

## THE DESCRIPTION OF A NEW MARMOSET GENUS, *CALLIBELLA* (CALLITRICHINAE, PRIMATES), INCLUDING ITS MOLECULAR PHYLOGENETIC STATUS

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### Abstract

This paper describes a new genus of Amazonian marmosets, the dwarf marmoset *Callibella*, formerly identified as *Callithrix humilis* (Van Roosmalen *et al.*, 1998), and reports its phylogenetic relationship to other callitrichines, based on 902 base pairs (bp) of the mitochondrial control region DNA sequence. The molecular data indicate an early divergence of *Callibella humilis*, prior to the divergence of *Cebuella pygmaea* from the ancestral Amazonian marmoset stock. The high degree of divergence of this new taxon warrants its placement in a distinct genus, *Callibella*. New observations from the morphology, physiology, ecology, and ethology of the dwarf marmoset are presented which support this classification.

**Key Words** – Primates, Callitrichidae, Callitrichinae, *Callibella humilis* - dwarf marmoset, *Cebuella* - pygmy marmoset, *Mico* - Amazonian marmosets, *Callithrix* - Atlantic marmosets, molecular genetics, phylogeny.

### Resumo

Descreve-se um novo gênero de sagüis da Amazônia Brasileira, o sagüi-anão *Callibella* antes identificado como *Callithrix humilis* (Van Roosmalen *et al.*, 1998), e relata-se a sua condição filogenética baseada em 902 pares de bases da região de controle mitocondrial. A seqüência da região de controle mitocondrial revela uma divergência cedo de *Callibella humilis*, antes da divergência de *Cebuella pygmaea* do sagüi Amazônico ancestral do *Mico*. O alto grau de divergência deste taxon novo justifica a sua colocação num gênero distinto, *Callibella*. Apresentam-se novas observações dos campos de morfologia, fisiologia, ecologia, e etologia do sagüi-anão os quais acrescentam a justificativa de ser classificado como o novo gênero *Callibella*.

**Palavras-Chave** – Primatas, Callitrichidae, Callitrichinae, *Callibella humilis* - 'sagüi-anão', *Cebuella* - 'mico-leãozinho', *Mico* - 'sagüis' da Amazônia, *Callithrix* - 'sagüis' Atlânticos, genética molecular, filogenia.

### Introduction

Platyrrhines, the New World monkeys, were until recently comprised of two families, the Cebidae and the Callitrichidae (formerly Hapalidae) (Rosenberger, 1981). Schneider *et al.* (1996), however, propose three families:

- 1) Cebidae, including three subfamilies: Cebinae (*Cebus*, *Saimiri*), Aotinae (*Aotus*), and Callitrichinae (*Callithrix*, *Cebuella*, *Saguinus*, *Leontopithecus*, *Callimico*);
- 2) Atelidae, including one subfamily: Atelinae (*Ateles*, *Brachyteles*, *Lagothrix*, *Alouatta*); and
- 3) Pitheciidae (*Pithecia*, *Chiropotes*, *Cacajao*, *Callicebus*).

As presented in this paper, the callitrichine subfamily consists of seven distinct genera, including *Saguinus* (tamarins), *Leontopithecus* (lion tamarins), *Mico* (Amazonian marmosets), *Callithrix* (Atlantic marmosets), *Cebuella* (pygmy marmosets), *Callibella* (dwarf marmoset), and *Callimico* (Goeldi's monkey). On morphological grounds, *Callimico* stands apart in the family for its retention of third molars, its

bearing single offspring rather than twins (although sharing this feature with *Callibella*), and its vocalizations (Snowdon, 1993). *Callimico* has been hypothesized as being basal to the callitrichid clade (Rosenberger, 1984), and some authors have proposed its placement in a separate family, Callimiconidae (Chiarelli, 1972; Hershkovitz, 1977).

Tamarins and lion tamarins have traditionally been viewed as sharing a common ancestry, separate from the marmosets and pygmy marmosets (Hershkovitz, 1977), but the molecular data do not support these arrangements. Cronin and Sarich (1978), based on the electrophoresis of plasma proteins, put *Callithrix* and *Callimico* in a clade that forms a trichotomy with *Saguinus* and *Leontopithecus*. Barroso *et al.* (1997), comparing the IRBP intron 1 sequences, and Schneider *et al.* (1993), comparing the  $\epsilon$ -globin nuclear gene sequences, found the order of clade separation should be (*Saguinus* (*Leontopithecus* (*Callimico*, *Callithrix*))), whereas Horovitz and Meyer (1995) reversed the order of separation between *Leontopithecus* and *Saguinus* based on the 16S mitochondrial DNA sequences. Morphological

and molecular studies universally agree that marmosets and pygmy marmosets are most closely related (e.g., Hershkovitz, 1977; Canavez *et al.*, 1999; Porter *et al.*, 1997).

The callitrichine subfamily represents a specialized clade of diminutive primates that underwent secondary phyletic dwarfing, probably due to its highly specialized diet and feeding strategy—one based on insectivory, consumption of small-seeded fruits (berries), and tapping exudates from a number of woody plant species (Garber, 1992). Callitrichines are generally found in disturbed, edge or patchy habitats, where a greater abundance of insects and berries may be found (Peres, 1991). The callitrichines are unique among New World primates in their vertically clinging posture, made possible by claw-like nails that are specialized for grasping onto the sides of large-diameter tree trunks. This allows for more effective exploitation of tree sap and insect larvae. Marmosets actively tap trees and lianas for exudates by gouging small holes in the bark, which may serve as an indefinitely reliable or keystone source of food. Tamarins, lacking the tusked condition of the mandibular incisors found in marmosets, do not actually gouge holes themselves; but they do feed on exudates from damaged tree stems (Egler, 1992) and *Parkia* fruits (Peres, 1991) as well as “parasitizing” the gouge holes produced by sympatric pygmy marmosets (*Cebuella*) (Soini, 1988). The life histories of all callitrichines therefore indicate a highly specialized clade of New World monkeys, instead of an ancestral or proto-platyrrhine relic, as suggested by Hershkovitz (1977).

