

Mitochondrial DNA-like sequence in the nuclear genome of *Saguinus* (Callitrichinae, Primates): transfer estimation

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Abstract

Mitochondrial DNA-like sequences have been found in the nuclei of a variety of organisms. These nuclear pseudogenes can be used to estimate relative evolutionary rates of mitochondrial genes, and can be used as outgroups in phylogenetic analyses. In this study, mitochondrial sequences with pseudogene-like characteristics, including deletions and/or insertions and stop codons, were found in tamarins (*Saguinus* spp., Callitrichinae, Primates). Phylogenetic analysis allowed estimation of the timing of the migration of these sequences to the nuclear genome, and also permitted inferences on the phylogeny of the genus. The choice of an inadequate outgroup (*Aotus infulatus*) prevented a good phylogenetic resolution of the subfamily Callitrichinae. The relatively ancient divergence of the Cebidae (Callitrichinae, Aotinae and Cebinae) may have favored confounding homoplasies.

INTRODUCTION

Mitochondrial DNA-like sequences have been observed in the nuclear genome of several vertebrates and invertebrates (Gellissen *et al.*, 1983; Jacobs *et al.*, 1983; Fukuda *et al.*, 1985; Thorness and Fox, 1990; Smith *et al.*, 1992; Lopez *et al.*, 1994; Arctander, 1995; Moreira, 1996; Sunnucks and Hales, 1996). When transferred to the nucleus, these sequences lose their function and become pseudogenes as a result of the different genetic code and to regulatory factors present in this organelle. Lopez *et al.* (1994) designated such sequences as *Numt* (nuclear mitochondrial) to distinguish them from *Cymt* (cytoplasmic mitochondrial) sequences.

When non-specific primers are used, *Numt* sequences may be preferentially amplified because of the better matches between primer and pseudogene (Smith *et al.*, 1992; Collura and Stewart, 1995; van der Kuyl *et al.*, 1995). As a result they may confound phylogenetic analyses. However, such pseudogenes can be used as a powerful tool to estimate the relative evolutionary rates of mitochondrial genes (Arctander, 1995; Lopez *et al.*, 1997). As these sequences evolve more slowly than their mitochondrial counterparts, and are thus generally more similar to the ancestral sequences, they can be used as outgroups in phylogenetic analyses (Zhang and Hewitt, 1996). In addition, the timing of migration to the nuclear genome can be estimated.

The tamarins, *Saguinus* spp., are the basal members of the Callitrichinae, which also includes the genera *Callithrix*, *Leontopithecus* and *Callimico* (Primates - Platyrrhini) (Schneider *et al.*, 1993, 1996; Almeida, 1995; Barroso *et al.*, 1997). Schneider *et al.* (1996) estimated that this subfamily emerged approximately 10 million years ago (Mya). According to the classic review of

HersHKovitz (1977), *Saguinus* is divided into three "sections" based on the facial pelage (hairy face, mottled face and bare face), and six species groups, with a total of 10 species (Table I). More recently, Rylands *et al.* (1993) recognized 12 species, including *Saguinus tripartitus* (Thorington Jr., 1988) and *Saguinus geoffroyi* (Mittermeier and Coimbra-Filho, 1982), and also suggested a new grouping of *Saguinus midas* ssp. and *Saguinus bicolor* spp. (see Natori and Hanihara, 1990; Ferrari, 1993; Meireles *et al.*, 1997).

Jacobs *et al.* (1995) and Cropp *et al.* (1999) sequenced the D-loop region and a small part of cytochrome *b* from the mitochondrial DNA to clarify the taxonomy of *Saguinus*. According to these authors, *Saguinus* is composed of two clusters, one including *S. fuscicollis*, *S. tripartitus* and *S. nigricollis* (called small-bodied group), and the other with the rest of the species that compose the genus (large-bodied group). This new grouping has some important differences from the classical taxonomy of this genus (HersHKovitz, 1977), some of which are supported by other authors. For example, *midas* and *bicolor* are grouped while the Colombian tamarins are a sister group.

