

A comparative study of eleven protein systems in tamarins, genus *Saguinus* (Platyrrhini, Callitrichinae)

Carla Meireles¹, Iracilda Sampaio¹, Horacio Schneider¹, Stephen F. Ferrari¹,
Adelmar F. Coimbra-Filho², Alcides Pissinatti² and Maria P.C. Schneider¹

ABSTRACT

The genetic variability of six tamarin taxa, genus *Saguinus*, was analyzed comparatively using protein data from eleven systems coded by 15 loci. *S. fuscicollis weddelli* and *S. midas midas* were the most polymorphic taxa, and *S. bicolor* the least. The results of the phylogenetic analyses (UPGMA and neighbor-joining) and the genetic distances between taxa were generally consistent with their geographic and probable phylogenetic relationships. Analyses of the *S. bicolor* and *S. midas* populations suggested that they represent no more than three subspecies of a single species, *S. midas*, with the *bicolor* forms belonging to a single subspecies, *S. midas bicolor*. If supported by additional studies, this would have important implications for the conservation of the *bicolor* form, which is endangered with extinction. The genetic similarity of *S. fuscicollis* and *S. mystax* was also consistent with their geographical and morphological proximity, although more data from a larger number of taxa will be required before the taxonomic relationships within the genus can be defined.

INTRODUCTION

The tamarins, *Saguinus* spp., are small-bodied platyrrhine monkeys distributed in Amazonia and northwestern Colombia/eastern Panama. *Saguinus* is the most diverse of New World monkey genera, with 33 currently recognized taxa, including at least 10 species (Hershkovitz, 1977). The classic reviews of Hershkovitz (1977, 1979, 1982) divide the tamarins into three "sections" based on the characteristics of the facial pelage (hairy face, mottled face and bare face), and six species groups (Table I). Natori and Hanihara (1992), Ferrari (1993) and Rylands *et al.* (1993) have all proposed minor alterations to these groupings (Table I).

Despite being the most diverse of platyrrhine genera, *Saguinus* has fewer species than either *Callicebus* (Hershkovitz, 1990) or *Callithrix* (de Vivo, 1991; Mittermeier *et al.*, 1992), even if the suggested modifications of Mittermeier *et al.* (1988), Thorington (1988) and Coimbra-Filho (1990) are incorporated. In addition to the 10 species identified by Hershkovitz (Table I), Mittermeier *et al.* (1988) recognize the *geoffroyi* form as a true species (see also Hanihara and Natori, 1987), and Thorington (1988) gives species status to *tripartitus*. Coimbra-Filho (1990) has also supported the more traditional classification of the bleached forms of the saddle-back tamarins (*acrensis*, *crandalli* and *melanoleucus*) as members of *Saguinus melanoleucus*.

The most recent overview of *Saguinus* taxonomy (Rylands *et al.*, 1993) recognizes 12 species, including *Saguinus geoffroyi* and *Saguinus tripartitus*, but excluding *S. melanoleucus*, although neither this

¹ Departamento de Genética, Centro de Ciências Biológicas, Universidade Federal do Pará, Campus Universitário do Guamá, Caixa Postal 8607, 66075-900 Belém, PA, Brasil. Tel: (091) 211-1627, Fax: (091) 211-1568. Send correspondence to M.P.C.S.

² Centro de Primatologia do Rio de Janeiro, CPRJ FEEMA, Rio de Janeiro, Brasil.

arrangement, nor that of Mittermeier *et al.* (1988), was based on the collection or analysis of quantitative data.

In the light of recent trends (e.g. Hershkovitz, 1990; de Vivo, 1991), Mittermeier *et al.* (1992) support the need for a detailed taxonomic revision of the genus *Saguinus*. The analysis of genetic variables has been an important element lacking, to a greater or lesser extent, in the most traditional studies of callitrichine subgeneric systematics. This is at least partly due to the relative lack of genetic data, at least until recently (Nagai *et al.* (1986); Melo *et al.* (1992); Dantas (1994); Nagamachi (1995), and Meireles, C., Sampaio, I., Schneider, H., Ferrari, S.F., Coimbra-Filho, A.F., Pissinatti, A. and Schneider, M.P.C., unpublished results).

