

TWO NEW SPECIES OF MARMOSET, GENUS *CALLITHRIX* ERXLEBEN, 1777 (CALLITRICHIDAE, PRIMATES), FROM THE TAPAJÓS/MADEIRA INTERFLUVIUM, SOUTH CENTRAL AMAZONIA, BRAZIL

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### Abstract

Two new species of marmoset, *Callithrix manicorensis* sp. n. and *Callithrix acariensis* sp. n., are described; the first from the interfluvium of the Rios Madeira, Aripuanã and Manicoré, and the second from the interfluvium of the Rios Acari and Sucundurí, south of their confluence with the Rio Canumã, both in the state of Amazonas, south central Amazonia, Brazil. The new species are members of the *Callithrix argentata* group of marmosets, *sensu* Hershkovitz (1977). *Callithrix manicorensis* appears to be most closely related to *C. nigriceps*, *C. marcai* and *C. aff. emiliae*, its neighbors to the south, and *Callithrix acariensis* appears to be most closely related to *Callithrix saterei*, its neighbor to the north, and *Callithrix melanura* to the south, but they are easily distinguished from them. The most noticeable features of *Callithrix manicorensis* are the almost white or silvery upper parts including the arms, hands, chest, mantle, nape and forehead, and the indistinct light gray (rather than black) cap on the head. The back is grayish and transversely white or silvery striped or mottled. The under parts are yellowish or orange. The legs and feet are orange. The tail is black. Those of *Callithrix acariensis* include the almost entirely snow white upper parts including the upper arms, chest, mantle, nape and forehead, and the lack of a dark cap on the head. The back is grayish. The under parts are white. The legs and feet are bright orange. The tail is dark black with a bright orange dorsal patch at the proximal end. Most strikingly, this species shows a broad white hip and thigh stripe running from the mid dorsum (almost continuing across the back) to the knee. The phylogenetic status of the two new marmoset taxa is presented based on 902 base pairs (bp) of the mitochondrial control region and combined with previously determined sequences from related species. Additionally, 635 bp of intron 2 of the nuclear  $b_2$ -microglobulin gene was sequenced for *C. manicorensis* and combined with sequences from related species. *Callithrix manicorensis* is shown to be a member of the *C. argentata* clade according to mitochondrial control region and  $b_2$ -microglobulin intron 2 sequences. *Callithrix acariensis* is found to be most closely related to *C. mauesi* and *C. humeralifera* (the tufted-ear marmoset clade) based on mitochondrial control sequence. The phylogeography of the marmosets is discussed, as well as possible scenarios for historic patterns of dispersal and speciation. With the discovery of these two marmosets, the number of species of *Callithrix* increases to 21, of which 20 are endemic to Brazil (considering *C. aff. emiliae* from Rondônia a valid species as it is widely separated from *C. emiliae*). The total number of primate species for the country as a whole increases to 79, 41 of which are endemic.

**Key Words** - Primates, Callitrichidae, marmosets, *Callithrix manicorensis* sp. n., *Callithrix acariensis* sp. n., phylogeny, phylogeography, Brazil, Amazonia.

### Resumo

Descreve-se duas novas espécies de sagüi, *Callithrix manicorensis* sp. n., do interflúvio entre os Rios Madeira, Aripuanã e Manicoré, e *Callithrix acariensis* sp. n., do interflúvio entre os Rios Acari e Sucundurí, afluentes do Rio Canumã, estado do Amazonas, Amazônia Central Sul, Brasil. As novas espécies são alocadas no grupo de sagüis *Callithrix argentata*, *sensu* Hershkovitz (1977). A nova espécie, *Callithrix manicorensis*, parece ter maior parentesco com *C. nigriceps*, *C. marcai*, e *C. aff. emiliae*, os seus vizinhos ao sul, ainda que muito distinta destas. *Callithrix acariensis* parece ter maior parentesco com *C. saterei*, o seu vizinho ao norte, e *C. melanura* ao sul da sua distribuição. As diferenças mais notáveis em *Callithrix manicorensis* são a metade anterior do corpo de cor branco ou branco-argênteo, incluindo os membros anteriores, as mãos, garganta, peito, manto, e nuca, e a presença de uma mancha cinza clara ou cinza-argêntea (não preta) no vértice. O dorso é cinzento ou marron-claro e transversalmente listrada ou mosqueado com branco ou branco-argênteo. O ventre e os lados interiores do corpo são amarelados ou alaranjados. As pernas e os pés são alaranjadas. A cauda é lisa, não anelada e preta. As diferenças mais notáveis da nova espécie *Callithrix acariensis* são a metade anterior do corpo quase branco neve inclusive os braços anteriores, peito, juba, nuca e vértice, sem uma mancha cinza ou preta no vértice. O dorso é cinzento. O ventre é branco. As pernas e os pés são claramente alaranjadas. A cauda é preta, proximalmente com uma mancha alaranjada. Esta espécie mostra uma mancha larga branca na coxa do joelho quase até o dorso. Apresenta-se uma filogenia que inclui as duas novas espécies, baseada em 902 pares de bases da região controle mitocondrial e 635 pares de bases do intron 2 do gene nuclear  $b_2$ -microglobulin. O arranjo cladístico sugere que *Callithrix manicorensis* seja um membro do grupo *C. argentata*, e que *C. acariensis* seja mais próxima a *C. mauesi*