In the present study, we sequenced 549 bp of the cytochrome *c* oxidase subunit II (COII) mitochondrial gene of *Saguinus*, and included additional sequences from *Callithrix*, *Callimico* and *Aotus*, in order to i) identify mitochondrial-DNA like sequences, ii) determine the timing of the migration of the COII gene to the nucleus, and iii) evaluate the phylogenetic relationships among the callitrichines.

MATERIAL AND METHODS

Blood samples were collected from seven tamarins representing six taxa (Table II). DNA was extracted using

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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>	<i>S. nigricollis</i> <i>S. fuscicollis</i>	<i>nigricollis</i> , <i>graellsii</i> , <i>hernandenzii</i> <i>fuscus</i> , <i>avilapirensis</i> , <i>fuscicollis</i> , <i>nigrifrons</i> , <i>illigeri</i> , <i>leucogenys</i> , <i>lagonotus</i> , <i>tripartitus</i> *, <i>wedelli</i> , <i>cruzlimai</i> , <i>crandalli</i> , <i>acrensis</i> , <i>melanoleucus</i> , <i>primitivus</i>
<i>S. mystax</i>	<i>S. mystax</i> <i>S. labiatus</i> <i>S. imperator</i>	<i>mystax</i> , <i>pileatus</i> , <i>pluto</i> <i>labiatus</i> , <i>thomasi</i> <i>imperator</i> , <i>subgrisescens</i>
<i>S. midas</i>	<i>S. midas</i>	<i>midas</i> , <i>niger</i>
Mottled-face tamarin group		
<i>S. inustus</i>	<i>S. inustus</i>	
Bare-face tamarin group		
<i>S. bicolor</i>	<i>S. bicolor</i>	<i>bicolor</i> , <i>martinsi</i> , <i>ochraceus</i>
<i>S. oedipus</i>	<i>S. oedipus</i> <i>S. leucopus</i>	<i>oedipus</i> , <i>geoffroyi</i> *

* Rylands *et al.* (1993) gave this subspecies a species status in agreement with Mittermeier and Coimbra-Filho (1982) and Thorington Jr. (1988).

Table II - The origin and identification of the samples used in this study.

Taxonomic identification (Code)	Origin
<i>S. m. midas</i> (SMM)	Centro Nacional de Primatas (unknown origin)
<i>S. m. niger</i> (SMN)	Tucuruí, Pará, Brazil
<i>S. b. bicolor</i> (SBB1 and SBB2)	Centro de Primatologia do Rio de Janeiro (unknown origin)
<i>S. oedipus</i> (SOE)	Bolívar, Colômbia
<i>S. m. mystax</i> (SMY)	Centro Nacional de Primatas (unknown origin)
<i>S. f. melanoleucus</i> (SFM)	Left bank of Rio Envira, Acre, Brazil
<i>C. jacchus</i> (CJA)	Extremós, Rio Grande do Norte, Brazil
<i>C. argentata</i> (CAR)	Rio Anauera, Cametá, Pará, Brazil
<i>C. aurita</i> (CAU)	Centro de Primatologia do Rio de Janeiro (unknown origin)
<i>Callimico goeldi</i> (CGO)	Acre, Brazil
<i>Aotus infulatus</i> (AI)	Amapá, Brazil

phenol extraction, followed by sodium acetate precipitation. After extraction, the samples underwent 30 cycles of the polymerase chain reaction (PCR) with the following conditions: denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1.5 min. The sequences of the primers used in PCR were $\Sigma 24$ - 5' CCA TCC AGC CCA ACT AGG CTT A 3' and $\Sigma 25$ - 5' GGC TCA TACTTC AAA GTCTTG G 3'.

The PCR product was electrophoresed in 1% agarose gels. The band corresponding to the COII gene was excised and purified using the Qiaex gel extraction protocol (Qiagen). The purified DNA was inserted into the vector PGEM^T (Promega) according to the manufacturers recommendations, and then cloned into *E. coli* using a pT7Blue kit (Novagen Corp.). The clones were infected

with Helper Phage M13K07 (Invitrogen) and the single-strand DNA (ssDNA) was purified by PEG-NaCl precipitation, followed by phenol-chloroform extraction and ethanol precipitation. The ssDNA was sequenced by the dideoxy chain-termination method (Sanger *et al.*, 1977) using a Sequenase kit (Amersham Life Science).

