Genetics and Molecular Biology, 23, 4, 729-737 (2000)

Molecular studies of *Callithrix pygmaea* (Primates, Platyrrhini) based on transferrin intronic and ND1 regions: implications for taxonomy and conservation

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

Abstract

Traditional classifications of Platyrrhini monkeys, based mainly on morphological features, are being contested by recent molecular data. The subfamily Callitrichinae (Platyrrhini, Primates) consists of a diverse group of species, many of them considered endangered. Our analysis of two DNA regions, a mtDNA gene (ND1) and a nuclear gene (intronic regions of the transferrin gene), suggests that *Callithrix pygmaea* may have sufficient variability to justify the existence of subspecies or even separate species. Phylogenetic dendrograms based on the ND1 region show that this species is more closely related to Amazonian than to Atlantic forest marmosets. These results reopen the discussion about diversity and conservation programs based exclusively on traditional classifications.

INTRODUCTION

According to Schneider *et al*. (1996) the New World monkeys can be classified into two families: the Atelidae and the Cebidae. The latter is divided into three subfamilies: Cebinae (*Cebus* and *Saimiri*), Aotinae (*Aotus*) and Callitrichinae (*Saguinus*, *Leontopithecus*, *Callimico* and *Callithrix*). Some authors grouped the *Callimico* genus within the family Callitrichidae but allocated it to a distinct subfamily, the Callimiconinae (Napier and Napier, 1967; Ford, 1986; Kay, 1994). Dollman (1933) and Hershkovitz (1977) classified *Callimico* in its own family, the Callimiconidae. Thomas (1913); Simpson (1945); Stirton (1951), Cabrera (1958), and Simons (1972) included *Callimico* in the Cebidae family. There is a consensus that *Callithrix*, *Leontopithecus* and *Saguinus* all belong to the same clade but the evolutionary relationships between these genera are still controversial (Rosenberger, 1981; Ford, 1986; Schneider *et al*., 1996; Pastorini *et al*., 1998). The genus *Callithrix* has a wide distribution and can be divided into three distinct groups of species: the *argentata* group, represented by marmosets from the Amazonian forest and part of the *cerrado* (a type of Brazilian savanna); the *jacchus* group, comprising marmosets from the Atlantic forest, the *cerrado* and the *caatinga* (a dry region of stunted vegetation and brushwood) (Hershkovitz, 1977), and the *pygmaea* group made up of pygmy marmosets (*C. pygmaea*) from the Amazonian forest (Barroso *et al*., 1997). The pygmy marmoset was first classified as *Cebuella* Gray, 1866, a genus distinct from the already described *Callithrix* Erxleben, 1777. However, recent molecular data indicate that the pygmy marmoset should be classified as *C. pygmaea* (Schneider *et al*., 1996; Barroso *et al*., 1997; Porter *et al*., 1997). Previously, Rosenberger (1981, 1984) had drawn attention to morphological similarities between pygmy marmosets and species of *Callithrix* that could justify their classification within the same genus.

Considering the system adopted by the World Conservation Union (WCU) (see Rylands *et al*., 1995, 1997), several species and/or subspecies of Callitrichinae are classified as critically endangered or endangered as a consequence of habitat destruction and illegal capture or hunting. According to Rylands *et al*. (1995, 1997) the following callitrichines may be classified as critically endangered: *Leontopithecus rosalia*, *L. chrysopygus*, and *L. caissara* whereas those on the endangered list are *L. chrysomelas*, *C. flaviceps*, *C. aurita*, *Saguinus bicolor bicolor*, and *S. oedipus*. Although *C. pygmaea* is affected by illegal international trade, particularly in the area of Iquitos in Peru, it is classified in the WCU's Lower Risk category (Rylands *et al*., 1995). *Callithrix pygmaea* is a small monkey and thus is not hunted for food by humans. Furthermore, it has a wide geographical distribution and is capable of existing in isolated forest patches near human settlements (see Hershkovitz, 1977; Rylands *et al*., 1993).

The purpose of the present study was to provide a molecular view of the relationships among the callitrichines with particular emphasis on *C. pygmaea* and to highlight the importance of the use of molecular studies in designing conservation programs.

MATERIAL AND METHODS

The scientific names, sample code and geographical origins of monkeys, as well as the fragments of DNA se-

[1]*Laboratório de Genética e Biologia Molecular, Campus de Bragança, UFPA, Bragança, PA, Brasil.*
Send correspondence to C.H.T. E-mail:tagliaro@ufpa.br
[2] *Laboratório de Polimorfismo de DNA, Departamento de Genética, Centro de Ciências Biológicas, UFPA, Belém, PA, Brasil.*
[3] *School of Biology and Biochemistry, Queen's University of Belfast, Belfast, Northern Ireland.*

quenced, appear in Table I. Monkeys were anesthetized with Ketalar (10 mg/kg body weight) and blood samples collected from femoral veins. Blood samples were centrifuged and DNA extracted from the isolated leukocytes according to the protocol of Sambrook *et al*. (1989).

DNA sequences of the transferrin (exon 4 to exon 6) and ND1 regions were determined by direct sequencing of PCR-amplified fragments. For the ND1 fragment, a second PCR (internal) was performed on the fragment resulting from the first PCR in order to eliminate false priming products that occasionally arise in the original genomic DNA PCR. Primers for the amplification of these regions are described in Table II. The DNA sequences were determined using dye terminator cycle sequencing reactions that were subsequently loaded onto an automatic sequencer (Applied Biosystems model 373A) according to the manufacturer's protocols. Additional sequencing primers were designed as necessary.