MATERIAL AND METHODS

Eleven protein systems were analyzed using 322 blood samples of six *Saguinus* taxa (Table II): *Saguinus mystax* (SMY), *Saguinus bicolor bicolor* (SBB), *Saguinus bicolor martinsi* (SBM), *Saguinus fuscicollis weddelli* (SFW), *Saguinus midas midas* (SMM) and *Saguinus midas niger* (SMN). Fifty blood samples of *Alouatta belzebul belzebul* (ABB) (family Atelidae *sensu* Schneider *et al.*, 1993) were also analyzed and used as an outgroup (Table II).

For the collection of blood samples, monkeys were anesthetized with Ketalar (ketamine chloride, Park Davis) at a dose equivalent to 10 mg/kg body weight. Samples were processed following Sampaio and Schneider (1986): centrifuged at 3,000 rpm for 10 min at room temperature, red cells were isolated and glycerolized, and both plasma and cells were stored at -20°C before being analyzed.

Horizontal electrophoresis was carried out in the following media: 0.8% agarose, 0.8% agarose 2% starch and 11% starch gels. Two types of starch were used: potato starch (Sigma) and corn starch (Penetrose 30). The following 11 protein systems coded by 15 loci were investigated:

- lactate dehydrogenase (*LDHA*, *LDHB*, E.C. 1.1.1.27);
- malate dehydrogenase 1 (*MDH1*, E.C. 1.1.1.37);
- isocitrate dehydrogenase 1 (*IDH1*, E.C. 1.1.1.42);
- phosphogluconate dehydrogenase (*PGD*, E.C. 1.1.1.44);
- superoxide dismutase 1 (*SOD1*, E.C. 1.15.1.1);
- phosphoglucomutase 1 (*PGM1*, E.C. 2.7.5.1);

- esterases (*ESD*, *ES1*, *ES2*, E.C. 3.1.1.1);
- acid phosphatase 1 (*ACP1*, E.C. 3.1.3.2);
- carbonic anhydrase 2 (*CA2*, E.C. 4.2.1.1);
- hemoglobin (*HBA*, *HBB*) and
- albumin (*ALB*).

Table I - Taxonomy of the genus *Saguinus*, according to Hershkovitz (1977, 1979, 1982).

Section/group	Species	Subspecies
Hairy-face tamarin group		
<i>S. nigricollis</i> (1)	<i>S. nigricollis</i>	<i>S. n. nigricollis</i> , <i>S. n. hernandezi</i> , <i>S. n. graellsii</i>
	<i>S. fuscicollis</i>	<i>S. f. fuscicollis</i> , <i>S. f. acrensis</i> <i>S. f. avilapiresi</i> , <i>S. f. crandalli</i> <i>S. f. cruzlimai</i> , <i>S. f. fuscus</i> <i>S. f. illigeri</i> , <i>S. f. lagonotus</i> <i>S. f. leucogenys</i> , <i>S. f. melanoleucus</i> <i>S. f. nigrifons</i> , <i>S. f. primitivus</i> <i>S. f. tripartitus</i> , <i>S. f. weddelli</i>
<i>S. mystax</i>	<i>S. mystax</i>	<i>S. m. mystax</i> , <i>S. m. pileatus</i> <i>S. m. plutus</i>
	<i>S. labiatus</i>	<i>S. l. labiatus</i> , <i>S. l. thomasi</i>
	<i>S. imperator</i>	<i>S. i. imperator</i> , <i>S. i. subgrisescens</i>
<i>S. midas</i> (2)	<i>S. midas</i>	<i>S. m. midas</i> , <i>S. m. niger</i>
Mottled-face tamarin group		
<i>S. inustus</i> (2)	<i>S. inustus</i>	
Bare-face tamarin group		
<i>S. bicolor</i> (2)	<i>S. bicolor</i>	<i>S. b. bicolor</i> , <i>S. b. martinsi</i> <i>S. b. ochraceus</i>
<i>S. oedipus</i>	<i>S. oedipus</i> <i>S. leucopus</i>	<i>S. o. oedipus</i> , <i>S. o. geoffroyi</i>

