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Research Article

Molecular phylogeny of the genus *Saguinus* (Platyrrhini, Primates) based on the ND1 mitochondrial gene and implications for conservation

Claudia Helena Tagliaro[1], Horacio Schneider[2], Iracilda Sampaio[2], Maria Paula Cruz Schneider[3], Marcelo Vallinoto[2] and Michael Stanhope[4]

[1]*Universidade Federal do Pará, Campus de Bragança, Laboratório de Conservação e Biologia Evolutiva, Bragança, Pará, Brazil.*
[2]*Universidade Federal do Pará, Campus de Bragança, Laboratório de Genética e Biologia Molecular, Bragança, Pará, Brazil.*
[3]*Universidade Federal do Pará, Centro de Ciências Biológicas, Departamento de Genética, Laboratório de Polimorfismo de DNA, Belém, Pará; Brazil.*
[4]*Queen's University of Belfast, Belfast, Northern Ireland, UK.*

Abstract

The systematics of the subfamily Callitrichinae (Platyrrhini, Primates), a group of small monkeys from South America and Panama, remains an area of considerable discussion despite many investigations, there being continuing controversy over subgeneric taxonomic classifications based on morphological characters. The purpose of our research was to help elucidate the phylogenetic relationships within the monkey genus *Saguinus* (Callitrichinae) using a molecular approach to discover whether or not the two different sections containing hairy-faced and bare-faced species are monophyletic, whether *Saguinus midas midas* and *Saguinus bicolor* are more closely related than are *S. midas midas* and *Saguinus midas niger*, and if *Saguinus fuscicollis melanoleucus* and *Saguinus fuscicollis weddelli* really are different species. We sequenced the 957 bp ND1 mitochondrial gene of 21 *Saguinus* monkeys (belonging to six species and nine morphotypes) and one *Cebus* monkey (the outgroup) and constructed phylogenetic trees using maximum parsimony, neighbor joining, and maximum likelihood methods. The phylogenetic trees obtained divided the genus *Saguinus* into two groups, one containing the small-bodied species *S. fuscicollis* and the other, the large-bodied species *S. mystax*, *S. leucopus*, *S. oedipus*, *S. midas*, *S. bicolor*. The most derived taxa, *S. midas* and *S. bicolor*, grouped together, while *S. fuscicollis melanoleucus* and *S. f. weddelli* showed divergence values that did not support the division of these morphotypes into subspecies. On the other hand, *S. midas* individuals showed divergence compatible with the existence of three subspecies, two of them with the same morphotype as the subspecies *S. midas niger*. The results of our study suggest that there is at least one *Saguinus* subspecies that has not yet been described and that the conservation status of *Saguinus* species and subspecies should be carefully revised using modern molecular approaches.

Key words: Saguinus, Callitrichinae, ND1, phylogeny, mtDNA, conservation.

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Introduction

South American and Panamanian small monkeys (marmosets and tamarinds) are classified within the subfamily Callitrichinae (Primates, Platyrrhini, Ceboidea, Cebidae) (Rosenberger, 1981). In their assessment of the diversity of the New World primates, Rylands *et al*. (2000) stated that the Callitrichinae contained the six genera *Callimico* (Goeldi's monkey)*, Callithrix* (Atlantic marmosets)*, Cebuella* (pygmy marmosets)*, Leontopithecus* (lion or golden tamarins)*, Mico* (Amazonian marmosets) *and Saguinus* (tamarins), although a previous molecular phylogeny study of the New World monkeys by Schneider *et al*. (1996) had grouped *Callithrix, Cebuella* and *Mico* together in the genus *Callithrix*. The basal genus of the Callitrichinae is *Saguinus* (Barroso *et al*., 1997; Chaves *et al*., 1999; Schneider *et al*., 1996), a widely distributed genus which occurs from the Amazon basin northward into Panama (Hershkovitz, 1977; Rylands *et al.*, 1993), with both *Leontopithecus* and *Saguinus* being commonly known as tamarins (Rosenberger, 1978).

Send correspondence to C.H. Tagliaro. Universidade Federal do Pará, Campus de Bragança, Laboratório de Conservação e Biologia Evolutiva, Bragança, Pará, Brazil. E-mail: tagliaro@ufpa.br.