Initial sequence alignments were performed using the Eyeball sequence editor (Cabot and Beckenback, 1989). Data were analyzed by the neighbor-joining (NJ) method using the MEGA program (Kumar *et al*., 1993) whereas maximum-parsimony (MP) and maximum-likelihood (ML) analyses were performed with PAUP 4.0 beta version (Swofford, 1998) using a branch and bound algorithm search (Hendy and Penny, 1982). The robustness of the phylogenetic hypothesis obtained by MP was tested by bootstrapping (Felsenstein, 1985) and the criterion adopted to evaluate it was that suggested by Hillis and Bull (1993) who consider bootstrap values equal or superior to 70% to be statistically significant. All bootstrap analyses of DNA sequence data involved at least 2000 replications and all gaps were considered as single events. NJ analyses of the DNA sequence data were performed using the method of Jukes and Cantor (1969) for the transferrin fragment and that of Tamura and Nei (1993) for the mtDNA region (ND1). The reliability of the topologies of the NJ tree branches were tested by the standard error test (SET), using the method of Jukes and Cantor (1969) for transferrin, the Kimura two-parameter distance values for the mtDNA region (Kimura, 1980), and the MEGA program (Kumar *et al*., 1993).

The estimated time since divergence was calculated by means of the branch lengths of the NJ trees and the method proposed by Sarich and Wilson (1973), using the estimated time of 13 million years ago (Mya) for the emergence of the tribe Callitrichini (Goodman *et al*., 1998).

RESULTS

The transferrin sequences determined for callitrichine representatives are 1662 bp in length but only the intronic regions, corresponding to 1472 bp, were used in the phylo-

Table I - The origin and identification of various New World monkeys for which DNA sequences were determined.

Identification code	Taxonomic identification	DNA region sequenced	Origin
CAR-021	*Callithrix argentata*	ND1	Rio Anauera, Cametá, PA, Brazil
CGE-083	*Callithrix geoffroyi*	ND1	Criadouro Barbuse Leal, Brasília, DF, Brazil*
CJA-033	*Callithrix jacchus*	ND1 TF	Extremós, RN, Brazil
CKU-093	*Callithrix kuhli*	ND1	Floresta Azul, BA, Brazil
CKU-094	*Callithrix kuhli*	ND1	Ilhéus, BA, Brazil
CKU-095	*Callithrix kuhli*	ND1	Una, BA, Brazil
CPE-089	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-090	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-091	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-092	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-127	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-128	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-129	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPE-130	*Callithrix penicillata*	ND1	Biotério da Universidade de Brasília, Brasília, DF, Brazil*
CPY-104	*Callithrix pygmaea*	ND1 TF	Centro Nacional de Primatas, Belém, PA, Brazil*
CPY-105	*Callithrix pygmaea*	ND1 TF	Centro Nacional de Primatas, Belém, PA, Brazil*
LCH-101	*Leontopithecus chrysomelas*	TF	Centro de Primatologia do Rio de Janeiro, RJ, Brazil*
LCH-110	*Leontopithecus chrysomelas*	ND1	Centro de Primatologia do Rio de Janeiro, RJ, Brazil*
CGO-111	*Callimico goeldii*	ND1 TF	Municipality of Lagoínha, Rio Juruá, AC, Brazil
SMY-045	*Saguinus mystax*	ND1	Iquitos, Peru
CAP-048	*Cebus apella*	ND1 TF	Centro Nacional de Primatas, Belém, PA, Brazil*

*Animals in captivity and unknown origin.

genetic analyses. The trees obtained by NJ, ML and MP analysis all agree in their topology. The phylogenetic tree (Figure 1) shows both *C. pygmaea* individuals joining together at the same branch-point, an arrangement statistically supported by bootstrap (96%) and SET (95%) analysis. *C. jacchus* and *C. pygmaea* form a sister group, also considered significant by both bootstrap (100%) and SET (99%) analyses. The next representative to join this sister group is *Callimico*, supported only by bootstrap (76%) analysis. *Saguinus* and *Leontopithecus* were the most basal genera, but it was not possible to determine which one was the first to split.

One 1321-bp fragment spanning the ND1 fragment and flanking regions was sequenced, although only sequences of 951 bp that corresponded exclusively to the ND1 gene were considered in the analysis. The two *Callithrix pygmaea* (CPY-104 and CPY-105) individuals grouped together; this topology being supported by bootstrap (93%) and SET (99%) analysis. Representatives of the *jacchus* group (*C. jacchus*, *C. penicillata*, *C. kuhli*, *C. geoffroyi*) grouped together (Figure 2); an observation strongly supported by bootstrap (100%) and SET (99%) analyses. The existence of a *Callithrix* clade (containing representatives of species of the *Callithrix* genus, including *C. pygmaea*) was also supported by bootstrap (99%) and SET (99%) analysis. The arrangement of the dendrograms (produced by the NJ, ML and MP methods) obtained using ND1 data are not significant and no inference can be made as to the relationships between the most basal genera of the subfamily Callitrichinae and the species of the *jacchus* group.