The sequences were aligned using the ESEE200b sequence editor (Cabot and Beckenbach, 1989) by constructing a common alignment relative to the sequence from *Aotus infulatus*, which was used as the outgroup. Divergence was estimated with the MEGA program (Kumar *et al.*, 1993), using the algorithm of Tamura and Nei (1993) which is generally employed in analyses involving mitochondrial sequences. The most parsimonious tree was obtained using the SEQBOOT, DNAPARS and CONSENSE programs of the Phylogenetic Inference Package (PHYLIP) (Felsenstein, 1993).

RESULTS

The nucleotide sequences of the cytochrome *c* oxidase subunit II (COII) mitochondrial gene in seven OTUs (operation taxonomic units) of *Saguinus*, three *Callithrix* (*C. aurita* (CAU), *C. argentata* (CAR), *C. jacchus* (CJA)), one *Callimico goeldii* (CGO) and one *Aotus infulatus* (AI) are shown in Figure 1. The SMM, SMN, SBB1 and SOE sequences (cluster 2) presented deletions at positions 197, 239 and 384. SOE also showed an insertion at position 519. Such mutations are characteristics of *Numt*. On the other hand, SMY, SFM and SBB2 (cluster 1) showed no such mutations.

The OTUs from cluster 2 had no nucleotide variation at any codon position, as is generally observed in noncoding sequences (Figure 2). The inferred amino acid sequences of the two clusters were highly homogeneous,

Figure 1 - Alignment of 549 base pairs of the cytochrome *c* oxidase subunit II (COII) mitochondrial gene in four New World primate genera (*Saguinus*, *Callithrix*, *Callimico* and *Aotus*). Dots indicate nucleotides identical to *Saguinus bicolor bicolor* (SBB1), “?” indicates missing data and “-” represents an insertion or deletion. SMM = *S. m. midas*, SMN = *S. m. niger*, SBB1, SBB2 = *S. b. bicolor*, SOE = *S. oedipus*, SMY = *S. m. mystax*, SFM = *S. f. melanoleucus*, CJA = *C. jacchus*, CAR = *C. argentata*, CAU = *C. aurita*, CGO = *Callimico goeldi*, and AI = *Aotus infulatus*.

				111	111	111	122	222	222	223	333	333	333	444	444	444	455	555
	123	456	789	012	345	678	901	234	567	890	123	456	789	012	345	678	901	234
SBB1	CAA	AAC	GCT	ACG	TCC	CCC	ATT	ATA	GAA	GAA	CTA	ATT	GAT	TTC	CAT	GAT	CAC	ATC
SMN
SMM
SOE?	???	???	???	???	???	???
SBB2AT	..C	T..	..C	..CCCT	GCT
SFMAT	..CT	..?	..CC	..T	..CT	GCT
SMYAT	..CC	..C	..CC	..T	..C	..C	..T	GCT
CGOACTCCC	..T	GC.
CARC	G.A	..T	..A	..CTCC	..C	...	GCT
CAUC	G.AA	..CTCCC	..T	GCT
CJAC	G.AA	..CTCCC	..T	GCT
AIATGT	..C	..C	..T	..C	..CC
																111	111	111
	555	556	666	666	666	777	777	777	788	888	888	888	889	999	999	999	000	000
	567	890	123	456	789	012	345	678	901	234	567	890	123	456	789	012	345	678
SBB1	CTC	ATA	ATT	ATT	TTC	CTA	ATT	AGC	TCT	CCA	GTC	CTA	TAC	ATT	ATT	TCC	CTA	ATA
SMN
SMM
SOE?
SBB2	..TTA	..T	..AT	G..	..CG
SFM	..ATT	..A	..T	..AT	G..G
SMY	..ATT	..A	..T	..AT	G..G
CGO	..TT	T..	TT.	..T	T..	..T	T..	...
CAR	..GC	T..C	..TTC
CAU	..AC	T..A	..T	..TT	..CT
CJA	..AC	T..ATC	..C	..T
AI	..T	..TCTG	..TT
	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111	111
	011	111	111	112	222	222	222	333	333	333	344	444	444	445	555	555	555	666
	901	234	567	890	123	456	789	012	345	678	901	234	567	890	123	456	789	012
SBB1	CTT	ACT	ACA	AAG	CTA	AAC	CAT	ACC	AGC	ACC	ATA	TAT	GCT	CAA	GAA	ATG	GAA	ATA
SMN
SMM
SOE
SBB2	..C	..A	..C	..A	T..	..CA	..C	..TAA	..CT
SFM	..C	..A	..T	..AC	..CAA	..C	..?T	...
SMY	..C	..A	..T	..AC	..CAA	..C	..CC
CGOA	..T	..CT	..C	..TAC
CAR	..C	..CA	..G	..CT	..CT	..GAC
CAUA	..G	..CA	..CTAC
CJAA	..G	..CA	..CTG	..A	..CCG
AI	..CA	T..	..C	..CA	..C	..C	G..T	...
	111	111	111	111	111	111	111	111	111	111	111	111	122	222	222	222	222	222
	666	666	677	777	777	778	888	888	888	999	999	999	900	000	000	001	111	111
	345	678	901	234	567	890	123	456	789	012	345	678	901	234	567	890	123	456
SBB1	AT?	?GA	ACG	ATC	CTA	CCC	GCC	ATT	ATT	CTT	ATT	A-A	ATT	GCC	CTC	CCA	TCC	CTA
SMN	G.C	T..
SMM	..C	T..
SOE	..C	T..G
SBB2	..T	T..	..T	..TT	..ACC	..TT	..T
SFM	..T	T..	..C	..TT	..ACC	..TT	..T
SMY	..T	T..	..C	..TT	..ACC	..TT	..T
CGO	..C	T.G	..C	..T	T..	G.CTT	..TT	T..
CAR	..C	T..	..TA	..C	..CTA
CAU	..C	T..	..CT	..A	..A	..C	..CTT
CJA	..C	T..	..TT	..A	..A	..C	..CTT
AI	..C	T..	..TGC	..C	..CTG