The traditional classification of Hershkovitz (1977, 1979, 1982) for the *Saguinus* tamarins contains 10 different species, some of which have been further divided into subspecies which now total 33 taxonomic morphotypes. Hershkovitz (1977) used facial pelage to divide the species *Saguinus* into three sections: the hairy-faced tamarin section containing the *Saguinus nigricollis* group or white-mouthed tamarins (*S. nigricollis*, *S. fuscicollis*) as well as the *Saguinus mystax* group or mustached tamarins (*S. mystax*, *S. labiatus*, *S. imperator*) and the *Saguinus midas* group or midas tamarins (*S. midas midas, S. midas niger*); the mottled-faced tamarin section containing *Saguinus inustus* only); and the bare-faced tamarin section containing the *Saguinus bicolor* group (*S. b. bicolor*, *S. b. ochraceus, S. b. martinsi*) and the *Saguinus oedipus* group (*S. oedipus*, *S. leucopus*). The most primitive group is believed to be the white-mouthed tamarins (Hershkovitz, 1977). Mittermeier *et al*. (1988) considered that there was the same total number of *Saguinus* taxonomic morphotypes (*i.e.* 33), although they divided them into 12 species instead of the 10 accepted by Hershkovitz (1977). The Brazilian taxa of Callitrichinae primates was studied by Coimbra-Filho (1990), who partially agreed with Hershkovitz (1977) but grouped the three *Saguinus* subspecies *S. fuscicollis melanoleucus*, *S. fuscicollis crandalli* and *S. fuscicollis acrensis* into one independent species, *Saguinus melanoleucus,* made up of three subspecies (*S. m. melanoleucus*, *S. m. crandalli*, *S. m. acrensis*). Rylands *et al*. (1993) generally followed Hershkovitz's (1977) classifications as regards sections and groups, except that they agreed with Natori and Hanihara (1992) that a new group should be formed by removing *S. midas* from the hairy-faced section and *S. bicolor* from the bare-faced section. In a subsequent paper, Rylands *et al.* (1995) accepted 12 distinct species and a total of 32 taxonomic morphotypes (*S. f. acrensis* was not considered a valid form) but still agreed with Hershkovitz (1977) that the *Saguinus* tamarins should be divided into three sections, although they modified the structure of the groups as follows: the hairy-faced tamarin section containing only the *nigricollis* group (*S. nigricollis*, *S. fuscicollis*, *S. tripartitus*) and the *mystax* group (*S. mystax*, *S. imperator*, *S. labiatus, S. midas*), formed by combining the mustached and midas groups; the mottled-faced tamarin section containing *S. inustus* only; and the bare-faced tamarin section consisting of the *bicolor* group, containing the three *S. bicolor* subspecies, and the *oedipus* group (*S. oedipus*, *S. geoffroyi*, *S. leucopus*). More recently, Rylands *et al*. (2000) re-evaluated the taxonomic status of some forms and reclassified the genus *Saguinus* into 15 distinct species with a total of 33 morphotypes, including *S. labiatus rufiventer*.

Along with traditional methods, molecular data obtained from primate DNA is providing a new approach to taxonomic classifications (Schneider *et al*., 1993; Goodman *et al*., 1998; Schneider *et al*., 1996; Schneider, 2000). Several fragments of mitochondrial DNA (mtDNA) have recently been sequenced from members of the Callitrichinae and have been shown to be useful for phylogenetic analysis between genera and at the subgeneric level (Jacobs *et al*., 1995; Tagliaro *et al*., 1997; Pastorini *et al*., 1998; Jacobs-Cropp *et al*., 1999; Tagliaro *et al*., 2000; Van Roosmalen *et al*., 2000). Phylogenetic trees based on cytochrome b and D-loop sequence data obtained by Jacobs *et al*. (1995) using eight *Saguinus* species (including six subspecies of *S. fuscicollis*) and Jacobs-Cropp *et al*. (1999) using 12 *Saguinus* species (some of the species subdivided, totalizing 23 subspecies) suggest that *Saguinus* should be divided into two groups. The trees obtained by Jacobs-Cropp *et al*. (1999) grouped the tamarins studied into a small-bodied clade (*S. fuscicollis* spp., *S. tripartitus* and *S. nigricollis*) and a large-bodied clade (*S. labiatus*, *S. imperator*, *S. mystax* spp., *S. inustus*, *S. geoffroyi*, *S. oedipus*, *S. leucopus*, *S. midas* spp. and *S. bicolor spp.*).