Comparison between the DNA sequences of the two pygmy marmosets sampled (CPY-104 and CPY-105) showed important differences. The intronic transferrin fragments of 1472 bp (exon 4 to 6) had five transitions and two transversions. Sixty-two transitions and three transversions were detected among the ND1 sequences of the two pygmy marmosets sampled, which generated nine differences in the amino acid sequence of the ND1 protein. Comparison of the two sequences of the D-loop and adjacent region (GenBank numbers U89009, U89010) showed that there were 59 transitions and 36 transversions, along with three deletions of 1 bp and one of 15 bp.

Tables III-V show the divergence matrices obtained for representatives of the subfamily Callitrichinae. Considering the ND1 fragment, both *C. pygmaea* individuals showed greater differences in their sequences and, consequently, in the divergence matrix (Table IV) than were observed in comparisons between the four species of the *jacchus* group. A previous study (Tagliaro *et al.*, 1997) showed that the D-loop divergence matrix (Table V) also indicated considerable divergence between the two *C.*

Table II - Primers used for the amplification of DNA fragments of transferrin, and ND1.

DNA fragments	Primers	Internal primers
Transferrin	5' GGCAGGTCCGCTGGGTGGAACATCCCCAT 3' (foward)	
	5' AAGGCCACATCCCCAGCACCATCCTTCA 3' (reverse)	
ND1	5' CTACGTGATCTGAGTTCAGACCGG 3' (foward)	5' CTACGTGATCTGAGTTCAGACCGG 3' (foward)
	5' AGGGTATAACCAACATTTTCGGGGTATG 3' (reverse)	5' CCCGATAGCTTATTTAGCTGACCTTAC 3' (reverse)

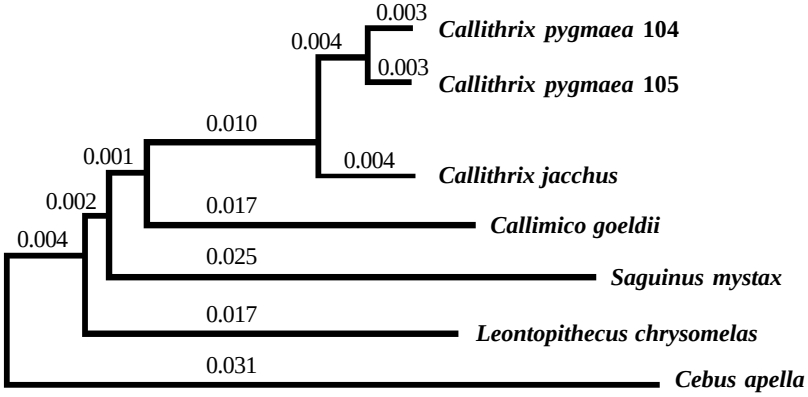

Figure 1 - Intronic transferrin sequence (exon 4 to 6) phylogenetic dendrogram showing branch-length values, constructed by the neighbor-joining method of Jukes and Cantor (1969).