(1) Ferrari (1993) places this species in the *S. mystax* group, and puts the *S. fuscicollis* forms in a separate group.

(2) Natori and Hanihara (1992) and Rylands *et al.* (1993) place *S. bicolor* in the *S. midas* group, to which Ferrari (1993) adds *S. inustus*.

Table II - Species and subspecies of tamarins analyzed, collecting sites and sample sizes.

Genus	Taxon	Code	Collecting sites	Sample size
<i>Saguinus</i>				
	<i>mystax</i>	SMY	Peru	22
	<i>fuscicollis weddelli</i>	SFW	Rio Jamari (RO)	138
	<i>midas midas</i>	SMM	Rio Amazonas (AP, AM, PA)	13
	<i>midas niger</i>	SMN	Rio Tocantins (PA)	123
	<i>bicolor bicolor</i>	SBB	INPA (AM)	22
	<i>bicolor martinsi</i>	SBM	Alto Trombetas (PA)	4
<i>Alouatta</i> (outgroup)				
	<i>belzebul belzebul</i>	ABB	Rio Tocantins (PA)	50

INPA - Instituto de Pesquisas da Amazônia.

States of Brazil: RO - Rondônia; AP - Amapá; AM - Amazonas; PA - Pará.

Electrophoretic conditions and staining procedures followed Harris and Hopkinson (1976). The medium, time of migration and the difference of potential used for each protein system are shown in Table III, and the buffers in Table IV. Gene frequencies were estimated by maximum likelihood using Reed and Schull's (1968) MAXLIK program, and genetic variability (average heterozygosity, proportion of polymorphic loci and mean number of alleles per locus) was calculated according to Nei (1987). The matrix of genetic distances (unbiased minimum distance) and identities (unbiased genetic identity) were calculated according to Nei's (1978) method. The trees were estimated by the neighbor-joining method (Saitou and Nei, 1987). All estimates were carried out using the DISPAN program (Kumar *et al.*, 1993).

RESULTS

The nomenclature of the electrophoretic phenotypes in this study was established by means of

Table III - The 11 protein systems investigated, with the electrophoretic conditions employed in the analysis.

Systems	Gel	Time of migration (hours)	Difference of potential (V/cm)
LDH, MDH1, PGD SOD1, ES	starch	18	4
IDH1	agarose	4	8
PGM1	agarose/starch	5	8
ACP1	agarose	3	10
CA2	agarose	4	10
HB	agarose/starch	4	10
ALB	starch	6	8

Table IV - Buffers used for the electrophoretic analysis.

Loci	Buffers		References
	Tank	Gel	
LDHA, LDHB, MDH1 IDH1	40 x PC	1 x PC	(Harris and Hopkinson, 1976)
PGD, SOD1, ESD, ES1, ES2, ACP1	20 x PCE	1 x PCE	(Harris and Hopkinson, 1976; Sampaio and Schneider, 1986)
PGM1, CA2	15 x TEMM	1 x TEMM	(Harris and Hopkinson, 1976; Sampaio <i>et al.</i> , 1986)
HBA, HBB	10 x TEB1	1 x TEB1	(Harris and Hopkinson, 1976)
ALB	6.2 x TEB2	1 x TEB2	(Franco and Salzano, 1985)