Although nuclear genes are usually too conservative to allow evaluation of close taxonomic relationships (Avise, 1994), Canavez *et al*. (1999) obtained significant results with respect to the phylogenetic relationships within the Callitrichinae at the subgeneric level by sequencing the first three exons and introns of the β_2-microglobulin nuclear gene. According to Canavez *et al*. (1999), *S. fuscicollis* appeared as the first divergent lineage within the genus *Saguinus* followed by an unresolved trichotomy (*S. mystax-S. imperator*, *S. midas-S. bicolor*, and *S. oedipus*), this research showing *S. midas midas* grouping with *S. bicolor* rather than *S. midas niger*. On the other hand, the biochemical analysis carried out by Meireles *et al.* (1997) and the mitochondrial results obtained by Jacobs-Cropp *et al.* (1999) showed that the *S. midas* subspecies (*S. m. midas* and *S. m. niger*) are closely related and are separated from the *S. bicolor* clade (*S. b. martinsi*, *S. b. bicolor, S. b. ochraceus*).

The work presented in this paper examined the phylogenetic relationships between species and subspecies of the genus *Saguinus* based on ND1 nucleotide sequences with the aim of discovering whether or not the two different sections containing the hairy-faced and bare-faced sections are monophyletic, whether *S. bicolor* and *S. midas midas* are more closely related to each other than to *S. midas midas* and *S. midas niger*, and if are *S. fuscicollis melanoleucus* and *S. fuscicollis weddelli* really are different species. The conservation status of *Saguinus* is also discussed in the light of the results of the phylogenetic analysis.

Materials and Methods

The *Saguinus* tamarin species and subspecies used (a total of 21) are shown in Table 1 and their geographical distribution in Figure 1. Since *Saguinus* is basal to the other Callitrichinae (Schneider *et al*., 1996; Barroso *et al*., 1997; Chaves *et al*., 1999;) the outgroup could not come from this

Table 1 - Taxonomic and geographical data concerning the *Saguinus* specimens used. Classification according to Hershkovitz (1977).

Section	Group	*Saguinus* species or subspecies	Number of specimens	Specimen code	Geographical origin
Hairy-faced	*nigricollis*	*S. fuscicollis weddelli*	6	77, 78, 79, 80, 112, 113	Rio Jamari, Rondônia, Brazil
		S. fuscicollis melanoleucus	1	137	Rio Envira (left bank), Acre, Brazil
	mystax	*S. mystax mystax*	2	45, 46	CNP[1*]
	midas	*S. midas midas*	2	133,134	CNP[1*]
		S. midas niger	4	37, 38, 39, 40	Tucuruí, Pará, Brazil
Bare-faced	*bicolor*	*S. bicolor bicolor*	2	117, 118	CPRJ[2*]
		S. bicolor martinsi	2	131, 132	CNP[1*]
	oedipus	*S. oedipus oedipus*	1	125	Colombia[*]
		S. leucopus	1	126	Colombia[*]

*Exact origin unknown.
[1]Centro Nacional de Primatas, Belém, Pará, Brazil.
[2]Centro de Primatologia do Rio de Janeiro, Rio de Janeiro, Brazil.

subfamily, because of which we used the monkey *Cebus apella* (Cebinae, Cebidae), a closely related taxon, as the outgroup. Blood samples (3 mL) were collected from the femoral vein by anesthetizing each animal using 10 mg kg^{-1} body weight of Ketalar. The collections were carried out by personnel (see acknowledgments) in the institutions holding the monkeys or from animals captured in the field and subsequently released, in either case the blood samples were kept in ice until analyzed.