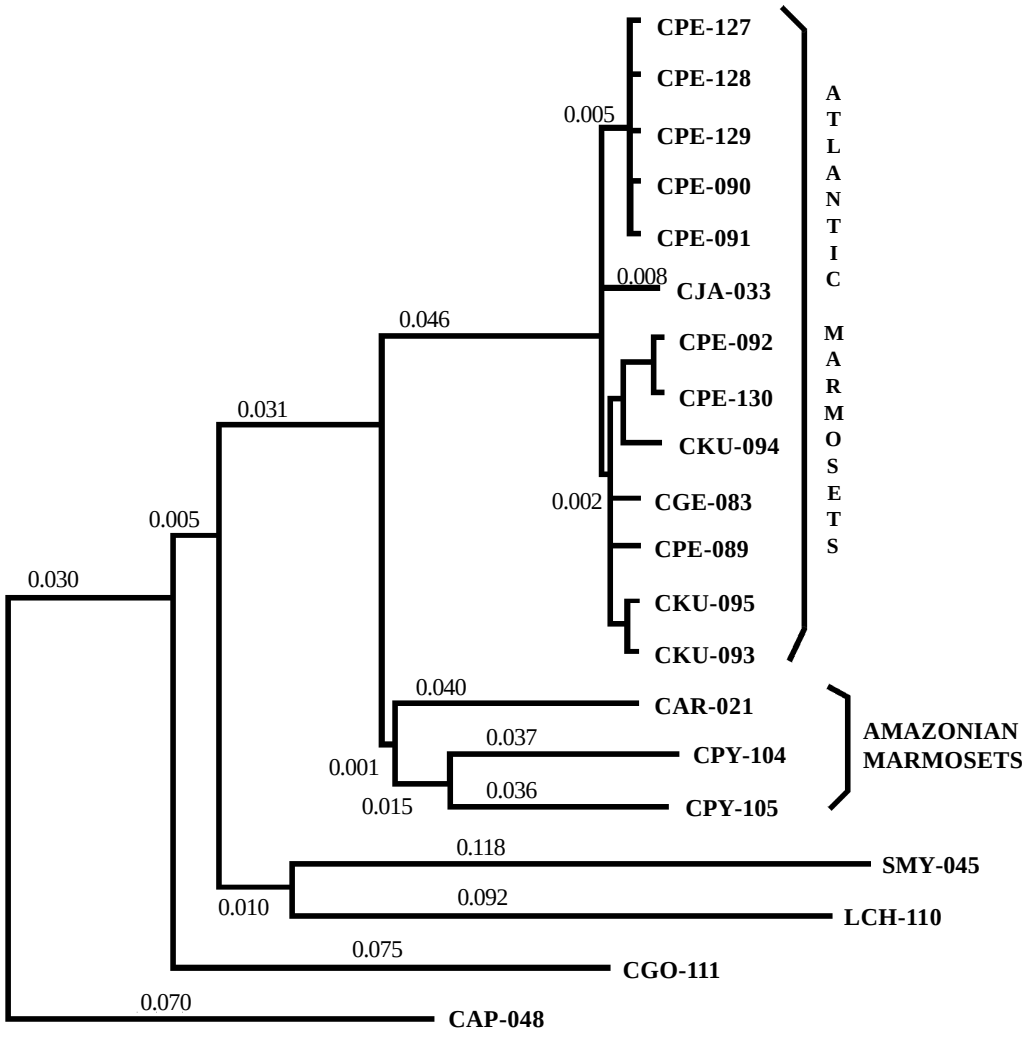

Figure 2 - ND1 gene-sequence phylogenetic dendrogram showing branch-length values, constructed by the neighbor-joining method of Tamura and Nei (1993).

Table III - Nucleotide divergence matrix for the transferrin fragment (x100) obtained by the method of Jukes and Cantor (1969).

Species	*Cebus apella*	*Callimico goeldii*	*Saguinus mystax*	*Leontopithecus chrysomelas*	*Callithrix pygmaea 105*	*Callithrix jacchus*
Callimico goeldii	5.56					
Saguinus mystax	6.33	4.30				
Leontopithecus chrysomelas	5.31	3.72	4.38			
Callithrix pygmaea 105	5.47	3.30	4.21	3.63		
Callithrix jacchus	5.14	3.14	4.05	3.47	1.11	
Callithrix pygmaea 104	5.56	3.38	4.30	3.55	0.55	1.03

pygmaea individuals. This value is comparable to values obtained between species of marmosets of different groups.

Based on the NJ dendrograms obtained for transferrin and ND1 sequences (Figures 1 and 2) and considering an estimate of 13 Mya (Goodman *et al*., 1998) as the time of the split of the Callitrichini from the other Platyrrhini, the timing of the separation of the lineages of the two *C. pygmaea* individuals was estimated as 1.8 Mya for transferrin and 5.4 Mya for ND1. The estimate of the time of separation between the two *C. pygmaea* individuals obtained by NJ dendrogram analysis (see Figure 3) of D-loop sequences was 4.0 Mya.

DISCUSSION

Molecular data from the ND1 region indicate that *C. pygmaea* is more closely related to other Amazonian marmosets (*argentata* group, represented in this study by *C. argentata*) than to those of the Atlantic forest or the *cerrado* and *caatinga* (*jacchus* group; represented by *C. jacchus*, *C. penicillata*, *C. kuhli*, *C. geoffroyi*). This agrees with the results obtained by Canavez *et al*. (1996) based on karyology, and Tagliaro *et al*. (1997), Porter *et al*. (1997) and Almeida (1995), based on DNA sequences. The studies of Barroso *et al*. (1997) and Sena (1998), also based on DNA analysis, are inconclusive in defining whether *C. pygmaea* is more closely related to the *argentata* group or to the *jacchus* group. Based on detailed studies of morphological features and feeding habits, Rosenberger (1981, 1984) considered that the pygmy marmoset should be classified within the genus *Callithrix*, although this researcher believed that the pygmy marmoset was closely related to the *jacchus* group, a theory which contrasts with the molecular evidence.

The two *C. pygmaea* individuals were more different than would be expected for a monospecific group in which no subspecies have yet been described. There was a divergence of 7.4% in the ND1 divergence matrix which corresponds to values observed between marmosets of different groups. In the D-loop divergence matrix (Table V) the divergence between the two *C. pygmaea* individuals was 11.2%. Similar values have been found only between *C. aurita* and other species of the *jacchus* group (*C. aurita* representing the most basal species of this group). All other intra-group divergence values were inferior to 8.0%.

The calculated timing of separation of the two lineages of *C. pygmaea* ranges from 1.8 Mya (transferrin) to 5.4 Mya (ND1). In relative terms these dates are informative, being earlier than those for the emergence of the present species of the amended *jacchus* group in the same studies. Hershkovitz (1977) considered the possibility of the existence of subspecies of *C. pygmaea*, but did not agree with the subspecies *C. p. niveiventris* Lonneberg 1940, and Van Roosmalen and Van Roosmalen (1997) reopened this debate after observing the *niveiventris* morphotype in areas where it had not been thought to occur. Meireles *et al*. (1998), investigating protein variability, found that *C. pygmaea* (N = 8) was the most variable species in a study involving 13 species and subspecies of callitrichines.

Apparently *Callithrix pygmaea* consists of two different subspecies or perhaps even two species. However, the individuals used in this study, like those in the study by Meireles *et al*. (1998), were captive animals from the "Centro Nacional de Primatas". These animals and their ancestors were of unknown geographical origin. In order to obtain more conclusive evidence, further molecular studies should be undertaken with more individuals of known geographical origin.

The 21st century is going to be a critical period for the conservation of biodiversity, particularly in richly biodiverse areas such as the neotropical forests. Descriptions of new

Table IV - Divergence matrix for ND1 region sequences (x100), obtained by the method of Tamura & Nei (1993).

