sambiranensis*, and *M. simmonsi*), four species being vulnerable (*M. lehilahytsara*, *M. myoxinus*, *M. rufus*, and *M. tavaratra*), and two species being of least concern (*M. griseorufus*, and *M. murinus*) (IUCN, 2015). The IUCN Red List has not yet recognized the five *Leontocebus* species we studied, thus these taxa are listed as being of "least concern" as *Saguinus fuscicollis* subspecies. However, the new species names should be adopted and these taxa's conservation status reevaluated.

Habitat loss is perhaps the greatest challenge for the conservation of global biodiversity, and prioritizing habitats for conservation often relies on the estimation of species richness and endemism (Bickford, 2006). This study shows that phylogenetic species named from genetic evidence corresponds with morphologically distinct taxa, thus these taxa warrant conservation programs which acknowledge them each as unique species.

References

Ackermann RR, Cheverud JM. 2002. Discerning Evolutionary Processes in Patterns of Tamarin (Genus *Saguinus*) Craniofacial Variation. *American Journal of Physical Anthropology* 117: 260-271.

Aguiar JM, Lacher TE. 2003. On the Morphological Distinctiveness of *Callithrix humilis* Van Roosmalen et al., 1998. *Neotropical Primates* 11(1): 11-18.

Aquino R et al. 2015. *Primates of Peru: Pocket Guide*. Series editors Mittermeier RA, Rylands AB. Arlington, Va: Conservation International.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2006. Cryptic Species as a Window on Diversity and Conservation. *Trends in Ecology and Evolution* 22(3): 148-155.

Buckner JC, Alfaro JL, Rylands AB, Alfaro ME. 2014. Biogeography of the Marmosets and Tamarins (Callitrichidae). *Molecular Phylogenetics and Evolution* 82: 413-425.

Cheverud JM. 1995. Morphological Integration in the Saddle-Back Tamarin (*Saguinus fuscicollis*) Cranium. *The American Naturalist* 145: 63-89.

Cracraft J. 1989. Speciation and the Ontology: the Empirical consequences of Alternative Species Concepts for Understanding Patterns and Processes of Differentiation. In: Otte D, Endler JA, editors. *Speciation and its Consequences*. Sunderland, MA: Sinauer Associates.

Darwin, CR. 1859. *On the origin of species by means of natural selection, or the preservation of favored races in the struggle for life*. London: John Murray.

Digby JL, Ferrari SF, Saltzman W. 2007. Callitrichines: The Role of Competition in Cooperatively Breeding Species. In: MacKinnon K.C., Panger M, Bearder S, Campbell C, Fuentes A, editors. *Primates in Perspective*. Oxford: Oxford University Press. p. 91-107.

Garber PA. 1991. A comparative study of positional behavior in three species of tamarin monkeys. *Primates* 32: 219–230.

Garber PA. 1992. Vertical clinging, small body size, and the evolution of feeding adaptations in the Callitrichinae. *American Journal of Physical Anthropology* 88: 469–482.

Garber PA. 1993. Feeding ecology and behavior of the genus *Saguinus*. In: Rylands AB, ed. *Marmosets and tamarins: systematics, behavior, and ecology*. Oxford: Oxford University Press, 273–295.

Garber PA, Porter LM, Spross J, Di Fiore A. 2016. Tamarins: insights into monogamous and non-monogamous single female social and breeding systems. *American Journal of Primatology in press*, doi: 10.1002/ajp.22370.

Goldizen AW. 1988. Tamarin and marmoset mating systems: unusual flexibility. *Trends in Ecology and Evolution* 3: 36–40.

Goldizen AW, Mendelson J, van Vlaardingen M, Terborgh J. 1996. Saddle-back tamarin (*Saguinus fuscicollis*) reproductive strategies: evidence from a thirteen-year study of a marked population. *American Journal of Primatology* 38: 57–83.

Groves C. 2001. *Primate Taxonomy*. Washington D.C.: Smithsonian Institute Press.

Groves C. 2012. Species Concept in Primates. *American Journal of Primatology* 74(8): 687-691.

Hershkovitz P. 1977. *Living New World Monkeys (Platyrrhini)*. Chicago: University of Chicago Press.