Total DNA was extracted from each blood sample using the protocol described by Sambrook *et al*. (1989). For each monkey sampled the sequence of a 1321 bp fragment spanning the ND1 fragment and flanking regions of the mitochondrial DNA was determined by the direct sequencing of PCR-amplified fragments obtained using a 5'-CTACGT GATCTGAGTTCAGACCGG-3' (forward) and 5'-AGGGTATAACCAACATTTTCGGGGTATG-3' (reverse) primer (both designed by Dr. M. Stanhope). In order to eliminate any false priming products that occasionally arise in the original genomic DNA, a second (internal) PCR was performed on the first PCR using the same forward primer and a 5'-CCCGATAGCTTATTTAGCTGACCTT AC-3' reverse primer, the internal primers used (Table 2) being designed by Dr. C. Tagliaro. The reaction protocol consisted of initial denaturing at 94 °C for 3 min, 30 cycles of one minute at 94 °C and three minutes at 65 °C followed by a final extension at 65 °C for ten minutes. The DNA sequences were determined using dye terminator cycle sequencing reactions and a model 373A automatic sequencer according to protocols supplied by the manufacturers (Applied Biosystems, Foster, CA, USA), additional internal sequencing primers being designed as necessary. Although the sequenced fragment was 1321 bp, only 957 bp sequences (including initiation and ending codons) corresponding exclusively to the ND1 gene were considered in the analysis.

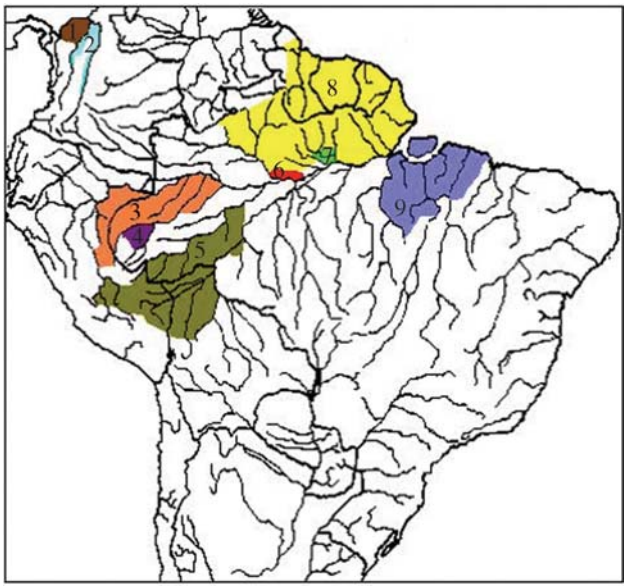

Figure 1 - Geographical distribution of the *Saguinus* specimens used in our study. 1: *S. oedipus*; 2: *S. leucopus*; 3: *S. mystax mystax*; 4: *S. fuscicollis melanoleucus*; 5: *S. fuscicollis weddelli*; 6: *S. bicolor bicolor*; 7: *S. bicolor martinsi*; 8: *S. midas midas*; 9: *S. midas niger*. Adapted from Rylands *et al.* (1993).

Initial sequence alignments were performed using the BioEdit (Hall, 1999) and Clustal W (Thompson *et al*., 1994) programs. The nucleotide frequencies and transition/transversion ratio were obtained using the Mega 2 software (Kumar *et al.* 2001) and the saturation test was performed using the DAMBE program (Xia and Xie, 2001). The nucleotide sequence data for the sequences used in this paper were deposited in GenBank under accession numbers AY579985 to AY579990, AY599494 to AY599497 and AY582798. The MODELTEST program, version 3.06 (Posada and Crandall, 1998) was used to identify the evolutionary model that best fitted the data, the divergence matrix being generated using the beta version of the PAUP 4.0 program (Swofford, 2002) based on the

Table 2 - Internal primers for the mitochondrial DNA ND1 flanking regions of *Cebus apella* and several *Saguinus* species.