Another hallmark of the callitrichine subfamily is its relatively high diversity of taxa. *Saguinus* includes 33 recognized taxa, most of which should merit full species status upon closer morphological, molecular and biogeographical examination (Van Roosmalen and Van Roosmalen, in prep.). *Callithrix* is composed of six species, while at least 14 species comprise the Amazonian *Mico*; four species are recognized in *Leontopithecus*; and *Cebuella*, *Callibella* and *Callimico* are monotypic genera. The callitrichine subfamily can thus be summarized as a highly adaptive, thoroughly diverse clade of primates that has successfully radiated throughout much of South America.

Despite this diversity, little is known about the evolutionary relationships among the various species of marmoset. In fact, most molecular phylogenies to date have found that *Mico*, the Amazonian marmoset clade, is more closely related to *Cebuella* (pygmy marmosets) than to *Callithrix*, the Atlantic clade (Barroso *et al.*, 1997; Canavez *et al.*, 1999; Porter *et al.*, 1997; Tagliaro *et al.*, 1997). This evidence led Rylands *et al.* (2000) and Groves (2001) to separate the larger marmosets into the genera *Mico* (Amazonian clade) and *Callithrix* (Atlantic marmosets or ouistitis). This approach retained *Cebuella* in a distinct genus rather than merging all marmosets—Amazonian, Atlantic and pygmy—into a single genus. In a previous study (Van Roosmalen *et al.*, 2000), the phylogenetic standing of two newly described Amazonian marmosets, *Mico manicorensis* and *M. acariensis*, was investigated using the mitochondrial

control region. Given the relatively low number of species represented in the few genetic studies on callitrichine interrelationships to date, the addition of the dwarf marmoset to the existing phylogenies should help resolve evolutionary patterns of radiation and speciation among the marmosets.

The purpose of this study is to determine the phylogenetic status of the dwarf marmoset, discovered in 1996 and published conservatively as *Callithrix humilis* Van Roosmalen *et al.*, 1998, based on mitochondrial control region (D-loop) sequences. These sequences were selected for their high rate of evolution, and have thus been considered optimal for phylogenetic resolution among closely related organisms by previous studies (Aquadro and Greenberg, 1983; Tagliaro *et al.*, 1997). The addition of the dwarf marmoset to the marmoset phylogenetic tree—as well as new information on its morphology, physiology, ontogeny, ecology and ethology—should shed new light on the relationships between marmoset clades, providing insight into patterns of evolution, radiation and dispersal, and the mechanisms that regulate isolation and speciation in the Amazon basin.

## Genus Description

### Genus *Callibella* Van Roosmalen and Van Roosmalen

*Callithrix* Erxleben, 1777 – type species *Callithrix humilis* Van Roosmalen, Van Roosmalen, Mittermeier and Fonseca, 1998

### *Callibella humilis* (Van Roosmalen, Van Roosmalen, Mittermeier and Fonseca, 1998)

**Holotype:** Museu Paraense Emílio Goeldi, Belém, Pará, Brazil, MPEG 24769, adult male, stuffed skin, skull. Specimens collected by Marc G. M. van Roosmalen and Tomas van Roosmalen on May 16, 1997, one km south of Nova Olinda, west bank of the lower Rio Aripuanã, right bank tributary of the Rio Madeira, south-central Amazonia, Brazil.

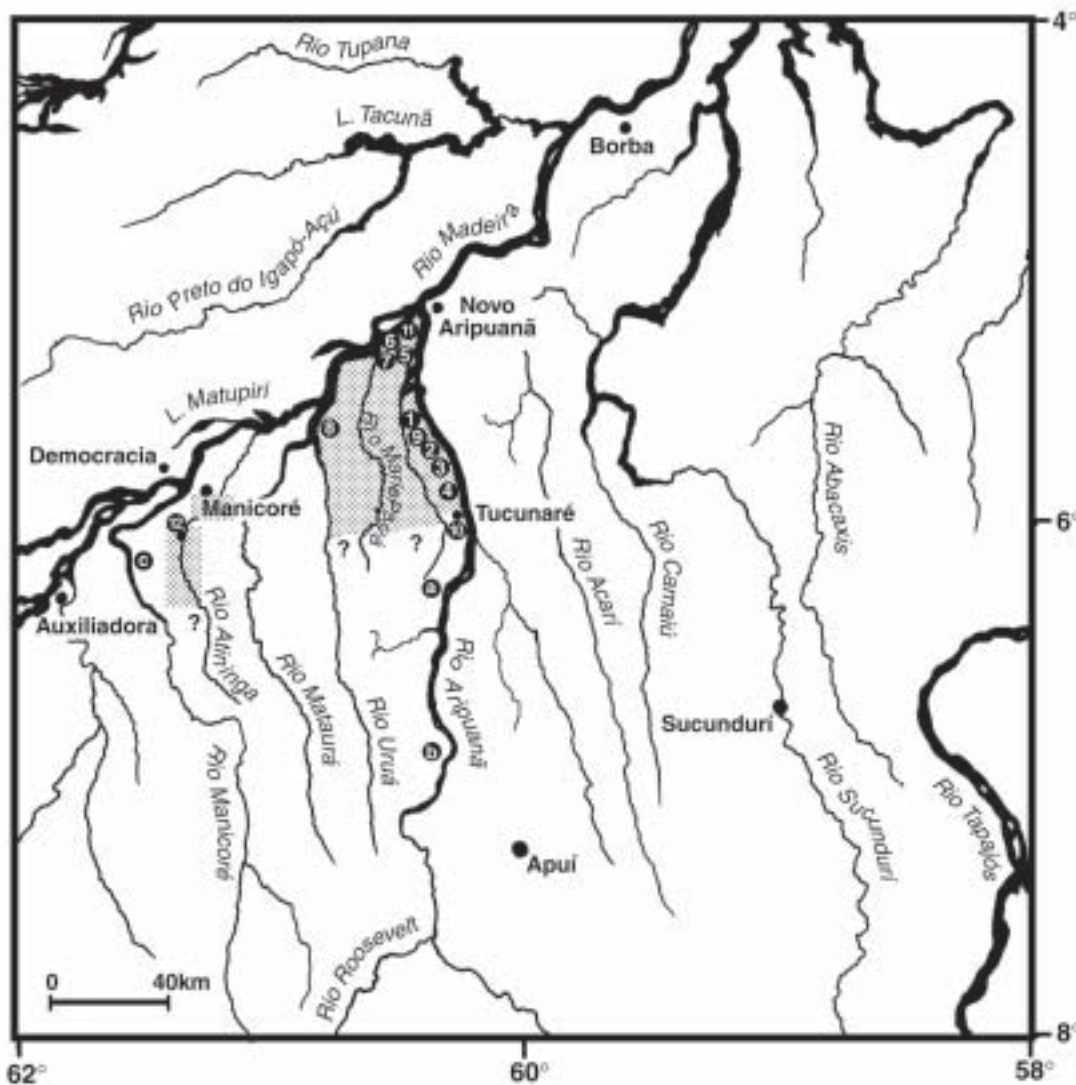
**Type locality:** West bank of the lower Rio Aripuanã, one km south of the settlement of Nova Olinda, 41 km SW of the town of Novo Aripuanã, Amazonas State, Brazil. This region is located in south-central Amazonia, Brazil, south of the Rio Amazonas and east of the Rio Madeira. Coordinates for the type locality are 05° 30' S, 60° 24' W. Altitude 45 m.