PC = pH 5.9, 245 mM monobasic sodium phosphate, 150 mM citric acid; PCE = pH 6.9, 110 mM monobasic sodium phosphate, 75 mM trisodium citrate, 2.5 mM EDTA; TEMM = pH 7.4, 100 mM Tris, 100 mM maleic anhydride, 10 mM EDTA, 10 mM magnesium chloride; TEB1 = pH 8.6, 180 mM Tris, 100 mM boric acid, 4 mM EDTA; TEB2 = pH 6.9, 30 mM Tris, 300 mM boric acid, 4.3 mM EDTA.

intra- and inter-populational comparisons of the observed patterns, using the notation for *A. belzebul* (Schneider, 1988) as standard. The new phenotypes were numbered sequentially in ascending order starting from the most anodal to the most cathodal, and were nominated in accordance with Shows *et al.* (1979).

Nine polymorphic loci (*MDH1*, *IDH1*, *PGD*, *SOD1*, *PGM1*, *ESD*, *ES2*, *CA2* and *ALB*) were observed in the six populations of *Saguinus*, while six loci were monomorphic: *LDHA*, *LDHB*, *ES1*, *ACP1*, *HBA* and *HBB*. Allelic frequencies are presented in Table V.

Genetic variability, measured using average heterozygosity, proportion of polymorphic loci and mean number of alleles per locus is shown in Table VI. *S. midas midas* and *S. fuscicollis weddelli* were the most varied of the tamarins, while the two *bicolor* subspecies exhibited the lowest variation.

Genetic distances (D) in *Saguinus* species varied from 0.1% (between the two subspecies from *Saguinus bicolor*, see Table VII) to 20% (between *S. b. bicolor* and *S. mystax*). The highest distance value observed between a tamarin (*S. b. bicolor*) and the atelid outgroup (*A. belzebul*) was 71% and the lowest 63% (*S. f. weddelli* x *A. belzebul*). Figures 1 (UPGMA) and 2 (neighbor-joining) show the phylogenetic trees for the seven populations obtained from the protein data.

DISCUSSION

The frequencies of some alleles differed significantly between subspecies, species, genera, and families, while others were found exclusively in either a single species or species group. Thus, these alleles are important biochemical markers for the analysis of callitrichine phylogeny, and can be divided into four categories:

(i) markers present in a single subspecies:

S. midas midas - PGD⁷ (4%) and SOD1⁴ (12%);

S. midas niger - PGD³ (8%), PGD⁶ (9%) and ES2⁴ (3%);

S. b. bicolor - IDH1³ (14%);

S. b. martinsi - PGD⁵ (13%);

(ii) markers present in a single species:

S. mystax - MDH1¹ (2%) and PGM1³ (11%);

S. fuscicollis - CA2³ (53%), CA2⁴ (47%) and ALB¹ (6%);

S. midas - ESD¹ (7%), and CA2⁷ (57%);

S. bicolor - ES1¹ (100%);

(iii) markers that differentiate species groups:

Hairy-face tamarin group - *ES1*²;
Bare-face tamarin group - *ES1*¹;

(iv) markers present in a single genus:

Saguinus (Callitrichinae) - *PGM1*², *ACP1*¹,
*HBA*¹, *HBB*¹ and *ALB*²;
Alouatta (Atelinae) - *PGM1*¹, *ACP1*², *HBA*²,
*HBB*² and *ALB*³.

S. f. weddelli (H = 4.1%) and *S. midas midas* (H = 5.6%) presented the highest H values, although in the case of the latter taxon, the small sample size (N = 13) may mean that this value is not representative of the levels of variability occurring in natural populations. The value of 5% for *A. belzebul*, the outgroup in the present study, was similar to that recorded for this species by Schneider et al. (1991). By contrast, the *S. bicolor* subspecies showed the lowest H values (Table VI), only slightly higher than those recorded for *Leontopithecus rosalia* (H = 0.7%; Forman et al., 1986) and *Cebus apella paraguayanus* (H = 1.2%; Sampaio et al., 1991). The matrix of genetic distances (Table VII) also shows that *S. b. bicolor* and *S. b. martinsi* are the most closely related, and in accordance with these values, we suggest that they represent a single taxon, *S. bicolor*.