Primers (5' to 3')	5' positions	Primer sequences	*Saguinus* specimens
A	-	5' CTA CGT GAT CTG AGT TCA GAC CGG 3'	all species sequenced
A2SF	62	5' CAC TCA CAG AAC GAA AAA TCC TAG GC 3'	*S. fuscicollis*
A2SO	64	5' CTC ACA GAA CGA AAA GTG CTA GGC 3'	*S. oedipus*
A2SL	61	5' ACA CTC ACA GAA CGA AAA GTA TTG GG 3'	*S. leucopus*
A2SM	61	5' ACA CTC ACA GAA CGA AAG ATC CTA G 3'	*S. mystax*
A2SB	61	5' ACA CTC ACA GAG CGA AAA GTA TTA GG 3'	*S. bicolor, S. midas*
A2APE	124	5' CCA TAT GGA GTA CTC CAA CCA ATC 3'	*Cebus apella*
A3SN	395	5' CAC TAC GAG CTG TAG CCC AAA CAA T 3	*S. midas, S. oedipus*
A3SF	395	5' CAC TAC GAG CTG TAG CTC AAA CAA T 3'	*S. fuscicollis, S. leucopus*
A3SB	414	5' GAC AAT CTC GTA CGA AGT TAC CCT 3'	*S. bicolor*
A3SM	396	5' GCT ACG AGC TGT AGC TCA AAC AAT 3'	*S. mystax*
A3APE	411	5' CCA GAC CAT TTC ATA CGA AGT CAC 3'	*Cebus apella*
C (3' to 5')	-	5' CCC GAT AGC TTA TTT AGC TGA CCT TAC T 3'	all species sequenced

model parameters selected by the MODELTEST program. Maximum-parsimony (MP), neighbor joining (NJ) and maximum-likelihood (ML) analyses were performed with the PAUP 4.0 program using an heuristic search. The robustness of the phylogenetic hypothesis obtained was tested by bootstrapping (Felsenstein, 1985) with 2000 pseudo-replicates for NJ and MP, and 500 for ML. The criterion adopted to evaluate robustness was to consider bootstrap values equal or superior to 90% as being statistically significant. The Bremer Decay indexes were obtained using the SEPAL program (Salisbury, 1999, 2000).

Results and Discussion

For the 21 *Saguinus* specimens investigated no insertions or deletions (indels) were detected in the alignments and the average percentage nucleotide composition was thymine (T) 26.5, cytosine (C) 30.1, adenine (A) 31.5 and guanine (G) 11.9. The ND1 nucleotide frequencies obtained for *Saguinus* were similar to the values previously obtained for other genera of Callitrichinae and *Cebus* (Tagliaro, 1997). The average transition/transversion rate was 3.6 (without the outgroup) and the saturation test using the ND1 *Saguinus* sequences detected no saturation at the intrageneric level at any of the codon positions.

The maximum-likelihood best-fit model selected by the MODELTEST program for the 22 samples was the Hasegawa-Kishino-Yano (HYK) model (Hasegawa *et al*., 1985), which takes into account the proportion of invariable sites (I), gamma (G) and the gamma distribution shape parameter (HKY+I+G). The settings from the best-fit model selected by hierarchical likelihood ratio tests (HLRTs) were: base frequencies (A = 0.3479, C = 0.3105, G = 0.0982, T = 0.2434); the proportion of invariable sites (Pinv = 0.5802); gamma distribution shape parameter (Rates = gamma, Shape = 3.5920); transition/transversion rate (Tratio = 7.1012); and number of substitution types (Nst = 2).

The small-bodied and large-bodied size division

The trees obtained in this study using different methods (MP, NJ and ML) showed the same general topology (Figure 2), with the genus *Saguinus* divided into two major clades, one containing the two *S. fuscicollis* subspecies and the other, the larger species (*S. oedipus*, *S. leucopus*, *S. mystax*, *S. midas* and *S. bicolor*). The small and large-bodied size division was initially proposed by Jacobs-Cropp *et al*. (1999) and it was also supported by the