**Geographical distribution:** The species is known from the west bank of the lower Rio Aripuanã, from the mouth with the Rio Madeira just SW of the town of Novo Aripuanã south at least to the village of Tucunaré on the west bank of the Rio Aripuanã, and along the east bank of the Rio Madeira south as far as the mouth of the Rio Maturá. The southern limit for the species, in the interfluvium delineated by the Rios Madeira, Maturá and Aripuanã, is probably the headwaters of the Rios Mariepauá and Arauá. A geographically isolated population has been found along the middle and upper reaches of the Rio Atininga, ca. 50 km southwest of the southern limit of the main population and

ca. 10 km east of the town of Manicoré, situated on the east bank of the Rio Madeira. A live specimen (INPA 4090) was collected by the locals when it fell off the mother's back, on the right bank of the Rio Atininga; coordinates for this locality are 05° 54' S, 61° 15' W. This individual was kept for two years in our breeding center in Manaus, where it died just two weeks after giving birth to a single infant. Fig. 1 shows the distribution and the localities where dwarf marmosets were seen in the wild.

**Habitat:** All observations of wild dwarf marmosets in their main distribution were in disturbed primary and secondary *terra firme* rain forest, in the immediate vicinity of plantations, fields and tree gardens managed by local

people (*caboclos*). In an earlier paper (Van Roosmalen *et al.*, 1998), we assumed that dwarf marmosets also occur away from human settlements, although at extremely low densities. However, four trained observers carried out an intensive survey along two transects laid out perpendicular on the left bank of the Rio Aripuanã, one measuring 5 km (Capimtuba) and the other 3.5 km (Monte Alegre). Accumulating 120 km of census observations, the survey revealed not a single sighting of dwarf marmosets in undisturbed primary *terra firme* forest nor in riparian *igapó* forest (Van Roosmalen and Peres, in prep.). Along both transects, trees known to be exudate sources for dwarf marmosets were carefully inspected, but only gouge holes made by *Mico manicorensis* were found. During the surveys, both



**Figure 1.** Distribution of *Callibella humilis*. Numbers indicate localities where we have observed dwarf marmosets in the wild: 1. Nova Olinda, type locality for *Callibella humilis* (05°30'63"S, 60°24'61"W); 2. Monte Alegre, opposite Ilha Monte Alegre, left bank of Rio Aripuanã (05°34'S, 60°23'W); 3. Novo Oriente, left bank of Lago Capimtuba (05°43'S, 60°17'W); 4. Terra Preta, left bank of Rio Aripuanã (05°45'S, 60°15'W) and Santa Maria, left bank of Rio Aripuanã; 5. Igarapé Arauzinho, left bank of Rio Aripuanã; 6. São Sebastião, east bank of Lago Paiucuru, right bank of Rio Madeira; 7. Santa Cruz, on right bank near mouth of Rio Mariepauá, right-bank tributary of Rio Madeira; 8. São Martin, right bank of lower Rio Maturá, right-bank tributary of Rio Madeira; 9. Gethal selective logging site, 1 km north of Monte Alegre; 10. Tucunaré, left bank of Rio Aripuanã, 2 km north of Lago Açai Grande; 11. Guariúba, west bank of Lago da Guariúba, left bank of Rio Aripuanã (05°13'03"S, 60°23'04"W); 12. Atininga, both banks of upper Rio Atininga, on mouth of Igarapé Santa Luzia and 5 km upstream from mouth of Ig. Santa Luzia (05°54'S, 61°15'W). (Map by Stephen Nash.)

**Note:** extensive surveys at (a) Rio Arauzinho (06°16'S, 60°20'W), (b) São Raimundo, (c) along both banks of the lower Rio Manicoré, and along the right bank of the lower Rio Aripuanã (Rio Aracá, Jatuarana, Frechal, Prainha, Cipotuba, Tabira) as far south as Rio Juma, Itapiranga and Prainha, did not reveal the presence of *Callibella*.