	CAP	SMY	LCH	CGO	CPE-089	CPE-090	CPE-091	CPE-092	CPE-127	CPE-128	CPE-129	CPE-130	CGE	CJA	CKU-093	CKU-094	CKU-095	CPY-104	CPY-105	CAR
SMY	23.13																			
LCH	21.24	21.19																		
CGO	17.67	20.36	19.10																	
CPE-089	19.46	22.09	19.04	16.80																
CPE-090	18.96	22.01	18.33	16.72	1.83															
CPE-091	19.26	22.04	18.63	16.67	1.62	0.21														
CPE-092	19.24	22.41	18.69	16.76	1.51	1.39	1.40													
CPE-127	19.12	22.04	18.65	16.86	1.83	0.21	0.21	1.39												
CPE-128	19.09	22.01	18.63	16.85	1.61	0.21	0.00	1.39	0.11											
CPE-129	19.06	22.00	18.43	16.65	1.72	0.11	0.11	1.29	0.11	0.11										
CPE-130	19.35	22.20	18.74	16.67	1.40	1.40	1.29	0.21	1.40	1.29	1.29									
CGE	19.05	22.14	18.69	16.88	1.61	1.61	1.62	1.40	1.72	1.71	1.61	1.40								
CJA	18.04	22.13	18.64	16.46	1.95	1.40	1.41	1.95	1.40	1.39	1.29	1.96	1.83							
CKU-093	19.02	22.27	19.67	17.50	1.17	1.50	1.51	0.96	1.50	1.50	1.39	1.18	1.28	1.61						
CKU-094	19.15	21.96	18.82	17.09	1.61	1.50	1.51	0.74	1.50	1.50	1.39	0.75	1.49	2.05	1.28					
CKU-095	18.76	22.00	19.41	17.50	1.17	1.50	1.51	0.96	1.50	1.50	1.40	1.18	1.28	1.61	0.21	1.28				
CPY-104	19.81	20.27	18.51	17.42	10.36	10.22	10.56	10.48	10.49	10.48	10.40	10.81	10.99	11.30	10.33	10.57	10.47			
CPY-105	19.11	19.29	18.90	16.72	10.86	10.45	10.80	10.71	10.72	10.71	10.63	11.04	10.96	10.61	10.69	10.80	10.70	7.40	CPY-105	
CAR	18.25	19.98	16.27	17.39	9.94	9.26	9.59	9.41	9.52	9.51	9.30	9.58	9.51	9.68	9.50	9.23	9.26	9.05	9.27	

See Table I for identification of the samples.

Table V - Divergence matrix for marmoset control region sequences (x100) obtained by the method of Tamura and Nei (1993).

	LCH																								
CAU-120	23.00	**CAU-120**																							
CAU-121	22.81	00.38	**CAU-121**																						
CJA-033	22.47	11.04	10.87	**CJA-033**																					
CJA-043	22.48	11.18	11.01	00.13	**CJA-043**																				
CKU-094	21.76	09.33	09.17	05.67	05.53	**CKU-094**																			
CKU-095	22.13	09.81	09.65	05.67	05.54	05.79	**CKU-095**																		
CKU-096	21.76	09.33	09.17	05.67	05.53	00.00	05.79	**CKU-096**																	
CKU-122	21.09	08.63	08.48	05.25	05.12	04.70	02.43	04.70	**CKU-122**																
CKU-123	21.76	09.33	09.17	05.67	05.53	00.00	05.79	00.00	04.70	**CKU-123**															
CGE-081	22.59	09.59	09.44	06.94	07.08	05.93	06.32	05.93	05.63	05.93	**CGE-081**														
CGE-083	22.41	09.59	09.44	06.94	07.08	05.93	06.32	05.93	05.63	05.93	00.13	**CGE-083**													
CGE-085	22.41	09.59	09.44	06.94	07.08	05.93	06.32	05.93	05.63	05.93	00.13	00.00	**CGE-085**												
CGE-087	22.59	09.59	09.44	06.94	07.08	05.93	06.32	05.93	05.63	05.93	00.00	00.13	0013	**CGE-087**											
CPE-089	21.07	09.64	09.48	05.81	05.67	04.56	05.23	04.56	04.15	04.56	05.37	05.23	05.23	05.37	**CPE-089**										
CPE-129	22.17	09.36	09.50	04.29	04.16	04.85	06.22	04.85	05.69	04.85	07.35	07.35	07.35	07.35	05.95	**CPE-129**									
CAR-021	23.52	14.27	14.10	12.88	13.02	12.10	13.01	12.10	11.77	12.10	11.92	11.92	11.92	11.92	12.06	12.26	**CAR-021**								
CAR-023	23.52	14.27	14.10	12.88	13.02	12.10	13.01	12.10	11.77	12.10	11.92	11.92	11.92	11.92	12.06	12.26	00.00	**CAR-023**							
CAR-098	23.70	14.76	14.59	13.18	13.33	12.42	13.18	12.42	12.55	12.42	12.08	12.08	12.08	12.08	12.52	12.56	00.89	00.89	**CAR-098**						
CMA-009	24.03	13.44	13.91	13.32	13.47	12.20	13.28	12.20	12.35	12.20	11.87	11.87	11.87	11.87	11.44	12.52	06.65	06.65	07.08	**CMA-009**					
CMA-010	24.03	13.44	13.91	13.32	13.47	12.20	13.28	12.20	12.35	12.20	11.87	11.87	11.87	11.87	11.44	12.52	06.65	06.65	07.08	00.00	**CMA-010**				
CMA-011	23.52	13.93	13.75	12.52	12.66	12.52	13.11	12.52	11.88	12.52	11.26	11.26	11.26	11.26	12.03	12.36	06.66	06.66	07.08	03.23	03.23	**CMA-011**			
CHU-029	24.57	14.06	13.89	13.42	13.57	13.44	13.72	13.44	12.49	13.44	12.62	12.62	12.62	12.62	13.09	12.95	07.35	07.35	07.93	04.17	04.17	02.31	**CHU-029**		
CHU-031	23.86	13.30	13.13	12.37	12.51	12.37	12.34	12.37	11.74	12.37	11.88	11.88	11.88	11.88	12.19	12.37	05.95	05.95	06.66	03.77	03.77	02.05	02.04	**CHU-031**	
CPY-104	24.14	14.67	14.50	12.55	12.40	13.19	13.01	13.19	12.23	13.19	12.67	12.67	12.67	12.67	12.67	12.07	10.44	10.44	10.74	11.77	11.77	11.32	12.54	11.78	**CPY-104**
CPY-105	22.57	18.20	18.00	16.41	16.26	15.43	16.20	15.43	15.74	15.43	16.33	16.33	16.33	16.33	15.73	15.93	13.71	13.71	14.02	13.84	13.84	13.54	14.66	14.64	11.23