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Table I - Continued

	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222
	111	222	222	222	233	333	333	334	444	444	444	555	555	555	566	666	666	667
	789	012	345	678	901	234	567	890	123	456	789	012	345	678	901	234	567	890
SBB1	AGA	ATC	CTG	TAC	ATA	ACA	GAC	G-G	TTT	AAT	AAA	CCC	TAC	TTA	ACC	CTT	AAA	GCA
SMN
SMM
SOE
SBB2	C.C	...	T.A	..TT	.AATC	...
SFM	C.C	...	T.AAAGTC	...
SMY	C.C	...	T.AAAGTC	...
CGO	C.C	..T	.A	..TT	.A.	..CT
CAR	C.T	..T	T.A	..T	..GT	.AAG	...	C..	..T
CAU	C.T	..T	T.A	..TA.A	...	C.T	..T
CJA	C.C	..T	T.A	..TA.A	..T	C..
AI	C.T	..T	..TT	.AAC	C..
	222	222	222	222	222	222	222	222	222	223	333	333	333	333	333	333	333	333
	777	777	777	888	888	888	899	999	999	990	000	000	000	111	111	111	122	222
	123	456	789	012	345	678	901	234	567	890	123	456	789	012	345	678	901	234
SBB1	ACT	GGT	CAC	CAA	TGA	TAC	TGA	AGC	TAC	GAA	TGC	TCA	GAC	TAT	GAA	GAC	TTA	GCT
SMNT
SMM
SOEA.?	?..
SBB2	.T.	..CT	..G	.A.	C..	..C
SFM	.T.T	..T	..G	.A.C
SMY	.T.	..CT	..T	..G	.A.C
CGO	.T.TT	..T	..T	..A.	..GT	C..	..A	...
CAR	.TC	..CGT	..G	.A.	..CG	..A	...
CAU	.T.	..CA.	..CA	...
CJA	.TC	..CTA.	..CA	...
AI	.T.	..C?T	..G	.A.	.T.	..TT	C..	..TC
	333	333	333	333	333	333	333	333	333	333	333	333	333	333	333	333	333	333
	222	223	333	333	333	444	444	444	455	555	555	556	666	666	666	777	777	777
	567	890	123	456	789	012	345	678	901	234	567	890	123	456	789	012	345	678
SBB1	TTT	GAT	TCT	TAT	ATT	ATA	TCA	GCA	TAT	TTT	CTT	GAA	CCC	GGC	GAA	TTT	CAA	CTT
SMN
SMMT
SOE	C..A
SBB2C	..C	..C	..C	..C	C.T	A..	..CCAC	..G	..C
SFM	..C	..C	..C	..C	..C	..C	C.T	A..	..CT	..AC	..G	..C	..C
SMYC	..C	..C	..C	..C	C.T	A..	..CT	..AC	..G	..C	..C
CGOC	..CCG	C..	A..	..C	..C	..A	..GGG	..C	..C
CAR	..C	..C	..C	C.C	A..	..C	..CT	..G	..GG	..C
CAU	..C	C..	A..CGGC	..G	..C
CJA	..C	..C	..C	..C	C..	A..	..C	..CGC	..G	..C	..C
AI	...	A.CT	C..	A..CGTG	...
	333	333	333	333	333	333	333	444	444	444	444	444	444	444	444	444	444	444
	788	888	888	889	999	999	999	000	000	000	011	111	111	112	222	222	222	333
	901	234	567	890	123	456	789	012	345	678	901	234	567	890	123	456	789	012
SBB1	CCT	GA-	GTA	GAT	AAC	CGA	ACC	ACC	TTG	CCA	ATA	GAA	GCA	GAT	ATC	CGT	ATG	CTA
SMN
SMM
SOE
SBB2	.T.	..AA	..T	C.A	..CA	...
SFM	.T.	..ACA	..T	C.A	..CC	..A
SMY	.T.	..ACA	..T	C.A	..CC	..A
CGO	.T.	..GAA	..T	..GC	..T	...	G.A	...
CAR	.T.	..A	..GA	...	C.A	..CC	..TA	T..
CAU	.T.	..ACAA	..TTA	T..	...
CJA	.T.	..ACAT	..G	G.A	T..	...
AI	..TA	..AGA	...	C..T	..C	..A