The genetic distance recorded between *S. midas midas* and *S. midas niger* (D = 0.05) is well within the threshold of 0.15 defined by Thorpe (1982) for allopatric subspecies, and supports their classification as such. It is interesting to note, however, that the value of 0.07 recorded between *S. midas midas* and the *S. bicolor* populations also falls within this range, whereas those recorded between the latter and *S. midas niger* are of the order of 0.14, as might be expected from the geographic distribution of these populations. *S. midas midas* and *S. bicolor* are parapatric in the north of the Amazon, while *S. midas niger* occurs to the south of this river, some 500 km east of the eastern limit of the range of *S. bicolor*.

The UPGMA (Figure 1) and in particular the neighbor-joining (Figure 2) trees also reinforce the close ties between *S. bicolor* and *S. midas* populations, especially the parapatric populations. In addition to supporting the species-group arrangements of Natori and Hanihara

Table V - Gene frequencies of 15 loci analyzed in seven populations of tamarins.

Locus	Allele	Populations (sample size)						
		SMY (22)	SFW (138)	SMM (13)	SMN (123)	SBB (22)	SBM (04)	ABB (50)
<i>LDHA</i>	<i>LDHA</i> ²	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>LDHB</i>	<i>LDHB</i> ¹	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>MDH1</i>	<i>MDH1</i> ¹	0.977	1.000	1.000	1.000	1.000	1.000	1.000
	<i>MDH1</i> ²	0.023	—	—	—	—	—	—
<i>IDH1</i>	<i>IDH1</i> ¹	1.000	1.000	1.000	1.000	0.864	1.000	0.930
	<i>IDH1</i> ²	—	—	—	—	—	—	0.070
	<i>IDH1</i> ³	—	—	—	—	0.136	—	—
<i>PGD</i>	<i>PGD</i> ¹	—	—	—	—	—	—	0.230
	<i>PGD</i> ²	—	—	—	—	—	—	0.770
	<i>PGD</i> ³	—	—	—	0.025	—	—	—
	<i>PGD</i> ⁴	1.000	1.000	0.962	0.947	1.000	0.875	—
	<i>PGD</i> ⁵	—	—	—	—	—	0.125	—
	<i>PGD</i> ⁶	—	—	—	0.028	—	—	—
	<i>PGD</i> ⁷	—	—	0.038	—	—	—	—
<i>SOD1</i>	<i>SOD1</i> ¹	—	—	—	—	—	—	0.020
	<i>SOD1</i> ²	1.000	1.000	0.885	1.000	1.000	1.000	—
	<i>SOD1</i> ³	—	—	—	—	—	—	0.980
	<i>SOD1</i> ⁴	—	—	0.115	—	—	—	—
<i>PGM1</i>	<i>PGM1</i> ¹	—	—	—	—	—	—	1.000
	<i>PGM1</i> ²	0.886	1.000	1.000	1.000	1.000	1.000	—
	<i>PGM1</i> ³	0.114	—	—	—	—	—	—
<i>ESD</i>	<i>ESD</i> ¹	—	—	0.125	0.164	—	—	—
	<i>ESD</i> ²	1.000	1.000	0.875	0.836	1.000	1.000	0.010
	<i>ESD</i> ³	—	—	—	—	—	—	0.990
<i>ES1</i>	<i>ES1</i> ¹	—	—	—	—	1.000	1.000	—
	<i>ES1</i> ²	1.000	1.000	1.000	1.000	—	—	—
	<i>ES1</i> ³	—	—	—	—	—	—	1.000
<i>ES2</i>	<i>ES2</i> ¹	—	—	—	—	—	—	0.020
	<i>ES2</i> ²	0.045	1.000	—	—	—	—	0.980
	<i>ES2</i> ³	—	—	1.000	0.975	1.000	1.000	—
	<i>ES2</i> ⁴	0.955	—	—	0.025	—	—	—
<i>ACP1</i>	<i>ACP1</i> ¹	1.000	1.000	1.000	1.000	1.000	1.000	—
	<i>ACP1</i> ²	—	—	—	—	—	—	1.000
<i>CA2</i>	<i>CA2</i> ¹	—	—	—	—	—	—	0.080
	<i>CA2</i> ²	—	—	—	—	—	—	0.920
	<i>CA2</i> ³	—	0.526	—	—	—	—	—
	<i>CA2</i> ⁴	—	0.474	—	—	—	—	—
	<i>CA2</i> ⁵	—	—	0.818	—	1.000	1.000	—
	<i>CA2</i> ⁶	1.000	—	0.045	—	—	—	—
	<i>CA2</i> ⁷	—	—	0.137	1.000	—	—	—
<i>HBA</i>	<i>HBA</i> ¹	1.000	1.000	1.000	1.000	1.000	1.000	—
	<i>HBA</i> ²	—	—	—	—	—	—	1.000
<i>HBB</i>	<i>HBB</i> ¹	1.000	1.000	1.000	1.000	1.000	1.000	—
	<i>HBB</i> ²	—	—	—	—	—	—	1.000
<i>ALB</i>	<i>ALB</i> ¹	—	0.057	—	—	—	—	—
	<i>ALB</i> ²	1.000	0.943	1.000	1.000	1.000	1.000	—
	<i>ALB</i> ³	—	—	—	—	—	—	1.000