CHU = *Callithrix humeralifera*; CMA = *Callithrix mauesi*; CAU = *Callithrix aurita*; LCH = *Leontophitecus chrysomelas*. See Table I for identification of the other samples.

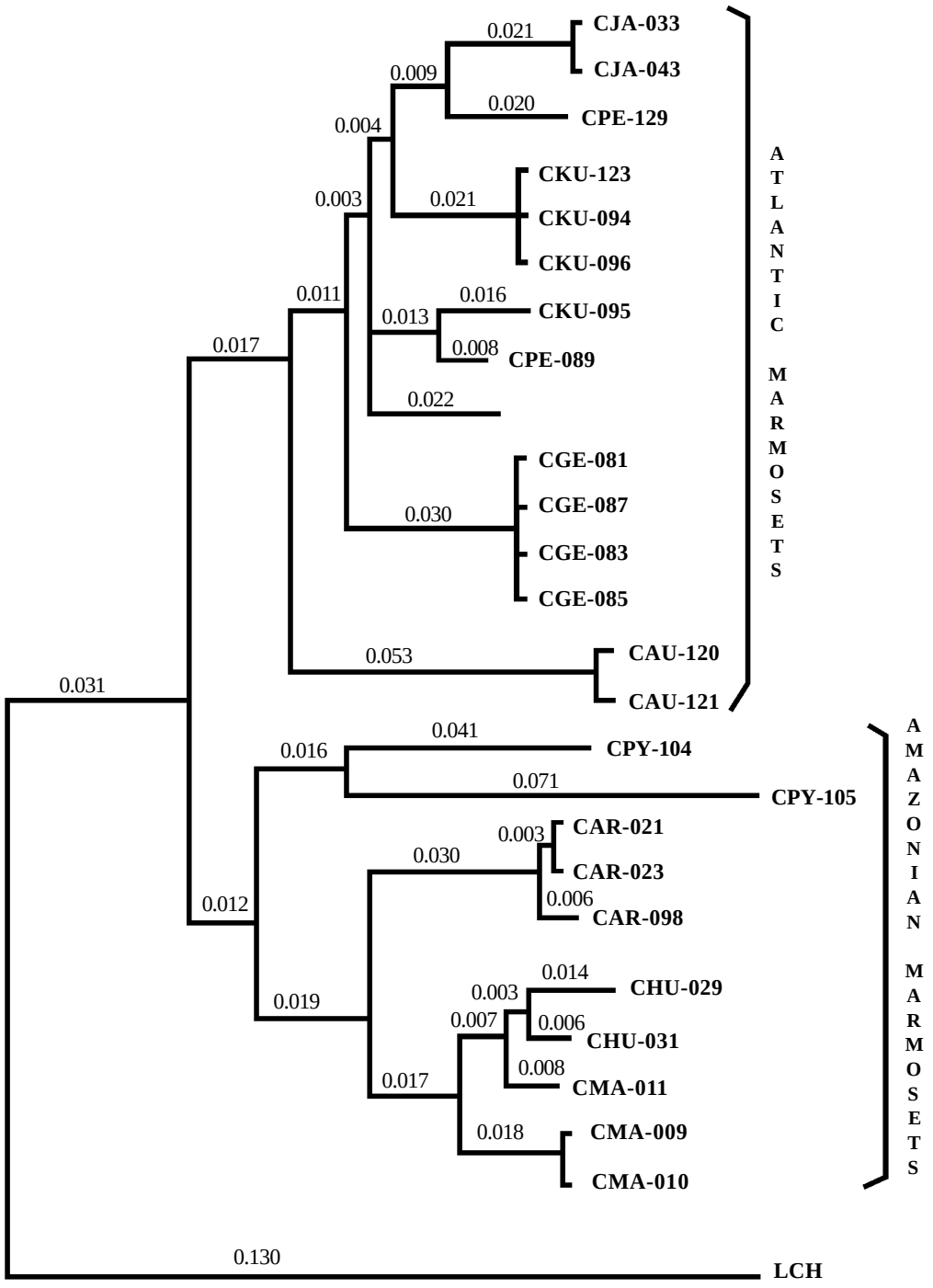

Figure 3 - D-loop phylogenetic dendrogram showing branch-length values, constructed by the neighbor-joining method of Tamura and Nei (1993). See Table I for identification of the samples. CHU = *Callithrix humeralifera*; CMA = *Callithrix mauesi*; CAU = *Callithrix aurita*; LCH = *Leontophitecus chrysomelas*.