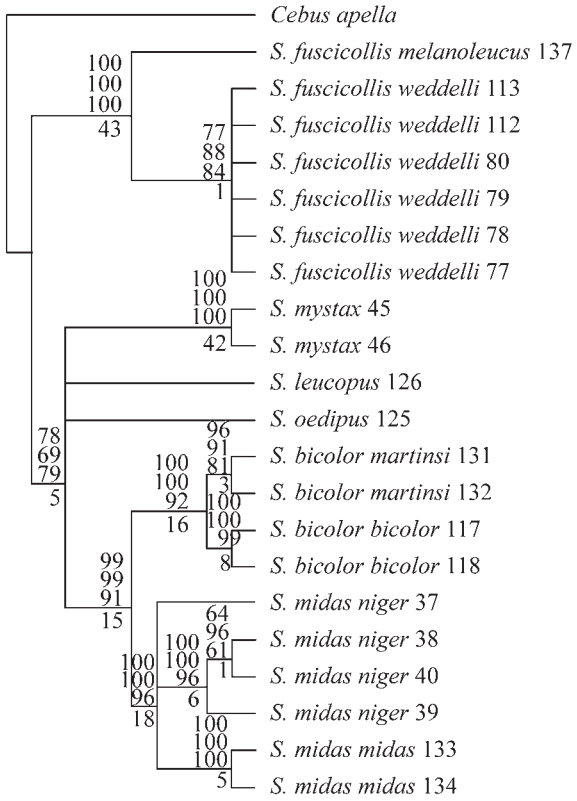

Figure 2 - Consensus maximum-parsimony (MP), neighbor joining (NJ) and maximum likelihood (ML) tree for the genus *Saguinus*. The bootstrap values are given above the branches: top = MP, 2000 replicates; middle = NJ, 2000 replicates; bottom = ML, 500 replicates. The Bremer Decay indices are given below the branches.

results of Canavez *et al.* (1999). Other studies based on morphological features (Hershkovitz, 1977; Natori and Hanihara, 1992) did not agree with this division based on body size, but molecular data indicate that it may be an important morphological character that should be used to clarify the evolutionary history of this genus.

The bare-face and hairy-face tamarin sections

The monophyletic origin of the bare-faced *S. leucopus* and *S. oedipus* was not evident in the present analysis since the bootstrap values were not significant in any of the three distinct topologies obtained by MP, NJ and ML. Although our data did not show significance joining *S. leucopus* and *S. oedipus*, other molecular and morphological studies (Jacobs *et al*., 1995; Jacobs-Cropp *et al.*, 1999; Natori, 1988; Natori and Hanihara, 1992) and the geographical distribution (Figure 1) of these species indicate that they probably are sister taxa and could be grouped together (*oedipus* group) as proposed by Hershkovitz (1977).

Our phylogenetic analysis strongly supports a clade joining the bare-faced *Saguinus bicolor* and the hairy-faced *S. midas* (bootstrap values: MP = 99%, NJ = 99%, ML = 91%; Decay index = 15), these species are geographically closely related (see Figure 1). The close relationship which we found between *S. midas* and *S. bicolor* was first noted by Natori and Hanihara (1992) and supported by Rylands *et al*. (1993), with these authors even proposing that *S. midas* and *S. bicolor* should together form an independent group. Our results show *S. midas-S. bicolor* grouping with the bare-faced tamarins of northwestern South America (*Saguinus oedipus oedipus, S. oedipus geoffroyi, S. leucopus*). Hershkovitz (1977), considering morphological features and geographical distribution, suggested that the hairy-faced trait is the most primitive and grouped all the bare-faced tamarins together, although he recognized that *S. bicolor* acquired the bare-faced condition independently of the bare-faced tamarins of northwestern South America. According to Hershkovitz (1977), the *S. bicolor* group may have gained the bare-faced condition from a founder colony of hairy-faced tamarins separated by a river-bend cutoff from the ancestral stock on the south bank of the Amazon west of the Rio Madeira. The *Saguinus oedipus* group arose independently from an upper Amazonian stock of hairy-faced tamarins, spread northward along the eastern base of the Andes, then west into northern Colombia and Middle America. Although our results do not support the division of *Saguinus* species based on the presence or absence of facial-hair, we agree with Hershkovitz that the bare-faced condition may have arisen in two different lineages by convergence.

Are *S. bicolor* and *S. midas midas* more closely related to each other than to *S. midas midas* and *S. midas niger*?

At the species level, all our ND1 phylogenetic trees (Figure 2) showed that all the representatives grouped according to the morphological classification of Hershkovitz (1977). The suggestion that *S. bicolor* and *S. midas* could be subspecies of the same species (Canavez *et al.,* 1999), was not supported by our results but they were consistent with those of Meireles *et al*. (1997) and Jacobs-Cropp *et al*. (1999), joining *S. bicolor* and *S. midas* as sister groups. One possible reason for this discrepancy could be the fact that both we and Jacobs-Cropp *et al*. (1999) used mitochondrial sequences which evolve faster than the nuclear sequences as used by Canavez *et al*. (1999).