Continued on next page

Table 1 - Continued

	444	444	444	444	444	444	444	444	444	444	444	444	444	444	444	444	444	444
	333	333	344	444	444	445	555	555	555	666	666	666	677	777	777	778	888	888
	345	678	901	234	567	890	123	456	789	012	345	678	901	234	567	890	123	456
SBB1	ATT	TCA	TCA	GAA	GAC	ATC	TTA	CAC	TCA	TGA	TCT	GTC	CCA	CCA	CTA	GGC	GTT	AAA
SMN
SMM
SOE
SBB2	..C	C..	...	G..	G.C	T..G
SFM	..C	C..	...	G..	..G	G??	..T	...	T..G
SMY	..C	C..	...	G..	..G	G??	..T	...	T..G
CGO	C..	...	G..	C..G	G.C	..A	...	G..T	..A	...
CAR	..CG	C..	...	G.T	C..	G.C	..A	...	T..T	..A	...
CAU	..C	C.G	..T	G.TG	..C	..C	T..G	...
CJA	..C	C..	...	G.T	G..	..A	..T	T..G	...
AI	...	A..	...	C..	...	G..	G..	T..	T.G	..T
	444	444	444	444	455	555	555	555	555	555	555	555	555	555	555	555	555	555
	888	999	999	999	900	000	000	001	111	111	111	222	222	222	233	333	333	334
	789	012	345	678	901	234	567	890	123	456	789	012	345	678	901	234	567	890
SBB1	ACA	GAT	GCA	ATC	CCC	AGA	CGT	TTA	AAT	CA?	GC-	TGC	CCT	AGC	CTC	TAT	ACG	ACC
SMNC
SMMC
SOE	G..	.A.A	..TC	..T
SBB2	G..T	G..	..CAAT	A..	G..	...	C.C
SFM	G..T	G..	..CC	..AAT	A..	G..	...	C..
SMY	G..T	G..	..CC	..AAT	A..	G..	...	C..
CGOT	..T	...	G..	..C	C..A	...	CAT	AT.	..A.C
CARA	G..	..CAAT	AG.	...	T..	C..	G..	...
CAU	G..	..CC	..A	...	CAT	AG.	...	T..	C..
CJA	..C	G..	..CA	...	CAT	AG.	...	T..	C..
AICAAT	A..A	C..
	555	555	555															
	444	444	444															
	123	456	789															
SBB1	AGG	CCT	ATT															
SMN															
SMM															
SOE															
SBB2	G..															
SFM															
SMY															
CGO	...	TT.	..A															
CARA															
CAUA															
CJAA															
AI	..C.															