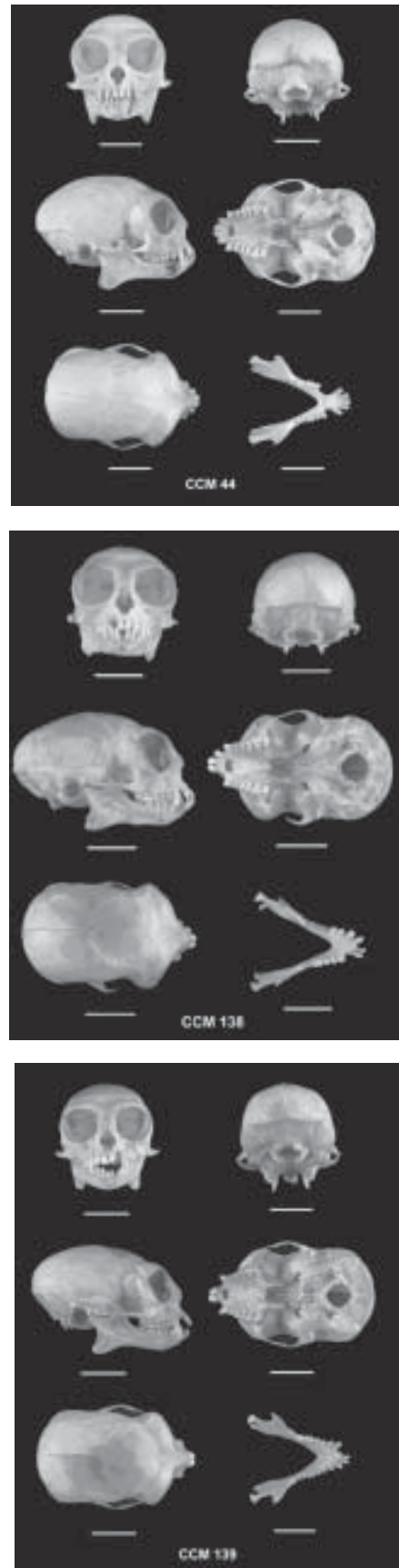
*Mico manicorensis* and *Callicebus bernhardi*—which share with *Callibella humilis* a preference for disturbed forest near human settlements, and abandoned or cultivated *terra preta* anthrosols—were regularly seen in natural secondary forest growing in treefall clearings away from human disturbance. Several groups of the disjunct southern dwarf marmoset population, however, were observed in both disturbed *terra firme* rain forest and seasonally inundated forest (*igapó*) of the Rio Atininga and its major tributaries. Its seasonal habitat preference for *igapó* in this area has been confirmed by the locals, but groups were also seen on and near the few *terra pretas* scattered along this river on higher ground behind the *igapó*. We assume that *Callibella* nowadays occurs almost exclusively on bluffs along blackwater and clearwater streams and lakes, where generations of ancient Indian farmers once lived and accumulated black-earth deposits (Mann, 2002). (For a fuller discussion of the anthropogenic black-earth areas known as *terra pretas*, please refer to Appendix I.)

**Diagnosis:** A very small marmoset (*sensu* Hershkovitz, 1977), slightly larger in size than *Cebuella* (adult head-body length = 160–170 mm; total length = 380–390 mm; weight 150–185 g), but sharing more physical and behavioral characteristics with *Mico*. Adults are distinguished from *Cebuella* by the following features: evenly non-banded hairs, dark olive brown (not tawny agouti) above, orange-yellow to golden to grayish-yellow below, including inner sides of limbs; upper surface of hands, feet and lower arms orange mixed with black; naked ears not concealed by a cape of hair; a black triangular crown; white “eyebrows” extending back to temples; triangular, naked face flesh-colored and unpigmented (lacking the white mustache or white dots beside the nostrils), except for blackish circumocular zone; larger inter-nostril distance; iris darker orange-brown; streaks of white hairs (avg. 12 mm long) growing from central pinna; tail longer, almost black, not or obscurely orangish-ringed and longer-haired; color patterns changing with age, particularly on head. Drastic color changes with age are unique among marmosets, as are hair tufts arising from the center of the pinnae (see Van Roosmalen *et al.*, 1998 for description and illustrations). Table 1 gives body measurements and weight, Figures 2a, -b, and -c provide adult cranial characteristics, and Table 2 lists cranial and dental measurements.

**Origin of the name:** *Callibella* is a combination of the Greek adjective *calo* or *calli* meaning ‘beautiful’ and the Portuguese/Italian adjective *belalbella* meaning ‘beautiful,’ thus meaning ‘double beautiful’; *humilis* means ‘small’ or ‘dwarf’ in Latin.

### Comparisons with Other Callitrichines

In an earlier paper (Van Roosmalen *et al.*, 1998), we offered five hypotheses for the relationships and origins of the new species, all equally parsimonious for lack of basic data on behavior, ecology, geographic range and molecular genetics:



**Figure 2.** Skulls of adult male holotype *Callibella humilis* (CCM 44 = MPEG 24769), adult female *Callibella humilis* (CCM 138 = INPA 4090), and adult male *Callibella humilis* (CCM 139 = INPA 4091). Photographs by Stephen D. Nash. Scale bar = 1 cm.

Table 1. Measurements (mm) and weight (g) of several captive *Callibella humilis*.

Coll. nr.	Gender	Age (mths)	Head-Body	Tail	Total	Hand	Foot	Hallux	Ear	Weight
INPA 4090	Female	24	167	220	387	24	55	-	-	150
INPA 4091	Male	36	161	209	370	20	54	-	-	150
MPEG 24769	Male	>24	150	240	390	35	50	8	22x11	120
MvR 52	Male	>24	162	220	382	40	50	6	22	122
MvR 50	Female	>24	160	240	400	28	41	6	20x18	185
MvR 51	Male	12	130	205	335	32	48	6	20	95
MvR 53	Male	13	145	235	380	31	48	5	21x15	136
MvR 54	Female	2 days	65	85	150	15	20	-	-	16

1. The new species is just a species or subspecies of *Cebuella* that managed to raft across the Rio Madeira;
2. The new species is a “missing link” that is intermediate between *Cebuella* and *Mico*;
3. The new species is a small, primitive form of *Mico*, possibly ancestral to some of the other species;
4. The new species is just another form of *Mico* in this region; and
5. The new species represents a separate callitrichine radiation, and deserves recognition as a distinct *genus* that happens to have a very small geographic range.