Heymann EW. 1997. The relationship between body size and mixed-species troops of tamarins. *Folia Primatologica* 68: 287–295.

Heymann EW. 2000b. The number of adult males in callitrichine groups and its implications for callitrichine social evolution. In: Kappeler PM, ed. *Primate males*. Cambridge: Cambridge University Press, 64–71.

Heymann EW. 2001. Interspecific variation of scent-marking behavior in wild tamarins, *Saguinus mystax* and *Saguinus fuscicollis*. *Folia Primatologica* 72: 253–267.

Heymann EW, Buchanan-Smith HM. 2000. The behavioral ecology of mixed-species troops of callitrichine primates. *Biological Reviews* 75: 169–190.

Marroig G, Cropp S, Cheverud JM. 2004. Systematics and Evolution of the Jacchus Group of Marmosets (Platyrrhini). *American Journal of Physical Anthropology*. 123: 11-22.

Marsh LK. 2014. A Taxonomic Revision of the Saki Monkeys, Pithecia Desmarest, 1804. *Neotropical Primates* 21(1): 1-165.

Matauschek C, Roos C, Heymann EW. 2011. Mitochondrial Phylogeny of Tamarins (*Saguinus*, Hoffmannsegg 1807) with Taxonomic and Biogeographic Implications for the *S. nigricollis* Species Group. *American Journal of Physical Anthropology* 144: 564-574.

Mayr E. 1942. *Systematics and the Origin of Species*. New York: Columbia University Press.

Nixon KC, Wheeler QD. 1990. An Amplification of the Phylogenetic Species Concept. *Cladistics* 6(3): 211-223.

Peres CA. 1993b. Diet and feeding ecology of saddle-back (*Saguinus fuscicollis*) and mustached (*S. mystax*) tamarins in Amazonian terra firme forest. *Journal of Zoology*, London 230: 567–592.

Peres CA, Patton JL, da Silva MNF. 1996. Riverine barriers and gene flow in Amazonian saddle-back tamarins. *Folia Primatologica* 67: 113–124.

Rylands AB. 2007. Lemur diversity: a recent efflorescence of species. *Lemur News* 12:12-7.

Rylands AB, Heymann EW, Alfaro JL, Buckner JC, Roos C, Matauschek C, Boubli JP, Sampaio R, Mittermeier RA. 2016. Taxonomic Review of the New World Tamarins (Primates: Callitrichidae). *Zoological Journal of the Linnean Society*: 1-26.

"IUCN 2015". *IUCN Red List of Threatened Species*. Version 2015.4. International Union for Conservation of Nature. Retrieved 6 March 2016.

Van Roosmalen MGM, Van Roosmalen T. 2003. The Description of a New Marmoset Genus, *Callibella* (Callitrichinae, Primates), Including its Molecular Phylogenetic Status. *Neotropical Primates* 11(1): 1-10.