although there were several differences. The OTUs from cluster 2 had two stop codons (nucleotide positions 217-219 and 502-504). The former codon and a deletion were located in membrane-spanning domains. Other important changes included a tyrosine (cluster 1) - cysteine (cluster 2) swap (301-303) in the sequence of aromatic residues in a region believed to favor electron transfer and which is highly conserved in eutherians (Adkins and Honeycutt, 1994; Adkins *et al.*, 1996).

The parsimonious consensus tree showed two monophyletic clades, supported by 68.1 and 100% of bootstrap percentages. One encompasses (((SMN,SBB1),SMM),SOE), and the others include ((SMY,SFM),SBB2) grouped

with (((CJA,CAU),CAR),CGO). *Saguinus* was split into two clusters: a basal lineage, cluster 1, with non-significant bootstrap values (68.1%), and a sister group of the Callitrichinae, cluster 2 (Figure 3).

The genetic divergence (Tamura and Nei, 1993) among *Saguinus* species ranged from 0.37% (SBB1 and SMN) to 22.29% (SOE and SBB2). However, when the other species were included, the maximum divergence increased to 23.56% (SMM and CAR). The divergence among cluster 1 OTUs ranged from 1.31% (SMY and SFM) to 6.14% (SBB2 and SMY), and in cluster 2, from 0.37% (SBB1 and SMN) to 2.33% (SMM and SOE). Divergence rates varied little when individuals of different clusters

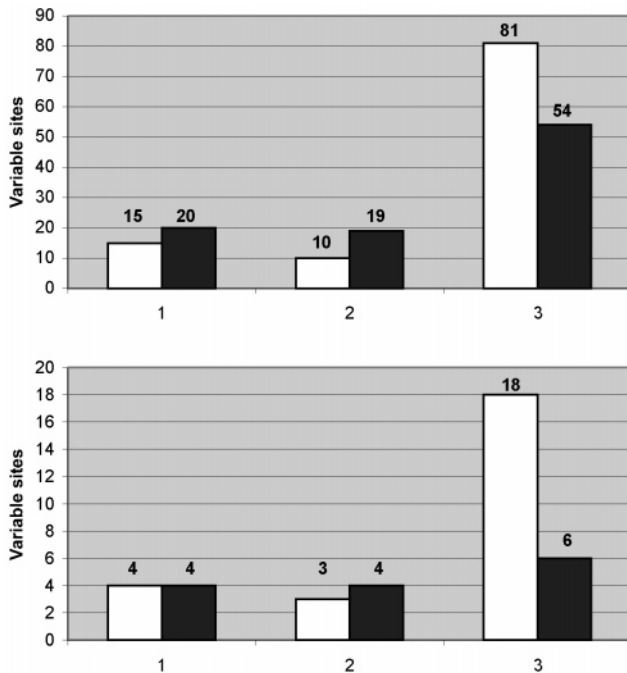


Figure 2 - Number of variable sites in the three codon positions in cluster 1 (open columns) and cluster 2 (filled columns) with (upper panel) and without (lower panel) the outgroup.