We took a conservative stance in describing this monkey as a dwarf form of *Callithrix* (now *Mico*), inclining most to Hypothesis 4 in considering it just another form of Amazonian marmoset in this region, one which happens to be considerably smaller than any of its relatives. Hypothesis 5, however, was given some validity as well, since the monkey shares only a few behavioral features with other Amazonian marmosets (Table 3, Van Roosmalen *et al.*, 1998), and is to be considered an anomaly among marmosets for its loss of twinning and territoriality, among other features. In view of our recent molecular research, Hypothesis 5 now seems most appropriate, and we consider this new species to be a separate and basal marmoset divergence.

Dwarf marmosets are remarkable for displaying the following characteristics:

- their diminutive body size and weight;
- their apparent lack of territoriality, exceptional among primates—although an average group contains 6-8 individuals, we have observed group aggregations counting over 30 individuals in which no antagonistic behavior was noted, and scent marking of a common home range using urine or gland secretions has never been observed in captivity or in the wild;
- male and female genitals are not hypertrophied as in *Cebuella*, *Mico* and *Callithrix*;
- they represent the only New World primate with long hair tufts growing from the *center* of the pinna;
- two singleton births took place in captivity from different mated pairs, and adults were never seen carrying twins in the wild, which together imply obligate singleton births;
- lack of female reproductive inhibition by pheromones in a female’s urine (a birth-controlling mechanism prominently developed in all other callitrichines);
- several observations in the wild of more than one reproductive female in a single group suggest that they do not perform an exclusive alpha-female reproductive system, although multiple reproductive females have been reported in some other callitrichid species (Masataka, 1981; Digby and Ferrari, 1994; French, 1996; Roda and Pontes, 1998);
- both in captivity and in the field we observed that only biological mothers carry the offspring on the back, and do not share transportation duties with the father or other adult/subadult group members, unlike other callitrichines;
- in captivity we observed so-called ‘parking behavior’ in which the mother, already as early as the fifth day after birth, leaves her infant behind on a twig or branch where it stays silent and immobile until it is picked up again, a behavioral pattern only known among some prosimians (lemurs, galagos, and lorises) and *Cebuella*;
- they perform a clearly different vocal repertoire, including a double-tone contact or distant call (Van Roosmalen *et al.*, in prep.);
- the ontogeny is unique, with three discrete stages of pelage coloration and hair length (Van Roosmalen *et al.*, 1998);
- exudate-gouging of tree and vine barks seems as important a foraging pattern as in allopatric *Cebuella* and *Callithrix*, the gum functioning as a keystone resource during the dry season when fleshy fruits are scarce, much more so than in sympatric *Mico manicorensis* (Van Roosmalen *et al.*, in prep.);
- extreme commensalism with humans, making the dwarf marmosets almost totally dependent for survival on multi-species managed forests, fruit orchards and gardens growing on so-called *terra pretas* (anthropogenic soils, or ‘black earth’).

### Molecular phylogenetics - Methods

Faecal samples were obtained from specimens kept in our Breeding Center for Endangered Amazonian Primates in Manaus, which were later deposited at the zoological museum of the Instituto Nacional de Pesquisas da Amazônia

**Table 2.** Cranial and dental measurements (mm) of three *Callibella humilis*: CCM 138 (= INPA 4090), adult female from Rio Atininga; CCM 139 (= INPA 4091), adult male, locality unknown; and CCM 44 (= MPEG 24769), holotype adult male from Novo Aripuanã.

Skull and Mandible (mm)	CCM 138	CCM 139	CCM 44
Greatest Skull Length (SL)	35.8	37.8	36.0
Zygomatic Width (ZW)	23.6 (broken)	25.7	25.3
Biorbital Width (BW)	22.0	22.3	20.9
Postorbital Width (PW)	18.3	19.5	18.9
Nasion-Basion	24.7	26.6	26.0
Basion-Prosthion	25.8	27.2	26.2
Biauricular Breadth	20.1	22.5	21.6
Height of Canine (CH)	3.3	4.3	3.8
Length of Mandible (ML)	21.3	25.1	23.8
Across First Molars (AM)	9.0	10.0	9.4
Across Canines	6.3	6.2	6.3
Dental Field (P2-M2)	8.3	9.1	8.9 (no P2)
Premaxillary Height	5.9	6.1	6.0
Intradentale Superior to Premaxilla-Maxilla Junction at Alveolus	3.6	3.6	3.4
Intradentale Superior to Nasion (IS-NA)	10.7	10.9	11.8
Intradentale Superior to Posterior Nasal Spine (IS-PNS)	11.9	12.9	12.7
Bregma to Nasion (BR-NA)	20.4	20.1	20.5
Nasion to Fronto-Malar Junction at Orbit (NA-FM)	11.7	11.9	12.0
Fronto-Malar Junction to Pterion	5.2	5.7	5.7
Fronto-Malar Junction to Zygomaxillare Superior (FM-ZS)	6.5	7.0	6.3
Fronto-Malar Junction to Maxillary Tuberosity (FM-MT)	10.2	10.9	11.8
Zygomaxillare Inferior to Premaxilla-Maxilla Junction at Alveolus	10.0	9.6	10.8
Zygomaxillare Inferior to Zygomaxillare Superior (ZI-ZS)	5.7	4.8	5.1
Zygomaxillare Inferior to Maxillary Tuberosity (ZI-MT)	3.4	4.2	4.2
<b>Anterior Teeth</b>			
I <sup>1</sup> Length	1.5	1.5	1.7
I <sup>1</sup> Breadth	1.2	1.1	1.2
I <sup>1</sup> Height	2.3	2.5	2.6
I <sup>2</sup> Length	1.5	1.6	1.6
I <sup>2</sup> Breadth	1.1	1.2	1.1
I <sub>1</sub> Length	1.0	1.2	0.9
I <sub>1</sub> Breadth	1.1	0.9	1.3
I <sub>1</sub> Height	2.2	2.8	3.1
I <sub>2</sub> Length	1.0	1.1	1.2
I <sub>2</sub> Breadth	1.7	1.5	1.7
C <sup>1</sup> Length	2.0	2.2	2.1
C <sup>2</sup> Breadth	1.3	1.2	1.1
<b>Cheek Teeth</b>			
P <sup>2</sup> Length	1.7	1.9	-
P <sup>2</sup> Breadth	1.2	1.2	-
P <sup>4</sup> Length	1.5	1.6	1.7
P <sup>4</sup> Breadth	1.3	1.5	1.6
M <sup>1</sup> Length	1.9	2.1	2.2
M <sup>1</sup> Width	1.5	1.6	1.8
M <sup>2</sup> Length	1.8	2.0	2.1
M <sup>2</sup> Width	1.4	1.4	1.6