e *C. humeralifera* (baseada na análise da região controle mitocondrial). Discuta-se a filogeografia dos sagüis amazônicos tanto quanto cenários possíveis para os padrões históricos de dispersão e especiação. Com essas descobertas, acrescenta-se mais duas espécies ao gênero *Callithrix*, sendo agora um total de 21, com 20 endêmicas ao Brasil (considerando *Callithrix* aff. *emiliae* de Rondônia uma espécie válida, geograficamente isolada e separada de *Callithrix emiliae*). O número de espécies de primatas existentes no Brasil elevou-se para 79. Destas, 41 são endêmicas ao país.

**Palavras-Chave** - Primatas, Callitrichidae, sagüis, *Callithrix manicorensis* sp. n., *Callithrix acariensis* sp. n., filogenia, filogeografia, Brasil, Amazônia.

## Introduction

All but one of the 19 marmosets, *Callithrix* Erxleben, 1777, recognized to date are endemic to Brazil. The black-tailed marmoset, *Callithrix melanura*, ranges into Bolivia and Paraguay (Stallings and Mittermeier, 1983; Brown and Rumiz, 1986). Two major revisions of this genus have been carried out in the last two decades, one by Hershkovitz (1977), who recognized just three species and 11 taxa, and the other by De Vivo (1988, 1991), who listed an additional form, *C. emiliae*, and elevated all the forms to species status. Mittermeier *et al.* (1988) agreed with the taxonomy suggested by De Vivo, but also recognized a sixth eastern Brazilian species, *C. kublii*, which was considered a hybrid by Hershkovitz (1977) and as merely a pelage color variant of *C. penicillata* by De Vivo (see Rylands *et al.*, 1993). Mittermeier *et al.* (1992) followed De Vivo's decision on the *Callithrix argentata* group, elevating all forms to full species status, and listed nine species within this group.

In this paper we describe two new species from the basin of the Rio Madeira, state of Amazonas, Brazil, increasing the number of *Callithrix* species to 21 (see Table 1), the number of Brazilian primate species to 79, 41 (52%) of them being endemic, and the number of callitrichid taxa to 59 (see Van Roosmalen *et al.*, 1998). Figure 1 (after Van Roosmalen *et al.*, 1998) shows the distribution of 14 species of the Amazonian *Callithrix argentata* group, including the two described here, *C. manicorensis* and *C. acariensis*, and also *Callithrix humilis* M. G. M. van Roosmalen, T. van Roosmalen, Mittermeier and Fonseca, 1998.

*C. manicorensis* is from the region confined by the Rios Madeira, Aripuanã and Manicoré, and *C. acariensis* occurs between the Rios Acarí and Sucundurí, both in the state of Amazonas in south central Amazonia, Brazil (Fig. 2). In its pelage *C. manicorensis* is most similar to *C. aff. emiliae* from Rondônia (see footnotes to Table 1), *C. nigriceps*, and *C. marcai* (Figs. 3 and 4), with which it is parapatric. However, *C. manicorensis* is quite distinct, and clearly deserves recognition as a full species. *C. acariensis* is most similar to *C. melanura* to the south, *C. intermedia* along the left (west) bank of the upper Rio Aripuanã, and *C. saterei* to the north (between the Rios Canumã and Abacaxis), all four sharing a very distinct pale thigh and hip stripe (Fig. 3).

*Callithrix manicorensis* was first discovered by Marc G. M. and Tomas van Roosmalen on 5 July, 1996, during a field trip to the region in search of a new species of dwarf marmo-

set, *Callithrix humilis* (Van Roosmalen *et al.*, 1998). The animal was being kept as a pet and, as they were informed, had been collected on the Rio Mariepauá. *Callithrix acariensis* was first discovered by Marc G. M. van Roosmalen on 29 August, 1996, when he obtained a baby pet monkey from locals living along the right bank of the lower Rio Acarí.

### *Callithrix manicorensis* sp. n.

**Holotype:** INPA 2511, juvenile female, one of two juveniles obtained alive by Marc G. M. van Roosmalen on 30 December 1996, from the vicinity of the town of Manicoré. The specimen INPA 2511 died soon after being acquired and is deposited in the mammal collection of the Instituto Nacional de Pesquisas da Amazônia (INPA) Museum, Manaus, Amazonas, Brazil.

**Paratype:** A juvenile male, obtained alive together with the holotype INPA 2511 by Marc G. M. van Roosmalen on 30 December, 1996, from the vicinity of the town of Manicoré. It is still alive in captivity at the time of this publication. It will eventually be placed in the mammal collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil.