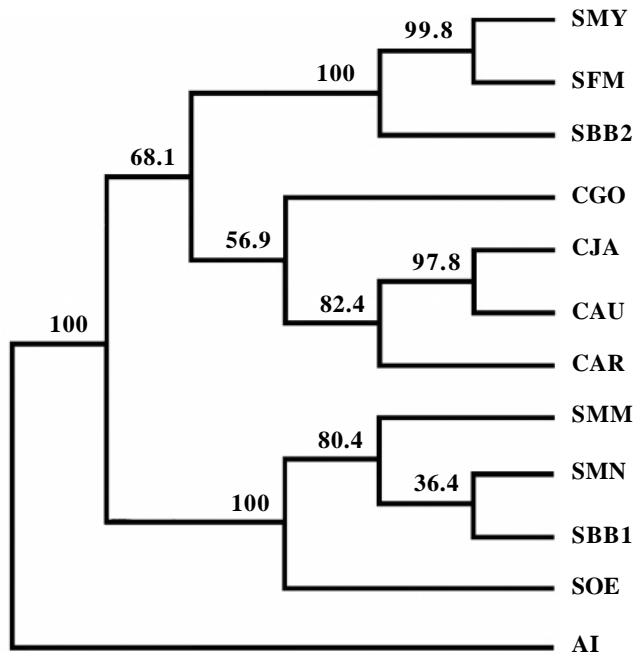


Figure 3 - Phylogenetic tree of the cytochrome *c* oxidase subunit II (COII) mitochondrial gene in four New World primate genera (*Saguinus*, *Callithrix*, *Callimico* and *Aotus*) obtained by parsimony analysis using PHYLIP 3.5c (Felsenstein, 1993). The score of the three most parsimonious trees was 348. Bootstrap percentages from 2000 replicates are shown above each node. For abbreviations, see legend to Figure 1.

were compared (from 20.99% for SFM and SBB1 to 22.29% for SOE and SBB2) (Figure 4).

There was a high degree of divergence (21.77%) between the two samples of *S. bicolor bicolor* used in the present study (SBB1 and SBB2), which was higher than divergences among distinct genera, such as *Saguinus* and *Callimico* (SBB2 and CGO) (19.73%).

DISCUSSION

The characteristics of the sequences of cluster 2 (SOE, SBB1, SMN, and SMM), such as the marked divergence (even among samples of the same species - *S. b. bicolor* samples), equivalent rates of change in all codon positions, deletions and/or insertions, stop codons and changes in highly conserved amino acids, lead us to conclude that we had obtained mitochondrial DNA-like nuclear sequences (pseudogenes). This is why SBB1 and SBB2 appear in different clades and consequently, the high divergence (Figure 4) should reflect the time of the migration of this sequence to the nucleus. However, other theories could account for this high degree of divergence, such as heteroplasmy (two different copies of the mitochondrial genome, one containing the intact COII gene and the other with mutations).

We excluded heteroplasmy as a possible explanation for these cases, because it can be maintained in an organism only if the differences between the two molecules are substitutions (which may even be non-synonymous, as long as protein function is unaffected), but not where deletions and/or insertions have occurred. Olivo *et al.* (1983) suggested that selective pressures resulted in the survival of only one of the heteroplasmic mitochondrial DNA molecules (the one without deletions or insertions) during the early development of an organism. However, this hypothesis is plausible only for functional mitochondrial sequences. In the case of noncoding sequences, such as the D-loop region, heteroplasmy can be maintained because there will be no selective pressure on molecules with deletions and/or insertions, except in regions where heavy chain replication is initiated (see Hayasaka *et al.*, 1991).

The number of substitutions in cluster 2 OTUs was much smaller than in cluster 1 OTUs, which would be expected when mitochondrial and nuclear sequences are compared (Figure 2). In addition, in the absence of the outgroup, cluster 2 OTUs exhibited no variation in nucleotides at any codon position.

Saguinus pseudogenes (cluster 2) and COII sequences (cluster 1) are phylogenetically distinct (Figure 3). The divergence between *Saguinus* of different clusters is greater than that between *Saguinus* (cluster 1) and *Callithrix*. According to our most parsimonious tree, the pseudogene would have appeared prior to the emergence of the Callitrichinae, that is, before 10 Mya (according to Schneider *et al.*, 1996), and the appearance of *Saguinus*. Consequently, all species in clade 2 possess the same se-

OTUs	SBB1	SMN	SMM	SOE	SBB2	SFM	SMY	CGO	CAR	CAU	CJA
SMN	0.37										
SMM	0.56	0.55									
SOE	1.95	2.14	2.33								
SBB2	21.77	22.17	22.24	22.29							
SFM	20.99	21.39	21.45	21.49	6.13						
SMY	21.74	22.14	22.21	22.27	6.14	1.31					
CGO	22.66	22.74	22.19	22.72	19.73	22.02	22.30				
CAR	23.41	23.50	23.56	23.16	17.68	18.71	18.39	19.36			
CAU	20.31	20.41	20.46	20.28	19.28	17.11	16.80	19.36	12.22		
CJA	21.20	21.29	21.35	20.89	18.40	17.40	17.09	18.40	12.25	5.59	
AI	18.10	18.21	17.44	19.05	19.09	20.10	19.18	22.52	19.22	20.60	22.61