Populations identified in Table II.

(1992), Ferrari (1993), and Rylands et al. (1993), the observed values all point to the possibility that these

Table VI - Average heterozygosity, proportion of polymorphic loci and mean number of alleles per locus in the seven populations of primates.

Population	N	L	H	SD	P	A
<i>Saguinus mystax</i>	22	15	2.3	1.5	20	1.2
<i>Saguinus fuscicollis weddelli</i>	138	15	4.1	3.4	13	1.1
<i>Saguinus midas midas</i>	13	15	5.6	2.8	27	1.3
<i>Saguinus midas niger</i>	123	15	2.8	1.9	20	1.3
<i>Saguinus bicolor bicolor</i>	22	15	1.6	1.6	7	1.1
<i>Saguinus bicolor martinsi</i>	4	15	1.7	1.7	7	1.1
<i>Alouatta belzebul belzebul</i>	50	15	5.0	2.5	40	1.4

N = Number of animals; L = number of loci; H(%) = average heterozygosity; SD(%) = standard deviation; P(%) = proportion of polymorphic loci; A = mean number of alleles per locus.

Table VII - Matrix of genetic distance (below) and genetic identity (above) between the *Saguinus* and *Alouatta* populations.

	SMY	SFW	SMM	SMN	SBB	SBM	ABB
SMY	—	0.885	0.877	0.867	0.797	0.798	0.274
SFW	0.112	—	0.887	0.879	0.810	0.811	0.343
SMM	0.119	0.107	—	0.951	0.927	0.929	0.277
SMN	0.130	0.117	0.047	—	0.861	0.862	0.273
SBB	0.199	0.185	0.070	0.136	—	0.999	0.263
SBM	0.198	0.184	0.069	0.135	0.001	—	0.272
ABB	0.700	0.627	0.685	0.699	0.713	0.704	—

populations represent subspecies of *Saguinus midas* Linnaeus, 1758, which, in accordance with the above observations on the status of the *S. bicolor* forms, would encompass a total of three subspecies, *S. midas midas*, *S. midas bicolor* and *S. midas niger*. If confirmed by additional data, this proposal would have significant implications for the conservation of the pied tamarins (*bicolor* forms), one of, if not the rarest and most endangered of Amazonian primates (Egler, 1993).