(INPA), Manaus, Amazonas, Brazil. Sampled marmosets included three *Callibella humilis* and two *Cebuella pygmaea niveiventris*, all wild-caught.

DNA was extracted from the faecal samples following the protocol provided by Gibco BRL DNAzol extraction kit. The PCR amplification and sequencing methodology was the same as has been used for *Mico* (*Callithrix*) *manicorensis* and *Mico* (*Callithrix*) *acariensis* (see Van Roosmalen *et al.*, 2000).

For the control region, sequences of three *Mico* (*Callithrix*) *argentatus*, three *Mico* (*C.*) *mauesi*, two *Mico* (*C.*) *humeralifer*, four *Callithrix geoffroyi*, two *Callithrix penicillata*, two *Callithrix jacchus*, five *Callithrix kuhlii*, two *Callithrix aurita*, two *Cebuella pygmaea*, and one *Leontopithecus chrysomelas* were obtained from GenBank. Upon alignment, sequences were entered into the PAUP program (Swofford, 1993) for phylogenetic analysis. A maximum parsimony (MP) algorithm was used to analyze the data, and bootstrap analyses (100 replicates) were performed on the resulting consensus trees. Pair-wise nucleotide distances were also determined for the species under consideration.

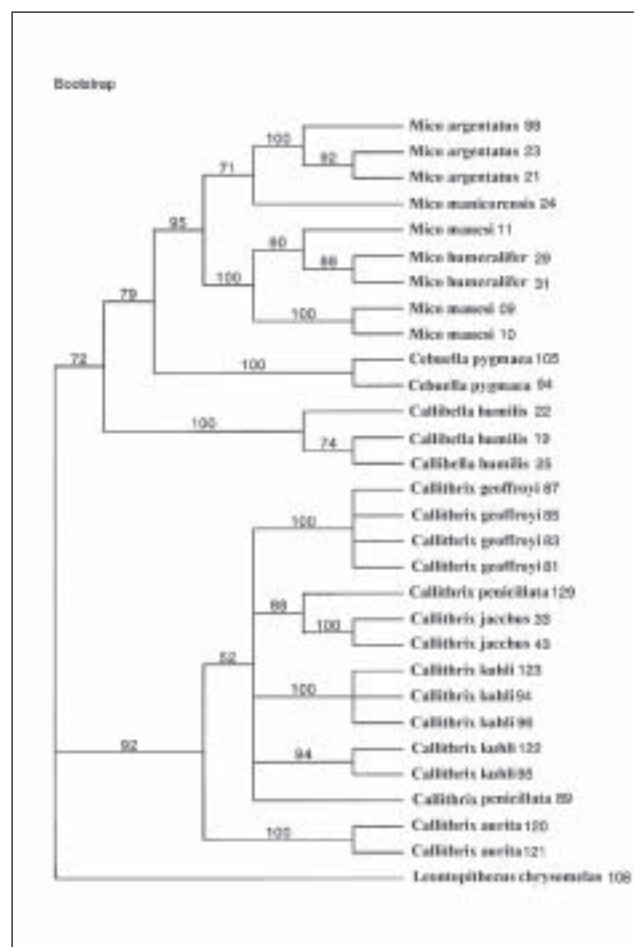
### Molecular phylogenetics – Results

Using PAUP, a maximum parsimony analysis with 100 bootstrap replications yielded the phylogenetic tree for the mitochondrial control region presented in Fig. 3. This tree does not include the shorter sequence of *Cebuella pygmaea* obtained in this study. When this sequence is included in the phylogeny, curtailed to only 532 bp for all species, bootstrap values are raised significantly for some nodes. Both trees agree, however, that *Callibella humilis* diverges before *Cebuella* from their common ancestor with the Amazonian marmoset clade. Pairwise divergences indicate a slightly greater genetic distance of *Callibella humilis* from *Mico* (*C.*) *argentatus* (approx. 13%) than the distance between *Cebuella* and *Mico* (*C.*) *argentatus* (11-12%). The status of the Atlantic marmosets (genus *Callithrix*), diverging before the radiation of Amazonian marmosets (including *Callibella humilis* and *Cebuella pygmaea*), remains in concordance with the phylogenetic trees presented by Tagliaro *et al.* (1997) before the addition of *Callibella humilis*.