The subspecies of *Saguinus midas*

Surprisingly, the *S. midas niger* clade was not strongly supported by MP (bootstrap = 64%) or NJ (bootstrap = 77%) analysis, although ML analysis (bootstrap = 96%) indicated that the four *S. midas niger* specimens were not joined together at all, with *S. m. niger* specimen 37 being isolated in its own branch. Distance values showed that specimen 37 diverged by 0.015 from the other *S. m. niger* specimens, this value being closely associated with the divergence found between subspecies of the same species, *i.e. S. bicolor bicolor* x *S. bicolor martinsi* (0.017 to 0.018), *S. midas midas* x *S. midas niger* (0.017 to 0.021) (Table 3). Since the distances observed between *S. m. niger* specimen 37, *S. midas midas* 133-134 and *S. midas niger* specimens 38-39-40 were almost the same, we concluded that probably there is a third subspecies with a similar morphotype to *S. midas niger*. Unfortunately, there were no details on which margin of the Tocantins river the *S. midas niger* specimens were captured.

Are *S. fuscicollis melanoleucus* and *S. fuscicollis weddelli* different species?

Although we sampled only one *S. fuscicollis melanoleucus* specimen we decided to use our ND1 mitochondrial DNA data to test Coimbra-Filho's proposal that this morphotype should be reclassified as a different species (Coimbra-Filho, 1990). In all the phylogenetic trees *Saguinus fuscicollis melanoleucus* was basal to *S. fuscicollis weddelli* but this arrangement was not statistically significant for any of the methods used to construct the trees. In fact, the distance matrix (Table 3) showed that the genetic distance values between this *S. f. melanoleucus* morphotype and *S. fuscicollis weddelli* range from 0.002 to 0.005, which are similar to the genetic distances obtained between the *S. fuscicollis weddelli* specimens (0 to 0.003). Contrasting with our results, Jacobs-Cropp *et al*., (1999) used cytochrome b and the D-loop mitochondrial DNA data to obtain genetic distance values agreeing with the traditional classification of Hershkovitz (1977), in which both *S. f. melanoleucus* and *S. f. weddelli* are considered as subspecies of *S. fuscicollis.* There are two possible explanations for these discrepancies, one being that in our study the *S. f. melanoleucus* specimen (specimen 137) was not correctly identified (improbable because the blood sample was

Table 3 - Hasegawa-Kishino-Yano (HYK) model (Hasegawa *et al.*, 1985) divergence matrix for the mitochondrial DNA ND1 flanking regions of *Cebus apella* and several *Saguinus* species.

	Cebus																					
SFM137	0.211	SFM137																				
SFW113	0.213	0.003	SFW113																			
SFW112	0.213	0.003	0.000	SFW112																		
SFW80	0.214	0.002	0.001	0.001	SFW80																	
SFW79	0.212	0.002	0.000	0.000	0.000	SFW79																
SFW78	0.214	0.003	0.002	0.000	0.001	0.001	SFW78															
SFW77	0.216	0.005	0.002	0.002	0.003	0.002	0.002	SFW77														
SMMY45	0.228	0.175	0.178	0.178	0.177	0.176	0.177	0.181	SMMY45													
SMMY46	0.226	0.180	0.182	0.182	0.182	0.181	0.182	0.186	0.003	SMMY46												
SL126	0.238	0.167	0.172	0.172	0.172	0.171	0.172	0.177	0.163	0.161	SL126											
SO125	0.239	0.168	0.166	0.166	0.165	0.165	0.166	0.170	0.156	0.158	0.144	SO125										
SBM131	0.238	0.162	0.168	0.168	0.166	0.166	0.168	0.170	0.160	0.161	0.154	0.112	SBM131									
SBM132	0.236	0.164	0.168	0.168	0.166	0.166	0.168	0.170	0.160	0.161	0.151	0.112	0.008	SBM132								
SBB117	0.231	0.168	0.172	0.172	0.170	0.171	0.172	0.174	0.167	0.168	0.161	0.112	0.017	0.018	SBB117							
SBB118	0.231	0.168	0.172	0.172	0.170	0.171	0.172	0.174	0.167	0.168	0.161	0.112	0.017	0.018	0.000	SBB118						
SMN37	0.228	0.150	0.155	0.155	0.153	0.153	0.154	0.157	0.161	0.163	0.161	0.117	0.066	0.067	0.066	0.066	SMN37					
SMN38	0.230	0.160	0.164	0.164	0.163	0.163	0.164	0.167	0.168	0.167	0.161	0.123	0.068	0.069	0.070	0.070	0.015	SMN38				
SMN39	0.228	0.159	0.163	0.163	0.161	0.162	0.163	0.165	0.167	0.166	0.157	0.122	0.068	0.069	0.070	0.070	0.015	0.002	SMN39			
SMN40	0.230	0.160	0.164	0.164	0.163	0.163	0.164	0.167	0.168	0.167	0.161	0.123	0.068	0.069	0.070	0.070	0.015	0.000	0.002	SMN40		
SMM133	0.220	0.153	0.158	0.158	0.156	0.156	0.157	0.160	0.163	0.161	0.153	0.120	0.070	0.072	0.073	0.073	0.017	0.021	0.021	0.021	SMM133	
SMM134	0.219	0.155	0.159	0.159	0.157	0.158	0.159	0.161	0.161	0.160	0.153	0.120	0.070	0.072	0.073	0.073	0.017	0.021	0.021	0.021	0.002	