**Type locality:** Seringal São Luis, east bank of the middle Rio Madeira, in the vicinity of the town of Manicoré, state of Amazonas, Brazil (Fig. 2). This region is located in south central Amazonia, Brazil, south of the Rio Amazonas, east of the Rio Madeira, and west of the lower Rio Aripuanã. Coordinates for the type locality are 05° 50' 28" S, 61° 18' 19" W. Altitude 45 m.

**Other specimens examined:** A specimen collected alive by Marc G. M. van Roosmalen on 5 July, 1996. It was reported to have been captured along the lower Rio Mariepauá. This specimen, an adult male, died a month later and only the skull and skeleton was preserved (see Table 3 for cranial measurements; Table 2 for intermembral, brachial and crural indices). It is deposited in the mammal collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil, under the number INPA 2512. It is depicted in Figure 5. Furthermore, a skull and a complete skeleton of an adult female that was kept for several years in the breeding center of the first author, has been preserved and is deposited in the mammal collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil, under the number INPA 3930. Figure 9 shows the skull of this specimen, and Table 4 gives the cranial and dental measurements.

Table 1. The taxonomic arrangements for the marmosets *Cebuella* and *Callithrix* in Hershkovitz (1977), De Vivo (1988, 1991), Mittermeier *et al.* (1988), Rylands *et al.* (1993), Van Roosmalen *et al.* (1998), and in this paper.

Hershkovitz (1977)	De Vivo (1988, 1991)	Mittermeier <i>et al.</i> (1988) Rylands <i>et al.</i> (1993)	Van Roosmalen <i>et al.</i> (1998)	This paper
<b>Pygmy marmosets – <i>Cebuella</i><sup>1</sup> (Amazonia in Colombia, Peru, Brazil, Bolivia)</b>				
<i>C. pygmaea</i> <sup>1</sup>	<i>C. pygmaea</i>	<i>C. pygmaea</i>	<i>C. p. pygmaea</i> <sup>2</sup> <i>C. p. niveiventris</i>	<i>C. p. pygmaea</i> <i>C. p. niveiventris</i>
<b>Dwarf marmoset – <i>Callithrix humilis</i> group (Brazilian Amazonia)</b>				
			<i>C. humilis</i> <sup>3</sup>	<i>C. humilis</i>
<b>Marmosets - <i>Callithrix jacchus</i> clade (Eastern Brazil)</b>				
<i>C. jacchus jacchus</i>	<i>C. jacchus</i>	<i>C. jacchus</i>	<i>C. jacchus</i>	<i>C. jacchus</i>
<i>C. j. penicillata</i>	<i>C. penicillata</i>	<i>C. penicillata</i>	<i>C. penicillata</i>	<i>C. penicillata</i>
<i>C. j. geoffroyi</i>	<i>C. geoffroyi</i>	<i>C. geoffroyi</i>	<i>C. geoffroyi</i>	<i>C. geoffroyi</i>
<i>C. j. aurita</i>	<i>C. aurita</i>	<i>C. aurita</i>	<i>C. aurita</i>	<i>C. aurita</i>
<i>C. j. flaviceps</i>	<i>C. flaviceps</i>	<i>C. flaviceps</i> <i>C. kublii</i> <sup>4</sup>	<i>C. flaviceps</i> <i>C. kublii</i>	<i>C. flaviceps</i> <i>C. kublii</i>
<b>Marmosets - <i>Callithrix argentata</i> clade (Brazilian Amazonia, Bolivia and Paraguay)</b>				
<i>C. argentata argentata</i>	<i>C. argentata</i>	<i>C. a. argentata</i>	<i>C. argentata</i>	<i>C. argentata</i>
<i>C. a. leucippe</i>	<i>C. leucippe</i>	<i>C. a. leucippe</i>	<i>C. leucippe</i>	<i>C. leucippe</i>
<i>C. a. melanura</i>	<i>C. melanura</i>	<i>C. a. melanura</i>	<i>C. melanura</i>	<i>C. melanura</i>
	<i>C. emiliae</i> <sup>5</sup>	<i>C. emiliae</i>	<i>C. aff. emiliae</i> <i>C. emiliae</i> <sup>5</sup>	<i>C. aff. emiliae</i> <i>C. emiliae</i>
		<i>C. nigriceps</i> <sup>6</sup>	<i>C. nigriceps</i>	<i>C. nigriceps</i>
			<i>C. marcai</i> <i>C. saterei</i> <sup>8</sup>	<i>C. marcai</i> <i>C. saterei</i>
				<i>C. manicorensis</i> <i>C. acariensis</i>
<i>C. humeralifer humeralifer</i>	<i>C. humeralifera</i>	<i>C. h. humeralifer</i>	<i>C. humeralifera</i>	<i>C. humeralifera</i>
<i>C. h. intermedius</i> <sup>9</sup>	<i>C. intermedia</i>	<i>C. h. intermedius</i>	<i>C. intermedia</i>	<i>C. intermedia</i>
<i>C. h. chrysoleuca</i>	<i>C. chrysoleuca</i>	<i>C. h. chrysoleuca</i>	<i>C. chrysoleuca</i> <i>C. mauesi</i> <sup>10</sup>	<i>C. chrysoleuca</i> <i>C. mauesi</i>

<sup>1</sup> Rosenberger (1981 see also Rosenberger and Coimbra-Filho, 1984) argued, on morphological terms, that the pygmy marmoset should correctly be included in the genus *Callithrix*. This argument was not maintained in Rosenberger *et al.* (1990), although recently Nagamachi *et al.* (1992), Barroso (1995), Schneider *et al.* (1996), Barroso *et al.* (1997), Tagliaro (1997), and Tagliaro *et al.* (1997) have also argued for this arrangement on the basis of their karyotype and molecular genetics.