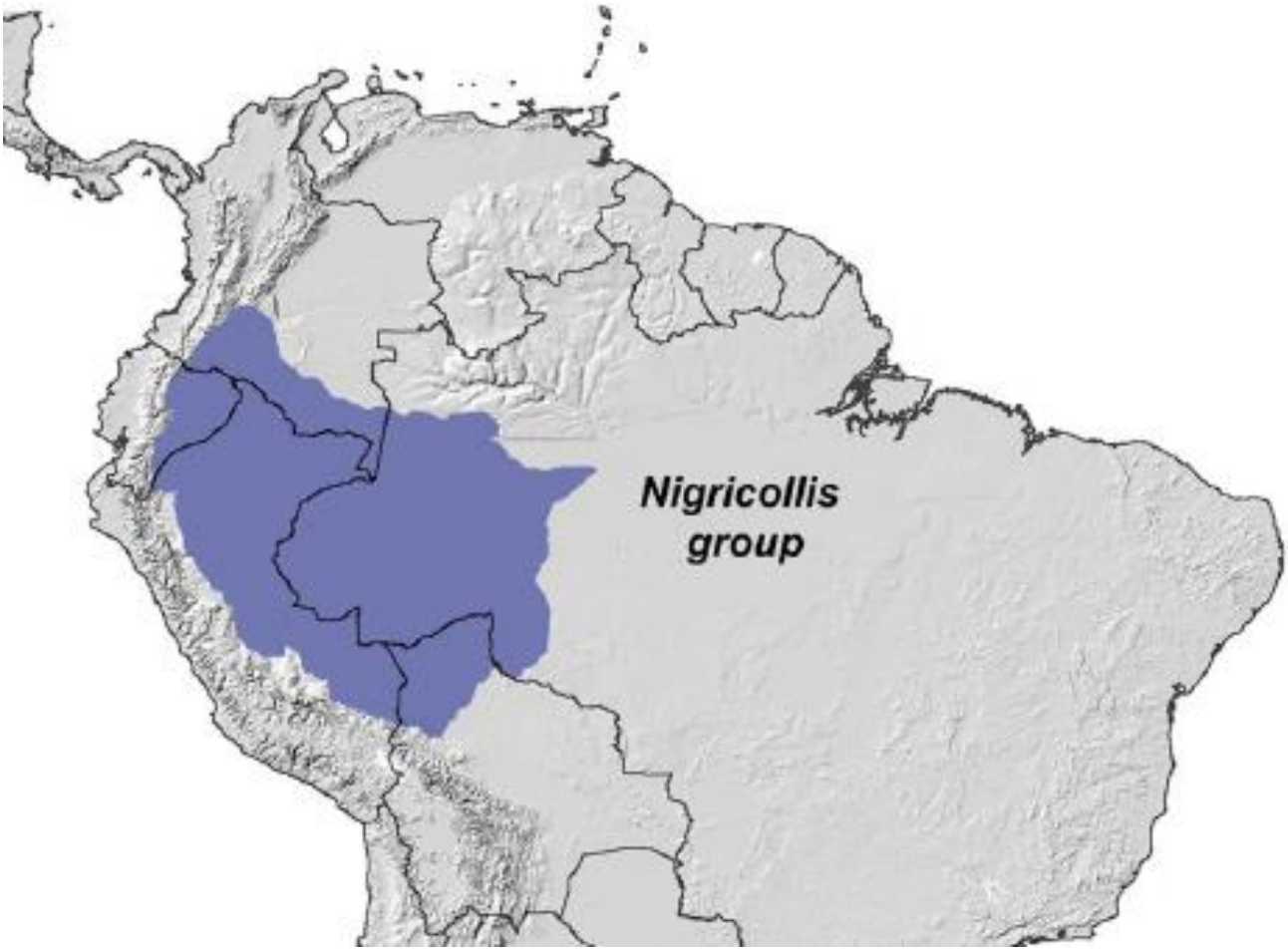

Figure 1. The geographical distribution of the nigricollis group tamarins. Map by Stephen D. Nash. © Conservation International. (Rylands, 2016).