Figure 4 - Genetic divergence calculated using the algorithm of Tamura and Nei (1993). For abbreviations, see legend to Figure 1.

quence that migrates to the nucleus. However, this does not mean that other *Saguinus* taxa, not present in this clade, do not have the same pseudogene. The PCR products were cloned; so, by chance, it is possible that one of the fragments amplified (the COII gene or its pseudogene counterpart) could have been cloned and subsequently sequenced. That is why some samples of *Saguinus* were sequenced for the COII gene (clade 1) and others for the COII pseudogene (clade 2).

However, the possibility that the migration occurred after the emergence of the callitrichines cannot be excluded, in which case divergent haplotypes may have existed, one of which migrated to the nucleus. This would explain the degree of divergence observed. If true, it may be possible to find haplotypes that are very similar to the pseudogenes in present-day *Saguinus* populations, if they have not been extinguished. The former hypothesis is, however, the most parsimonious.

The poor phylogenetic resolution of the present study may reflect the choice of *Aotus* as the outgroup, given that Schneider *et al.* (1996) estimated the emergence of the Cebidae at approximately 17.5 Mya, which might favor homoplasy between the Callitrichinae and Aotinae. The divergence rate between *Saguinus* (cluster 1) and *Aotus* (or even *Callithrix* and *Aotus*) is slightly higher than that between *Saguinus* (cluster 1) and *Callithrix*. This probably reflects saturation of the bases as a consequence of the relatively ancient divergence between the Callitrichinae and Aotinae-Cebinae. The use of the *Aotus* haplotype may thus favor the appearance of reverse mutations, turning potentially informative sites into homoplastic ones.

Other studies have shown that COII is a useful gene for resolving phylogenetic questions within platyrrhine genera (Anselmo, 1996; Sena, 1998; Figueiredo, 1999), but not among very divergent platyrrhine genera (von Dornum, 1997). Despite these problems, it is possible to draw two conclusions from our data. First, the close relationship between *S. mystax* and *S. fuscicollis* indicated by Hershkovitz (1977) was confirmed (cluster 1), contrary to Jacobs *et al.* (1995) and Cropp *et al.* (1999).

However, more data should be added to conclude if these two forms are really close taxa or not. Second, *S. midas* and *S. bicolor* were placed in the same group (cluster 2), as suggested by Natori and Hanihara (1990), Ferrari (1993), Rylands *et al.* (1993), Jacobs *et al.* (1995), Meireles *et al.* (1997) and Cropp *et al.* (1999), although a more detailed analysis will be required before the relationship between these two forms, i.e., whether *S. bicolor* is a subspecies of *S. midas*, can be defined.

In spite of its limitations, the present comparison of mitochondrial DNA in two different evolutionary scenarios (nucleus and mitochondria) offers a useful tool for tracing the evolutionary history of COII in callitrichines.

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RESUMO

Seqüências tipo mitocondriais têm comumente sido encontradas no genoma nuclear de diversos organismos. Quando acidentalmente incluídas em estudos de seqüências mitocondriais, diversas conclusões errôneas podem ser obtidas. No entanto, estes pseudogenes nucleares tipo mitocondriais podem ser usados para a estimativa da taxa relativa de evolução de genes mitocondriais e também como grupo externo em análises filogenéticas. No presente trabalho, seqüências mitocondriais com características do tipo de pseudogene, tais como deleções e/ou inserções e có-dons de parada, foram encontradas em *tamarins* (*Saguinus* spp., Callitrichinae, Primates). A análise filogenética permitiu a estimativa do tempo da migração da seqüência mitocondrial para o genoma nuclear e algumas inferências filogenéticas. A escolha de um grupo externo não adequado (*Aotus infulatus*) não permitiu uma reconstrução filogenética confiável da subfamília Callitrichinae. A divergência bastante antiga de Cebidae (Callitrichinae, Aotinae e Cebinae) pode ter favorecido o aparecimento de homoplasias, obscurecendo a análise.

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