<sup>2</sup> Lönnberg (1940) argued for two subspecies of *Cebuella pygmaea*, *C. p. pygmaea* and *C. p. niveiventris*. Although they were listed by Napier (1976), Hershkovitz (1977) did not recognize them. Van Roosmalen and Van Roosmalen (1997) and Van Roosmalen *et al.* (1998) argued that the two forms are distinct and valid subspecies. J. Hernández-Camacho (pers. comm.) believes they should be regarded as distinct species.

<sup>3</sup> Described by Van Roosmalen *et al.* (1998).

<sup>4</sup> Coimbra-Filho (1984, 1985, 1990), Rylands (1989), Mittermeier *et al.* (1988), Natori (1990), Rylands *et al.* (1993), and Groves (1993) recognize *Callithrix kublii* from southern Bahia as a valid form. Hershkovitz (1977) regarded it as a hybrid between *C. j. geoffroyi* and *C. j. penicillata*. De Vivo (1991) considered it to be indistinguishable from *C. penicillata*.

<sup>5</sup> *Callithrix emiliae* was first described by Thomas (1920) from the Rio Irirí, southern Pará. It was not recognized by Hershkovitz (1977) who regarded it as merely a dark form of *C. argentata argentata*. De Vivo (1985; see also De Vivo, 1991), revalidated this form on the basis of specimens from the state of Rondônia. However, Rylands *et al.* (1993) argued that the Rondônia marmosets described by De Vivo (1985) should be considered a distinct species based on the fact that the distribution of this form and that of the *C. emiliae* described by Thomas (1920) from Maloca on the Rio Curuá (see De Vivo, 1985; Ávila Pires, 1986) are disjunct, and separated by *C. melanura*. *C. emiliae* was not listed by Groves (1993). Here we recognize the Rondônia marmoset (*C. aff. emiliae* of De Vivo, 1985) and, provisionally, *C. emiliae* Thomas, 1920 from the Rio Irirí until further research is carried out on these marmosets.

<sup>6</sup> Described by Ferrari and Lopes (1992).

<sup>7</sup> *Callithrix marcai* was described as a subspecies of *Callithrix argentata* by Alperin (1993). It is listed here as a species to conform with the view that all *Callithrix* should be considered species (De Vivo, 1985, 1991; Mittermeier *et al.*, 1992). It is known only from the type locality, "Foz do Rio Castanho (= Rio Roosevelt), afluente esquerda do rio Aripuanã, Estado do Amazonas, Brasil" (Alperin, 1993). The type locality as described by Alperin is confused in that the Rio Castanho is not a synonym of the Rio Roosevelt, and is a left bank affluent of the Rio Roosevelt, not the Rio Aripuanã.

<sup>8</sup> Described by Silva Jr. and Noronha (1998).

<sup>9</sup> *Callithrix intermedia* was described as an intermediate color form of *C. humeralifer* by Hershkovitz (1977). Pelage color and pattern, and its geographic distribution, however, would place it as a subspecies of *C. argentata* if, following Hershkovitz (1977), the Amazonian marmosets are considered subspecies of just two species, *C. argentata* and *C. humeralifer* (see Rylands *et al.*, 1993).

<sup>10</sup> Described by Mittermeier *et al.* (1992).

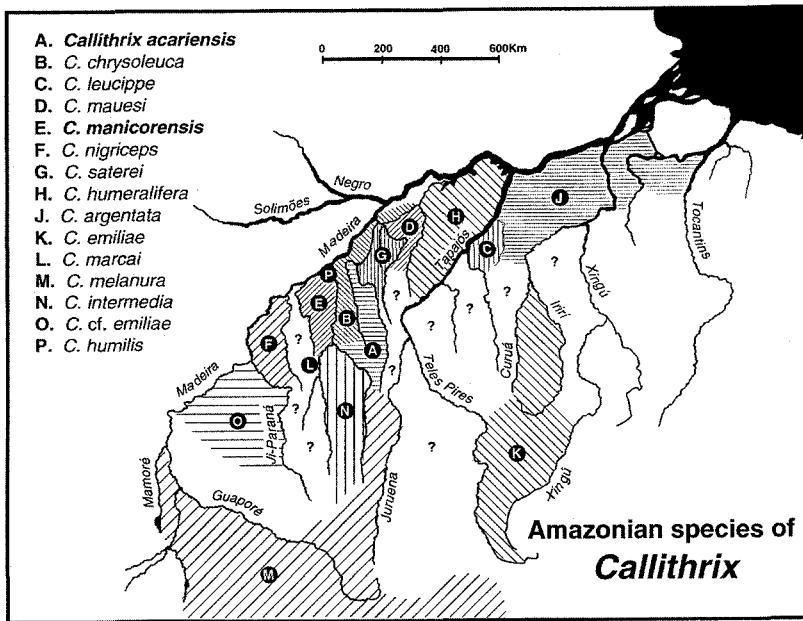


Figure 1. Distribution of 14 species of the Amazonian *Callithrix argentata* group, including *C. manicorensis* and *C. acariensis* described in this paper, and also of *Callithrix humilis* Van Roosmalen *et al.*, 1998. Map by Stephen D. Nash.