The positioning of the dwarf marmoset further suggests that it should be elevated from its original generic status in *Callithrix* (Van Roosmalen *et al.*, 1998) to a distinct genus, here proposed as *Callibella*. We therefore suggest renaming the dwarf marmoset as *Callibella humilis*. Its divergence from the ancestral stock of the Amazonian marmosets (*Callithrix*, now *Mico*) prior to the divergence of the pygmy marmosets (*Cebuella*) is strongly suggested by these data. Morphological, physiological, ecological and ethological observations as described in Van Roosmalen *et al.* (1998), and above in this paper, corroborate the finding that *C. humilis* represents a new genus.

This conclusion is supported by the pairwise divergences

calculated for *Mico*, *Callithrix*, *Cebuella*, and *Callibella*. For the mitochondrial control region, pairwise divergences between the species of Amazonian marmosets (*Mico*) range from approximately 3% to 7%, with 6-7% between the two main subclades (*argentatus* and *humeralifer/mauesi*) and 2-4% between species within each subclade. Divergences between *Cebuella* and any species of *Mico* are on the order of 11-12%, while the average divergence between the Amazonian marmosets and *Callibella* is approximately 12-13%. It is also noteworthy that the pairwise divergence between *Callibella* and *Cebuella* is 13-14%, suggesting that they share no common ancestry after their divergence from the ancestral *Mico* stock. Within-species divergences for the species analyzed in this study range from 0-3%. Pairwise divergences between the Atlantic marmosets (*Callithrix*) and the Amazonian marmosets (*Mico*) range from 12-13%, while the divergences between *Callithrix*, *Cebuella* and *Callibella* range from 14-15%. Divergence between all marmosets (including pygmy and dwarf marmosets) and the outgroup, the lion tamarin *Leontopithecus*, is over 20%. It is important to keep in mind that these differences are reflective only of a single DNA sequence, the mitochondrial control region. Such divergences may differ if other sequences were to be analyzed. The authors also recognize



**Figure 3.** Callitrichid gene tree based on 902 base pairs (BP) of the mitochondrial control region, analyzed using maximum parsimony. Bootstrap values are given above branches.

that it would be more informative to include additional specimens from each species. Unfortunately, the remoteness of their geographic ranges, as well as their status as endangered species under Brazilian law, has precluded more intensive sampling.

In essence, these data suggest an early, almost simultaneous divergence of the genera *Callibella*, *Cebuella*, and *Mico*. These genetic distances indicate that, contrary to suggestions by previous molecular studies (e.g., Barroso *et al.*, 1997; Tagliaro *et al.*, 1997) to subsume *Cebuella* within the genus *Callithrix*, the Amazonian and Atlantic marmoset clades should be separated into different genera. Given a 3-7% divergence for species within either clade, and a 10-11% divergence between clades, it seems taxonomically more informative to group the two clades into separate genera in recognition of their evolutionary distinctiveness. This separation would uphold the generic status of *Cebuella*, which is also much more divergent from *Mico* than any among-species divergences found within *Mico*. With this in mind, the comparatively greater divergence (twice that between any two given *Mico* species) of *Callibella humilis* from any other known marmoset or pygmy marmoset warrants its placement in a distinct genus as well, assuming that taxonomic classifications should reflect actual evolutionary distinctiveness. Similarly, the separation of Atlantic marmosets ('ouistitis') from Amazonian marmosets has been proposed by Groves (2001), using the subgenera *Mico* Lesson, 1840 and *Callithrix* Erxleben, 1777, respectively. Rylands *et al.* (2000) already treat them as distinct genera, a classification with which we fully concur.

### Conservation Status of the New Genus

The area in which *Callibella humilis* is confirmed to occur is only 250,000 to 300,000 hectares in size (Van Roosmalen *et al.*, 1998), perhaps the smallest distribution of any primate in the Amazon. The interfluvial basins of the secondary tributaries that drain this part of the interfluvium (as delineated by the Rios Madeira and Aripuanã) are uninhabited. Since pre-Columbian Indians never settled far inland from riverbanks, no *terra pretas* are to be found there. Our analysis of Landsat images of the entire area of distribution, recognizing occupied as well as abandoned *terra pretas* by their specific green color, reveals that the total surface area of *terra pretas* accounts for less than 1% of the region as a whole. Suitable habitat therefore amounts to less than 3,000 ha. Since average home range size and group size is approximately known for a handful of *terra pretas*, an educated guess would be a total population of around 10,000 individuals.

Local people living nowadays on *terra pretas* alongside rivers and creeks consider the dwarf marmoset too small to hunt for food. The monkeys may be regularly seen crossing open areas, running over the ground to reach isolated trees in the middle of house gardens or orchards in order to gouge their bark for gum. In this way they expose themselves to predators such as domestic dogs and cats—as well as birds

of prey, which are drawn to mice, rats and poultry and often perch in nearby trees. A more serious threat to the dwarf marmosets' survival may come from the fact that, as commensals, they are often exposed to forest fires, since farmers regularly burn secondary growth to clear their *terra preta* fields. These fires may run out of control and destroy entire orchards and house gardens, including the forest edges where the monkeys spend most of their time. In addition, the future survival of *Callibella humilis* could be dramatically affected if locals were to commercialize the *terra preta* soil as humus or peat for gardening, a practice commonly seen in the vicinity of Amazonian towns and cities.

The State Department for the Protection of the Natural Environment in the State of Amazonas (IPAAM) has been repeatedly informed about the necessity of implementing protected areas in the municipalities of Novo Aripuanã and Manicoré, but thus far without results. In the meantime, our Brazilian Civil Non-Profit Entity A.A.P. (the Amazon Association for the Preservation of High Biodiversity Areas), based in Manaus, is supporting the creation of Private Natural Heritage Reserves (Reserva Particular de Patrimônio Natural, or RPPN), which are private nature reserves with a perpetually protected status. This will not only safeguard significant samples of Amazonian ecosystems, but will also guarantee the maintenance of their biodiversity. Our Association purchases legal land titles and transforms these private properties into RPPNs through the authority of IBAMA, the Brazilian Institute for the Environment and Renewable Natural Resources. The status of these reserves is guaranteed the most rigid protection under the Brazilian Environmental Law of June 5<sup>th</sup>, 1996 (Decree #1922). Therein, the President decrees the following articles, among others:

*Art. 1.* The Private Reserve RPPN is an area of private ownership that is specially protected, by initiative of its owner, through the recognition of the government because of its relevant importance in terms of biodiversity, or its natural beauty, or its environmental characteristics that justify actions for its recuperation.