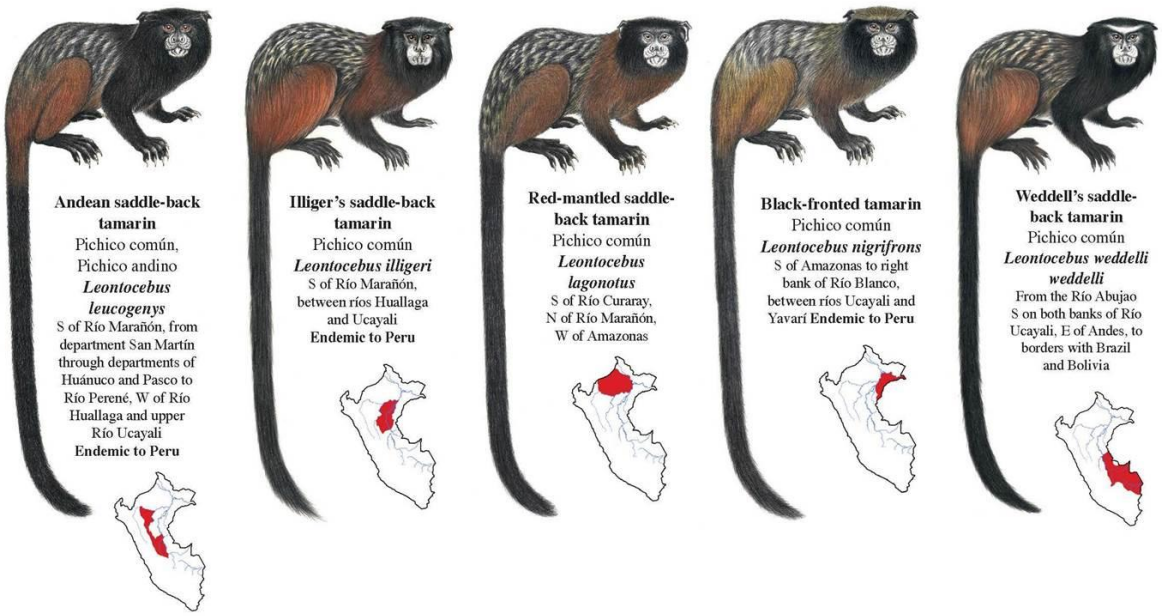

Figure 2. Tamarin species used in study. Drawings by Stephen Nash, in Aquino et al (2015).

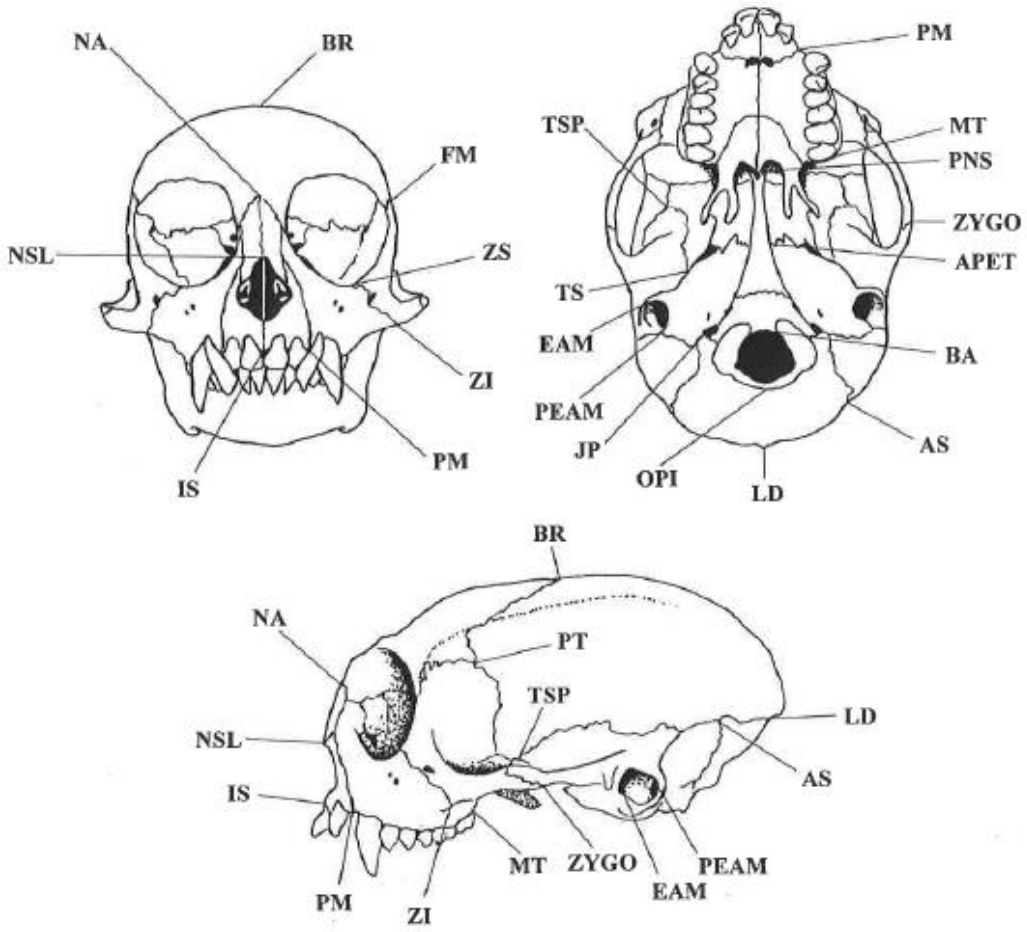

Figure 3. Tamarin craniofacial landmarks. Landmark abbreviations are spelled out in Table 1. Figure Taken from Ackermann (2002).