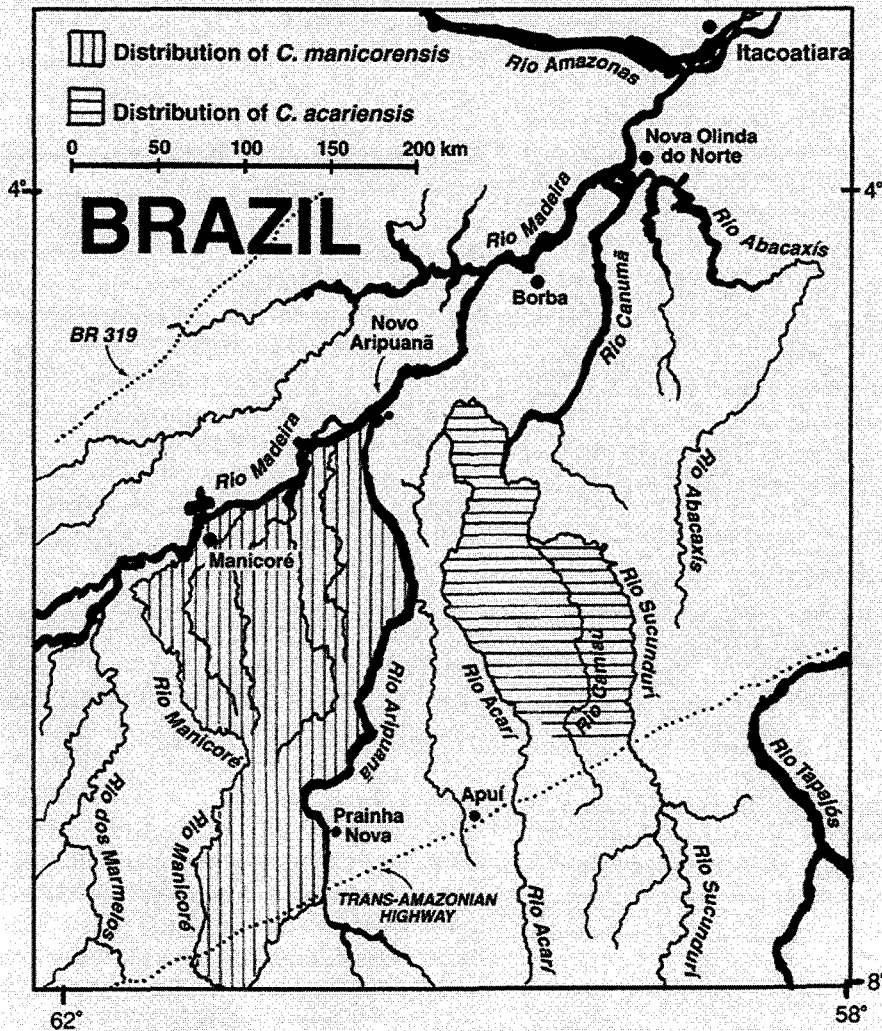


Figure 2. Distribution of *Callithrix manicorensis* and *Callithrix acariensis*. Map by Stephen D. Nash.

**Table 2.** The intermembral (humerus + radius)/(femur + tibia) x 100, brachial (radius/humerus) x 100 and crural (tibia/femur) x 100 indices of one adult *Callithrix manicorensis* compared with those of *Callithrix argentata*, *Callithrix humilis* and *Cebuella pygmaea* (measurements taken by Lesa A. Davis and Susan Ford).

Intermembral Index	Brachial Index	Crural Index
<i>C. argentata</i>	76	<i>C. humilis</i>
<i>C. manicorensis</i>	78	<i>C. argentata</i>
<i>C. humilis</i>	79	<i>C. manicorensis</i>
<i>Cebuella pygmaea</i>	82	<i>Cebuella pygmaea</i>

**Table 3.** Cranial measurements (mm) of the adult male (Registration number INPA 2512) of *Callithrix manicorensis* from the Rio Mariepauá.