*Art. 2.* The objective of RPPNs will be the protection of the environmental resources representative for the region.

*Art. 3.* The RPPNs can be used for activities whose aim is scientific, cultural, educational, recreational, but always in line with the stated aim of the previous article. These activities must be authorized or licensed by the responsible organ for the recognition of the RPPN and executed in such a manner that they will not compromise the ecological balance or endanger the survival of the existing species populations, in view of the carrying capacity of the area that is determined by the management plan.

In addition to covering habitat for *Callibella humilis*, the RPPNs proposed by the A.A.P. will also include viable populations of eight other primates along the left bank of the Rio Aripuanã, and 11 primates along the right bank—of



which seven (including the genera *Ateles*, *Pithecia*, *Lagothrix*, *Saimiri*, and *Aotus*) are species new to science, such as the recently described *Mico manicorensis* and *Callicebus bernhardi* (Van Roosmalen *et al.*, 2000; Van Roosmalen *et al.*, 2002).

### Acknowledgements

The authors thank Betty van Roosmalen-Blijenberg for taking care of the captive dwarf marmosets in Manaus, John Fleagle for taking the measurements of the skulls, and Stephen Nash for illustrating this paper. We also thank John Aguiar for his careful editing of a prior version of this manuscript. Support for fieldwork and for publication of this paper was kindly provided by the Margot Marsh Biodiversity Foundation and Conservation International, Washington, D.C.

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## Appendix I: Terra Pretas

*Terra pretas* (literally “black earth”) are exceptionally fertile soils which are thought to have been manufactured in pre-Columbian times by native Brazilian Indian farmers, who disappeared before the first European immigrants arrived (Mann, 2002). These farmers left their *terra pretas* on the bluffs above white-, black-, and clearwater rivers and streams, and along the margins of lakes with access to lime deposits (Van Roosmalen, in prep.). These ancient Indian farmers seem to have decomposed organic material to humus and then mixed it with large quantities of charcoal, calcium and phosphates. The charcoal may have been obtained from burning down large tracts of riparian forest (*igapó*), which is susceptible to fire during the peak of the dry season. Lime could have been obtained in large quantities from game and fish bones, freshwater turtle carapaces and molluscs (freshwater oysters, snails and mussels), commonly found in clear-water and white-water rivers.

Most *terra pretas* are currently occupied by families or small communities of *caboclos*, the descendants of acculturated Indians and immigrant settlers, who grow crops such as manioc, maize, sweet potatoes, sugar cane, bananas, and papaya on these almost inexhaustibly fertile soils. These current inhabitants do not know how to manufacture this black earth themselves. *Terra pretas* which have remained uncultivated by *caboclos* since their creators abandoned them are usually covered with an open type of primary rain forest, very different in composition from the matrix *terra firme* forest which covers the unmodified, extremely poor podzolic soils found elsewhere in Amazonia. Vines and twiners may dominate locally, or else dense stands of palms may be found, including species such as *babaçú* (*Attalea speciosa*), *inajá* (*A. maripa*), *caiaué* (*Elaeis oleifera*), *bacaba* (*Oenocarpus bacaba*), *bacabinha* (*O. minor*), and *tucumã* (*Astrocaryum vulgare* and *Astrocaryum aculeatum*).

Moreover, quite a few tree species found elsewhere only on richer soils, such as in whitewater floodplains (*várzea*), are known to thrive on *terra pretas*, such as *Spondias mombin*, *Tapirira guianensis*, *Annona montana* and other sour sop species, *Duguetia* spp., *Rollinia mucosa*, *Didymopanax morototoni*, *Astrocaryum aculeatum*, *Astrocaryum murumuru* var. *ferrugineum*, *Astrocaryum vulgare*, *Elaeis oleifera*, *Ceiba pentandra* (the kapok tree, considered sacred by most indigenous Amazonian peoples), *Carica papaya*, *Platonia insignis*, *Rheedia* spp., *Vismia* spp., *Cochlospermum orinocense*, *Acacia* spp., *Enterolobium schomburgkii*, *Inga* spp., *Parkia multijuga*, *Theobroma cacao* and several species of wild cacao.

Among these trees, many offer the dwarf marmosets exudates and/or edible fruits. Species such as *Didymopanax morototoni*, *Spondias mombin*, *Enterolobium schomburgkii*, *Parkia multijuga*, *Inga ingoides* and *I. alba*, as well as *Acacia* vines, often serve as aseasional keystone resources for the monkeys. These are especially important during the end of the wet season and the first half of the dry season, when fruits are scarce, and they offer the dwarf marmosets (and, west of the Rio Madeira, the pygmy marmosets) reliable sources of exudate. These marmosets literally live in the locals' backyards and orchards, and are tolerated because they do very little damage to their fruit crops. Since the larger Amazonian marmosets (*Mico*)—and, in the case of *Cebuella*, tamarins (*Saguinus*)—do not venture into the house gardens, intergeneric interactions are seldom seen. It is possible that *Callibella* and *Cebuella* have “dwarfed” in response to competition with other, more opportunistic callitrichines, and have become more successful after the introduction of man in Amazonian moist forest regions around 12,000 BP. The consequent reduction in size, coupled with a change in foraging behavior (*Cebuella* and *Callibella* seem to be more specialized in gouging tree barks than *Mico*), has led to sufficient ecological divergence to allow for their co-existence with larger callitrichines.