Figure 4. Photos of pelage. Species from left to right are *Leontocebus leucogenys, L. illigeri*, *L. lagonotus*, *L. nigrifrons*, and *L. weddelli weddeli*.

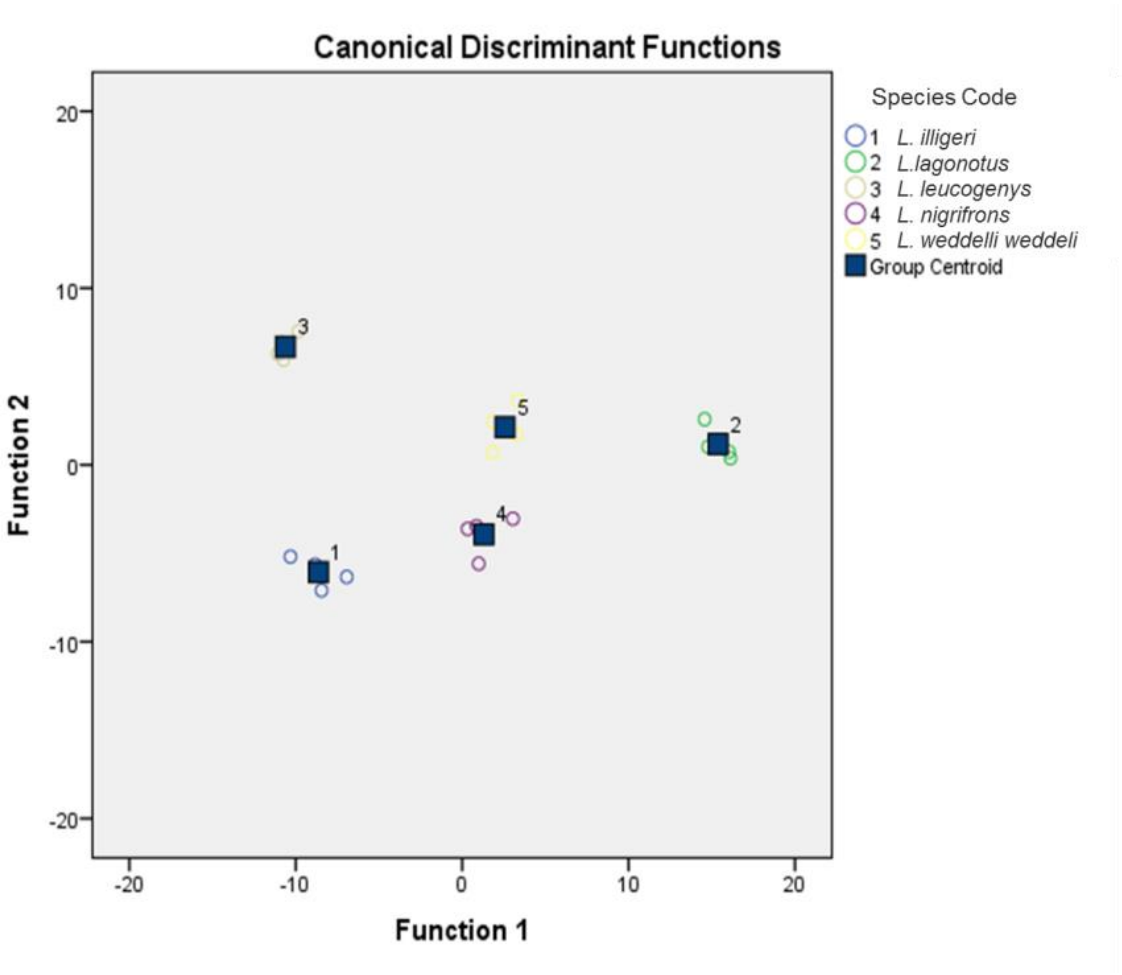

Figure 5. Discriminant analysis plot of tamarin taxa along gradients of size (Function 1) and shape (Function 2).

Table 1. Classification of tamarins examined in Matauschek et al. 2011 study, following Hershkovitz (1997) and Groves (2001).