Skull and mandible	mm
Greatest Skull Length (SL)	45.36
Condylbasal Length	36.92
Zygomatic Width (ZW)	30.31
Biorbital Width (BW)	27.71
Braincase Length	40.31
Braincase (Postorbital) Width (PW)	26.72
Across First Molars (AM)	15.55
Length of Mandible (ML)	30.45
Coronoidal Height	18.30
pm <sup>2</sup> -m <sup>2</sup>	9.27
i-m <sup>2</sup>	15.62
c-m <sup>2</sup>	11.72
pm <sub>2</sub> -m <sub>2</sub>	10.07
i-m <sub>2</sub>	15.80
c-m <sub>2</sub>	12.56

**Geographic distribution:** The species is known from the west bank of the lower Rio Aripuanã from the mouth, south to the confluence with the Rio Roosevelt, and along the east bank of the Rio Madeira from the mouth of the Rio Aripuanã, just south of the town of Novo Aripuanã, south to the mouth and right bank of the Rio Manicoré. The southern limit for the species is probably the headwaters of the Rio Manicoré, close to the Rio Roosevelt.

**Habitat:** The various groups observed in the wild by Marc G. M. van Roosmalen were in dense primary *terra firme* rainforest and in secondary forest surrounding plantations and fields. Very high densities were found in so-called *seringal*, a high riverbank forest, managed since the early days of rubber exploitation in the Amazon, where the density of rubber trees was increased artificially. *Seringais* are an early form of agroforestry, in which areas of high riverbank forests were turned into productive multi-species forests, rich in a number of commercially valuable and edible fruit producing native trees, including rubber (*Hevea brasiliensis*), Brazil nut (*Bertholletia excelsa*), 'orelha de macaco' (*Enterolobium schomburgkii*), wild cocoa (*Theobroma* spp.), 'bacuri' (*Rhedia* spp., *Platonia insignis*), 'ingá' (*Inga* spp.), 'taperebá' (*Spondias mombin*), 'biribá' (*Rollinia squamosa*), 'soursop' (*Annona* spp.), and a number of tree species belonging to the Sapotaceae and Arecaceae (palm) families. The *seringais* ap-

pear to offer optimal habitat and a year-round food supply for small monkeys, such as marmosets, pygmy marmosets, dwarf marmosets, titi and night monkeys.

**Diagnosis:** A marmoset of the *Callithrix argentata* group (*sensu* Hershkovitz, 1977), characterized by a black tail and a grey cap on the head, but quite distinct from *C. nigriceps*, *C. marcai*, and *C. aff. emiliae*. The lightest of these four species and most advanced phaeomelanically (*sensu* Hershkovitz, 1977), with the least pigmented face and ears. The cap on the head is light gray instead of black in the other members of this subgroup, and the naked face and eyes are surrounded by white fur. The general appearance of this monkey when spotted in the field is an overall drab whitish marmoset with orange legs and a pinkish to orange naked face and ears, irregularly brown mottled in the adults, and a slightly transversely striped gray back. This marmoset is easy to distinguish from the recently described black-capped dwarf marmoset (Van Roosmalen *et al.*, 1998), with which it is sympatric in the northern part of its distribution. The dwarf marmoset is almost one-third the size of *Callithrix manicorensis*, has an overall dark brown appearance with light brown to orange brown under parts. It spends more time gouging bark on tree trunks, in an upright squirrel-like posture, than true marmosets. The differences between *C. manicorensis*, *Callithrix humilis* and *C. marcai*, can be seen in the color illustrations in Figures 3 and 4.

**Description of the holotype:** General coloration of upper parts white, transversely mottled with gray; tail black; crown on head triangular, gray; sides of upper arms, shoulders and neck light grayish white, not mottled; back and rump grayish white, mottled with darker gray, giving a dark gray, slightly transversally striped appearance; outer sides of legs and feet orange; upper surface of lower arms and hands slightly orange, lower surface of lower arms ochraceous to orange; lower surface of legs orange; white rim on forehead above the eyes continuing onto the temples; face naked; facial skin and cheeks orange, pigmented dark brown around the eyes and on the lower lip and chin; nose and circumbuccal area naked, pink to flesh-colored, dark brown mottled around the nostrils, with a narrow dark brown vertical nose stripe, without moustache; auricle non-pigmented, pink or flesh-colored, slightly hairy (only in juveniles); throat, ventral surface of neck and chest white; belly and inner surface of legs bright orange; exposed genitals non-pigmented, white, hypertrophied (Fig. 6); tail black, proximal end slightly mottled with orange and gray; eyes with black pupil and dark brown iris. Weight of juvenile

female holotype: 135 g. Body measurements: head-body length - 160 mm; tail length - 270 mm; total length - 430 mm; hind foot - 51 mm; hand - 34 mm; hallux - 8 mm, other toes - 12-15 mm, pollex - 9 mm, other fingers - 13-15 mm.

**Origin of the name:** This marmoset was first collected and observed in the wild along the right bank of the Rio Manicoré, municipality of Manicoré. It is therefore named after the river Manicoré, a right bank tributary of the Rio Madeira, south central Amazonia. The name places it geographically relative to its congeners.

**Vernacular name:** This marmoset is referred to as *sagüi*, *sauím* (*souím*), or 'branquinho' (= little white fellow, in Portuguese) by the local people. For an English name, we suggest the Rio Manicoré marmoset.