As expected, the analyses also supported relatively close ties between *S. fuscicollis* and *S. mystax* (Figures 1 and 2) which are clearly sister taxa as expected from their geographical and morphological proximity, although the distance values between them and the *S. midas* subspecies were also smaller than might be expected (Table VII), reflecting the genetic homogeneity of the callitrichines in general (Nagai *et al.*, 1986; Melo *et al.*, 1992; Dantas, 1994; Nagamachi, 1995; Meireles, C., Sampaio, I., Schneider, H., Ferrari, S.F., Coimbra-Filho, A.F., Pissinatti, A. and Schneider, M.P.C., unpublished results). Clearly, more data from a larger number of taxa will be needed before the taxonomic relationships within the genus can be defined with precision.

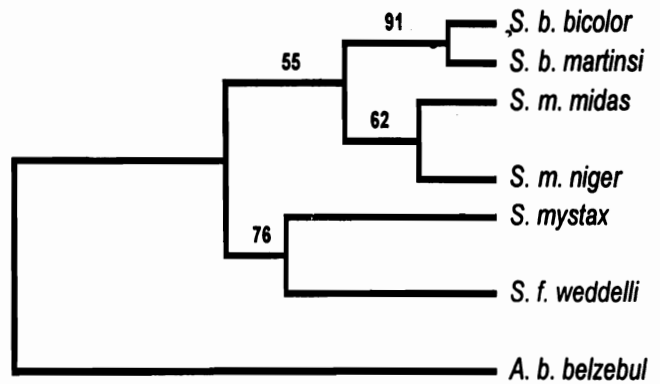


Figure 1 - UPGMA tree showing the relationships among the *Saguinus* species and *Alouatta belzebul*. Numbers at the nodes are bootstrap values from 2000 replicates.

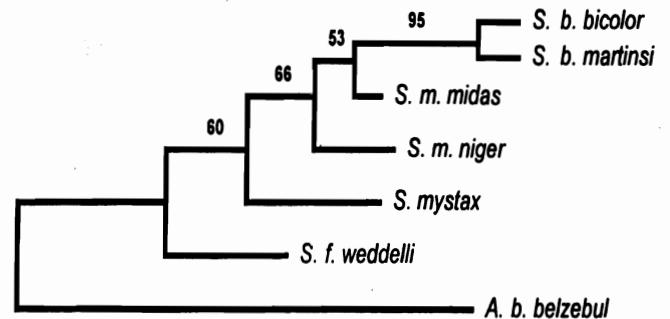


Figure 2 - Neighbor-joining tree showing the relationships among the *Saguinus* species and *Alouatta belzebul*. Numbers at the nodes are bootstrap values from 2000 replicates.

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RESUMO

A variabilidade genética de seis taxa de tamarins, gênero *Saguinus*, foi analisada comparativamente usando-se dados protéicos de onze sistemas codificados por quinze loci. *S. fuscicollis weddelli* e *S. midas midas* foram os taxa mais polimórficos, enquanto *S. bicolor* foi o menos. Os resultados da análise filogenética (UPGMA e neighbor-joining) e as distâncias genéticas entre os taxa foram em geral consistentes com suas relações geográficas e filogenéticas. As análises das populações de *S. bicolor* e *S. midas* indicaram que eles podem representar não mais do que três subspecies de uma única espécie, *S. midas*, com as formas de *bicolor* pertencendo a uma única subspecie, *S. midas bicolor*. Se apoiado por estudos

adicionais, este fato teria implicações importantes para a conservação da forma de *bicolor*, que está em perigo de extinção. A similaridade genética de *S. fuscicollis* e *S. mystax* foi também consistente com sua proximidade geográfica e morfológica, embora mais dados sobre um número maior de taxa seriam necessários antes de se definirem as relações taxonômicas dentro do gênero.

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