species and subspecies of plants and animals, some of which are restricted to small geographical areas or are from peculiar habitats, continue to be published, and species already described are having their taxonomic status revised, while habitat destruction is occurring at such a rate that many species become extinct before even being described.

Molecular genetics is a new approach to taxonomic classification. It is helping to evaluate biodiversity and aid decision-making concerning to the conservation priorities of many different taxa. The recognition of groups that exhibit very little evolutionary differentiation and those that are phylogenetically distinct allows direction of conserva-

tion efforts to protect the maximum biological diversity so that priority can be given to those taxa most at risk of extinction. The possibility of the existence of different species and/or subspecies of *Callithrix pygmaea* surely necessitates a re-evaluation of their conservation status, particularly in the light of the trade in these small monkeys in certain areas such as the Iquitos region of Peru (Rylands *et al*., 1993).

ACKNOWLEDGMENTS

We would like to thank Dr. José Augusto Pereira Carneiro Muniz (Centro Nacional de Primatas, Belém, PA, Brazil), Dr. Adelmar Coimbra-Filho and Dr. Alcides Pissinati (Centro de Primatologia do Rio de Janeiro, RJ, Brazil), Arlindo Pinto de Souza Jr., Milton Thiago de Melo, and Criadouro Barbuse Leal for the samples used in this research. We thank Dr. Colin Robert Beasley for checking the English. C.H.T. was supported by the Brazilian research agency CNPq. This research was made possible by grants to M.J.S. from the Nuffield Foundation, the Royal Society, and Northern Ireland Development and Research.

RESUMO

As classificações tradicionais envolvendo os macacos da infraordem Platyrrhini, principalmente baseadas em características morfológicas, têm sido contestadas por dados moleculares recentes. A subfamília Callitrichinae (Platyrrhine, Primates) engloba um diverso grupo de espécies, muitas das quais consideradas em perigo de extinção. A presente análise de duas regiões do DNA, um gene mitocondrial (ND1) e um gene nuclear (regiões intrônicas da transferrina), sugerem que *Callithrix pygmaea* apresenta variabilidade suficiente para justificar a existência de subespécies ou até mesmo de espécies distintas. As árvores filogenéticas baseadas na região do ND1 indicam que esta espécie está relacionada mais proximamente aos marmosets amazônicos do que aos da mata Atlântica. Estes resultados reabrem a discussão sobre diversidade e programas de conservação baseados apenas em classificações taxonômicas tradicionais.

REFERENCES

Almeida, R.A.C. (1995). Estudo das relações intergenéricas na subfamília Callitrichinae usando o intron 11 do gene do fator de von Willebrand. Master's thesis, Universidade Federal do Pará, Museu Paraense Emílio Goeldi & Empresa Brasileira de Pesquisas Agropecuárias, Belém, PA.

Barroso, C.M.L., Schneider, H., Schneider, M.P.C., Sampaio, I., Harada, M.L., Czelusniak, J. and **Goodman, M.** (1997). Update on phylogenetic systematics of New World monkeys: further DNA evidence for placing the pygmy marmoset (*Cebuella*) within the marmoset genus *Callithrix*. *Int. J. Primatol. 18*: 645-668.

Cabot, E.L. and **Beckenbach, A.T.** (1989). Simultaneous editing of multiple nucleic acid and protein with ESEE. *Comput. Appl. Biosci. 5*: 233-234.

Cabrera, A. (1958). Catálogo de los mamíferos de America del Sur. *Rev. Mus. Argent. Cienc. Nat. "Bernadino Rivadavia" 4*: 1-307.

Canavez, F., Alves, G., Fanning, T.G. and **Seuánez, H.N.** (1996). Comparative karyology and evolution of the Amazonian *Callithrix* (Platyrrhini, Primates). *Chromosoma 104*: 348-357.

Dollman, G. (1933). *Primates, Series 3*. British Museum (Natural History), London.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution 39*: 783-791.

Ford, S.M. (1986). Systematics of the New World monkeys. In: *Comparative Primate Biology* (Swindler, D.R. and Erwin, J., eds.). Alan R. Liss Inc., New York, pp. 73-135.

Goodman, M., Porter, C.A., Czelusniak, J., Page, S.L., Schneider, H., Shoshani, J., Gunnel, G. and **Groves, C.P.** (1998). Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. *Mol. Phylogenet. Evol. 9*: 585-598.

Hendy, M.D. and **Penny, D.** (1982). Branch and Bound algorithms to determine minimal evolutionary trees. *Math. Biosci. 59*: 277-290.

Hershkovitz, P. (1977). *Living New World Monkeys (Platyrrhini); With an Introduction to Primates.* The University of Chicago Press, Chicago.

Hillis, D.M. and **Bull, J.J.** (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol. 42*: 182-192.