Hershkovitz 1977	Groves 2001	Proposed classification
S. nigricollis nigricollis	*S. nigricollis nigricollis*	*S. nigricollis nigricollis*
S. nigricollis graellsi	*S. graellsi*	*S. nigricollis graellsi*[a]
S. fuscicollis fuscicollis	*S. fuscicollis fuscicollis*	*S. fuscicollis*
S. fuscicollis illigeri	*S. fuscicollis illigeri*	*S. illigeri* (including northern *leucogenys*)
S. fuscicollis leucogenys	*S. fuscicollis leucogenys*	*S. leucogenys*
S. fuscicollis nigrifrons	*S. fuscicollis nigrifrons*	*S. nigrifrons*
S. fuscicollis lagonotus	*S. fuscicollis lagonotus*	*S. lagonotus*
S. fuscicollis weddelli	*S. fuscicollis weddelli*	*S. weddelli weddelli*
S. fuscicollis melanoleucus	*S. melanoleucus melanoleucus*	*S. weddelli melanoleucus*[a]
S. fuscicollis tripartitus	*S. tripartitus*	*S. tripartitus*

[a] Differences in fur coloration support distinct status, but genetic data do not. For these taxa, additional studies are required.

Table 2. Specimens obtained from the Mammals Collection at the Field Museum of Natural History in Chicago, Illinois. Their taxonomic names are listed as they appear in the museum's catalog.

Catalog Number	Collector Number	Collector(s)	Scientific Name used byThe Field Museum	Country	Latitude	Longitude	Gender
87147	2116	C. Kalinowski	*Saguinus fuscicollis illigeri*	Peru	-4.833333333	-74.21666667	F
122754	9046	P. Hershkovitz	*Saguinus fuscicollis illigeri*	Peru	-3.30578	-74.62296	M
87146	2115	C. Kalinowski	*Saguinus fuscicollis illigeri*	Peru	-4.833333333	-74.21666667	F
87145	2114	C. Kalinowski	*Saguinus fuscicollis illigeri*	Peru	-4.833333333	-74.21666667	M
122757	9203	P. Hershkovitz	*Saguinus fuscicollis lagonotus*	Peru	-4.283333333	-74.31666667	M
86963	1917	C. Kalinowski	*Saguinus fuscicollis lagonotus*	Peru	-3.766666667	-73.51666667	F
122756	9202	P. Hershkovitz	*Saguinus fuscicollis lagonotus*	Peru	-4.283333333	-74.31666667	M
122753	9003	P. Hershkovitz	*Saguinus fuscicollis lagonotus*	Peru	-3.833333333	-73.26666667	F
55410	3183	C. C. Sanborn	*Saguinus fuscicollis leucogenys*	Peru	-8.85	-74.73333333	F
24191	6866	E. Heller	*Saguinus fuscicollis leucogenys*	Peru	-9.3	-75.98333333	F
62071	665	J. M. Schunke	*Saguinus fuscicollis leucogenys*	Peru	-8.3	-74.6	M
62072	665	J. M. Schunke	*Saguinus fuscicollis leucogenys*	Peru	-8.3	-74.6	M
88874	2433	C. Kalinowski	*Saguinus fuscicollis nigrifrons*	Peru	-4.45	-71.78333333	F
86965	2155	C. Kalinowski	*Saguinus fuscicollis nigrifrons*	Peru	-3.433333333	-72.76666667	F
88873	2357	C. Kalinowski	*Saguinus fuscicollis nigrifrons*	Peru	-4.216666667	-70.28333333	M
86958	2210	C. Kalinowski	*Saguinus fuscicollis nigrifrons*	Peru	-3.433333333	-72.76666667	F
65669	49	C. Kalinowski	*Saguinus fuscicollis weddelli*	Peru	-13.4	-70.71666667	F
84231	1655	C. Kalinowski	*Saguinus fuscicollis weddelli*	Peru	-12.78333333	-71.21666667	M
79880	155	H. H. Heller	*Saguinus fuscicollis weddelli*	Peru	-14	-69	F
84230	1654	C. Kalinowski	*Saguinus fuscicollis weddelli*	Peru	-12.78333333	-71.21666667	M

Table 3. Craniofacial landmarks recoded 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 in. Landmarks are also identified in Figure 3. Adapted from Cheverud (1995).

Table 4. Thirty linear craniofacial measurements calculated from the landmarks in Table 1

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 1 and Figure 3.