### *Callithrix acariensis* sp.n.

**Holotype:** A live female infant obtained by Marc G. M. van Roosmalen on 29 August 1996 on the right bank of the Rio Acarí near its confluence with the Rios Sucundurí and Canumã. It was being kept as a pet and was only 1-2 months old. It was kept in captivity in the endangered primate breeding center in Manaus (managed by the first author). It died when adult and is deposited in the mammal collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil, registration number INPA 3931. The skull is shown in Figure 10; cranial and dental measurements are given in Table 4.

**Type locality:** A small settlement on the right bank of the lower Rio Acarí close to the confluence with the Rios Sucundurí and Canumã, state of Amazonas, south central Amazonia, Brazil. Coordinates for the type locality are 05° 07' 08" S, 60° 01' 14" W.

**Geographical distribution:** The species is known from the right bank of the lower Rio Acarí, and assumed to occur in the entire interfluvium of the Rios Acarí and Sucundurí, south perhaps to a contact zone with *Callithrix melanura* somewhere between the Rio Aripuanã and the Rio Juruena.

**Habitat:** This new species has not been observed in the wild, but according to local settlers living along the right bank of the Rio Acarí it is found mainly in dense primary *terra firme* rainforest and in secondary forest surrounding their plantations and manioc fields.

**Diagnosis:** A marmoset of the *Callithrix argentata* group (*sensu* Hershkovitz, 1977), it is one of the most colorful of the Amazonian marmosets with its bright orange lower back, under parts, legs and proximal end of the black tail, and predominantly white upper parts and chest contrasting with the partly black pigmented muzzle, narrowly triangular nose patch and ocular rings of the otherwise pink face. Its ears are dark pigmented, partially covered with white hairs (Figs. 7 and 8). It is the lightest of its closest relatives, *C. saterei* and *C. melanura*, easy to distinguish from its neighbor to the north,

*C. saterei*, which has a much darker back, lower arms and legs, an almost totally naked head, asymmetrically black and non-pigmented naked ears, a narrower yellowish orange thigh and hip stripe, and a large black triangular patch on and above the nostrils; its black tail lacks the orange proximal ring or spot (Fig. 3). It is also very easy to distinguish from *C. melanura*, its neighbor to the south, which is predominantly gray and brown lacking orange and white in its pelage, and lacking the white fur surrounding the face (Fig. 3).

**Description of the holotype:** General coloration of upper parts white, distally grading into gray to dark gray mixed with orange; tail black with a very distinct orange proximal end or spot; crown on head light gray or white; sides of upper arms, shoulders, and neck white or white with grayish hair tips; outer sides of legs and feet bright orange, with a strikingly white, 2 cm wide thigh and hip stripe; upper surface of lower arms and hands gray mixed with orange, lower surface of lower arms white with orange; lower surface of legs white distally grading into orange; forehead white continuing onto the temples, nape, chest and upper arms; face naked; facial skin pink or flesh-colored, with black pigmented patches between the eyes, aside of the nostrils, and on the lower lip and chin; nose and circumbuccal area naked, pink to flesh-colored, with a narrowly triangular black patch on the nose, without moustache; auricle dark pigmented, grayish, covered with quite a few white hairs; throat, ventral surface of neck and chest white, giving the appearance of a white mantle; belly and inner surface of legs bright orange; exposed genitals non-pigmented, white, pudenda hypertrophied, 2.2 x 2 x 1.5 cm (Fig. 7); tail black, proximal end bright orange forming a 2 cm wide ring; eyes with black pupil and orange brown iris. Weight of adult female holotype: 420 g. Body measurements: head-body length - 240 mm; tail length - 350 mm; total length - 590 mm; hind foot - 63 mm; hand - 45 mm; hallux - 10 mm, other toes - 15-20 mm; pollex - 12 mm, other fingers - 15-20 mm.

**Origin of the name:** This marmoset was collected along the right bank of the Rio Acarí. It is therefore named after this river, the only reference to this remote, largely uninhabited part of south central Amazonia, in order to place it geographically relative to its congeners.

**Vernacular name:** This marmoset is referred to as *sagüi* or *sauím* (*souím*) by the local people. For an English name, we suggest the Rio Acarí marmoset.

### Phylogenetic Placement of *Callithrix manicorensis* and *Callithrix acariensis* Based on Nuclear and Mitochondrial Sequences