Sfm: *Saguinus fuscicollis melanoleucus*, Sfw: *Saguinus fuscicollis weddeli*, Smmy: *Saguinus mystax mystax*, Sl: *Saguinus leucopus*, So: *Saguinus oedipus*, Sbb: *Saguinus bicolor bicolor*, Sbm: *Saguinus bicolor martinsi*, Smn: *Saguinus midas niger*, Smm: *Saguinus midas midas*.

taken from this animal in its natural forest environment) and the other being that *S. f. melanoleucus* and *S. f. weddelli* are in fact the same taxon with regional morphological differences. Unfortunately, there is no other molecular study published using more than one individual of the same *S. fuscicollis* subspecies, which means that the range of divergence values of each of these subspecies cannot be verified.

Conservation status of *Saguinus*

Primates are important indicators for tropical forests and are a fundamental component for the establishment of biodiversity conservation strategies in biomes, regions and countries (Rylands *et al.*, 1997; Sechrest *et al*., 2002). According to the Mace-Lande system, recently adopted by the International Union for Conservation of Nature and Natural Resources (IUCN), 35 percent of Neotropical primate taxa are threatened (Rylands *et al.,* 1997). Brazil is not only the country with the highest diversity of primates (76 species and a total of 128 species and subspecies) but also has the highest number of threatened species and subspecies of primates, with 38 species and a total of 69 species and subspecies being considered as threatened (Rylands *et al.* 1997). Due to an almost complete lack of information on the populations of most of the callitrichids, decisions on their conservation status are based almost entirely on the size of their geographic range and a gross overview of the state of destruction and development of the regions involved. Such decisions are also based on taxonomic classifications that depend on morphological studies including pelage color (Rylands *et al.*, 1993). Two genera of Callitrichinae (*Leontopithecus* and *Callimico*) and 16 of the total of 55 species and subspecies are considered threatened, including five *Saguinus* species and subspecies, with *S. bicolor bicolor* and *S. oedipus* being considered endangered and *S. nigricollis hernandezi*, *S. imperator imperator* and *S. leucopus* vulnerable (Rylands *et al.*, 1997).

The study presented in this paper reinforces the value of molecular techniques in confirming traditional taxonomic classifications, our results pointing to the existence of a subspecies which has similar morphology to *S. midas niger* but which can be differentiated from *S. midas niger* on the basis of molecular data. None of the *S. midas* subspecies are at present considered threatened, mainly because of their wide geographical distribution (Figure 1), but since our molecular data indicates the possible existence of a new *S. midas* subspecies we should be alert to the conservation needs of this new subspecies because we have no idea as to its distribution or population size. Furthermore, the distribution area of *S. midas niger* is suffering pressure from deforestation for agriculture and cattle raising, which may mean that new taxa can disappear before even being described. There have been few molecular studies of New World monkeys using several individuals of the same species or subspecies but detailed studies at this level may re-

veal that the richness of the Amazonian primates is being underestimated.

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