Jukes, T.H. and **Cantor, C.R.** (1969). Evolution of protein molecules. In: *Mammalian Protein Metabolism* (Munro, H.N., ed.). Academic Press, New York, pp. 21-132.

Kay, R.F. (1994). Giant tamarin from the Miocene of Colombia. *Am. J. Phys. Anthropol. 95*: 333-353.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol. 16*: 111-120.

Kumar, S., Tamura, K. and **Nei, M.** (1993). *MEGA: Molecular Evolutionary Genetics Analysis.* The Pennsylvania State University, University Park.

Meireles, C.M., Czelusniak, J., Sampaio, I., Schneider, H., Ferrari, S.F., Coimbra-Filho, A.F., Pissinatti, A., Muniz, J.A., Ferreira, H.S. and **Schneider, M.P.** (1998). Electrophoretic polymorphisms and their taxonomic implications in Callitrichini. *Biochem. Genet. 36*: 229-244.

Napier, J.S. and **Napier, P.H.** (1967). *A Handbook of Living Primates*. Academic Press, New York.

Pastorini, J., Forstner, M.R.J., Martin, R.D. and **Melnick, D.J.** (1998). A reexamination of the phylogenetic position of *Callimico* (Primates) incorporating new mitochondrial DNA sequence data. *J. Mol. Evol. 47*: 32-41.

Porter, C.A., Czelusniak, J., Schneider, H., Schneider, M.P.C., Sampaio, I. and **Goodman, M.** (1997). Sequences of the primate ε-globin gene: implications for systematics of the marmosets and other New World primates. *Gene 205*: 59-71.

Rosenberger, A.L. (1981). Systematics: the higher taxa. In: *Ecology and Behavior of Neotropical Primates* (Coimbra-Filho, A.F. and Mittermeier, R.A., eds.). Academia Brasileira de Ciências, Rio de Janeiro, pp. 9-27.

Rosenberger, A.L. (1984). Aspects of the systematics and evolution of the marmosets. In: *A Primatologia no Brasil* (Mello, M.T.D., ed.). Sociedade Brasileira de Primatologia, Brasília, pp. 159-180.

Rylands, A.B., Coimbra-Filho, A.F. and **Mittermeier, R.A.** (1993). Systematics, geographic distribution, and some notes on the conservation status of the Callitrichidae. In: *Marmosets and Tamarins. Systematics, Behaviour and Ecology* (Rylands, A.B., ed.). Oxford University Press, Oxford, pp. 11-77.

Rylands, A.B., Mittermeier, R.A. and **Rodriguez-Luna, E.** (1995). A species list for the New World primates (Platyrrhini): distribution by country, endemism, and conservation status according to the Mace-Land system. *Neotropical Primates 3* (Suppl.): 113-160.

Rylands, A.B., Mittermeier, R.A. and **Rodriguez-Luna, E.** (1997). Conservation of neotropical primates: threatened species and an analysis of primate diversity by country and region. *Folia Primatol. 68*: 134-160.

Sambrook, J., Fritsch, E.F. and **Maniatis, T.** (1989). *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sarich, V.M. and **Wilson, A.C.** (1973). Generation time and genomic evolution in primates. *Science 179*: 1144-1147.

Schneider, H., Sampaio, I., Harada, M.L., Barroso, C.M.L., Schneider, M.P.C., Czelusniak, J. and **Goodman, M.** (1996). Molecular phylogeny of the New World monkeys (Platyrrhini, Primates) based on two nuclear genes: IRBP intron 1 and ε-globin sequences. *Am. J. Phys. Anthropol. 100*: 153-179.

Sena, L. (1998). Filogenia do gênero *Callithrix Erxleben* 1777 (Callitrichinae, Platyrrhini) baseada em seqüências do gene mitocondrial da citicromo oxidase II (CoII). Master's thesis, Curso de Pós-graduação em Ciências Biológicas da Universidade Federal do Pará, Belém.

Simons, E.L. (1972). *Primate Evolution: An Introduction to Man's Place in*

Nature. Macmill, New York.

Simpson, G.G. (1945). The principles of classification of mammals. *Bull. Am. Mus. Nat. Hist. 85*: 1-350.

Stirton, R.A. (1951). Ceboid monkeys from the Miocene of Colombia. *Univ. Calif. Publ., Bull. Depart. Geol. Sci. 28*: 315-356.

Swofford, D.L. (1998). *PAUP*. Phylogenetics Analysis Using Parsimony (*and other methods). Version 4*. Sinauer Associates, Sunderland, Massachusetts.

Tagliaro, C.H., Schneider, M.P.C., Schneider, H., Sampaio, I.C. and **Stanhope, M.** (1997). Marmoset phylogenetics, conservation perspectives, and evolution of the mtDNA control region. *Mol. Biol. Evol. 14*: 674-684.

Tamura, K. and **Nei, M.** (1993). Estimation of the number of nucleotide substitutions in the control region of the mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol. 10*: 512-526.

Thomas, O. (1913). On some rare Amazonian mammals from the collection of the Pará Museum. *Ann. Mag. Nat. Hist. (Series 8) 11*: 130-136.

Van Roosmalen, M.G.M. and **Van Roosmalen, T.** (1997). An eastern extension of the geographical range of the pygmy marmoset, *Cebuella pygmaea*. *Neotropical Primates, 5*: 3-6.

(Received May 26, 2000)