#### *Materials and Methods*

Hair, skin, and liver samples were obtained from deceased specimens from the Breeding Center for Endangered Amazonian Primates in Manaus, Amazonas, Brazil, which were deposited at the zoological museum of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Bra-

zil. Sampled marmosets included three *Callithrix humilis*, one *Callithrix manicorensis*, one *Callithrix acariensis*, and one *Cebuella pygmaea niveiventris*. DNA was extracted from the tissues following the protocol provided by Gibco BRL DNAzol extraction kit. The mitochondrial control region (also known as the D-loop) and intron 2 of the  $\beta_2$ -microglobulin gene were chosen for amplification because they had previously been characterized for various related callitrichids, and because they arguably proffered some of the greatest phylogenetic resolution for mitochondrial and nuclear genomes in these organisms, respectively. Primers were designed based on previously sequenced callitrichid mitochondrial control regions (Tagliaro *et al.*, 1997). A heavy strand primer was designed in the CSB1 region of the control region, as well as a shorter primer in the same region for sequencing purposes. In addition, primer L15926 (Kocher *et al.*, 1989) was used for light strand amplification. Primers used for  $\beta_2$ -microglobulin intron 2 PCR amplification were the same used in Canavez *et al.*, 1999. Polymerase chain reaction conditions consisted of: 80° C (1 min), 94° C (2 min), 35 cycles of {94° C (1 min), 50° C (1 min 30 sec), 72° C (2 min)} and 72° C (10 min) for the mitochondrial control region. PCR conditions for the  $\beta_2$ -microglobulin intron 2 were the same used by Canavez *et al.*: 95° C (30 sec); 30 cycles of {95° C (20 sec), 60° C (30 sec), 72° C (30 sec)} and 72° C (5 min). PCR reactions were on a 1% agarose gel, since the control region amplifications were expected to be around 900 bp long, and the  $\beta_2$ -microglobulin amplifications not longer than 700 base pairs, taking interspecific variation in length into account.

Upon successful amplification, PCR products were transformed into pCR®4-TOPO® plasmids which were subsequently transformed into One Shot® *Escherichia coli* cells using the TOPO TA Cloning® Kit for Sequencing (Invitrogen Corporation). Cells were plated on LB-agar plates 50 µg/ml ampicillin, and left to grow at 37° C overnight. Any cells transformed with the plasmid were conferred resistance to ampicillin. Ten to fifteen colonies were picked for each species, and cultured overnight in 2 ml of LB-amp both at 37° C. Plasmids were then isolated using the QIAprep® Spin Miniprep Kit (Qiagen Inc.). EcoRI sites at both ends of the insertion site of the plasmid allowed for digestion and subsequent screening for plasmids with inserts on a 1% agarose gel. Plasmids containing inserts were selected for sequencing. Primers M13 (forward and reverse), provided by Qiagen Inc., allowed for sequencing of the inserts from plasmids ends. The cloning and transformation process allowed for separation of desired sequences from any non-specific amplifications, or "smears", that would otherwise muddle sequencing performance. Amplifications were sequenced with an ABI Prism 310 Spectrum sequencer.

Sequences were aligned by eye, using the program Sequencer. Additionally, the sequences obtained in this study were combined with those obtained from GenBank using accession numbers given in Tagliaro *et al.* (1997) for the mitochon-

drial control region, and Canavez *et al.* (1999) for the nuclear intron. For the control region, sequences of three *Callithrix argentata*, three *C. mauesi*, two *C. humeralifera*, two *C. geoffroyi*, two *C. penicillata*, two *C. jacchus*, five *C. kuhli*, two *C. aurita*, one *Cebuella pygmaea*, and one *Leontopithecus chrysomelas* were obtained from GenBank. For the  $\beta_2$ -microglobulin intron 2, sequences were obtained for one *Callithrix humeralifera*, one *C. emiliae*, one *C. kuhli*, one *C. penicillata*, one *Cebuella pygmaea*, one *Callimico goeldii*, one *Saguinus midas*, one *S. imperator*, and one *Leontopithecus chrysopygus*. Upon alignment, sequences were entered into the PAUP program (Swofford, 1994) 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.

### Results

DNA extraction and PCR amplification proved to be equally successful for tissues stored at either freezing conditions or 100% alcohol before deposition in RNA later. For the mitochondrial control region, an alignable 902 base pair (BP) segment including both hypervariable regions and the conserved central domain was amplified and sequenced for all specimens, except *Cebuella pygmaea*. This sequence constitutes approximately 90% of the entire mitochondrial control region. For *Cebuella pygmaea*, only a 532 bp segment including the central conserved domain and the primate insertion sequence was obtained. For the  $\beta_2$ -microglobulin intron 2, a 607 bp segment was obtained for *Callithrix manicorensis* alone. These sequences were aligned with corresponding sequences from GenBank.

Pair-wise divergences for *Callithrix* and *Cebuella* were calculated. For the mitochondrial control region, those among Amazonian marmosets (*Callithrix*) ranged from approximately 3% to 7% (2-4% within each subclade, 6-7% between the *argentata* and *mauesi* subclades). Divergences between any Amazonian marmoset and *Cebuella* were on the order of 11-12%. Within-species divergences ranged from 0-3%. *C. mauesi* and *C. humeralifera* showed the greatest within-species divergence, possibly due to hybridization between these two species. Their separation by the Rio Maués-Açú, a minor tributary of the Rio Amazonas and contact zone with *C. humeralifera* between the headwaters of the Rio Maués and the Rio Abacaxis, may explain why hybridization still occurs between these two species, having never become completely isolated from one another. These findings call for a closer investigation into hybridization rates between populations of these two species, and whether they actually represent distinct species.

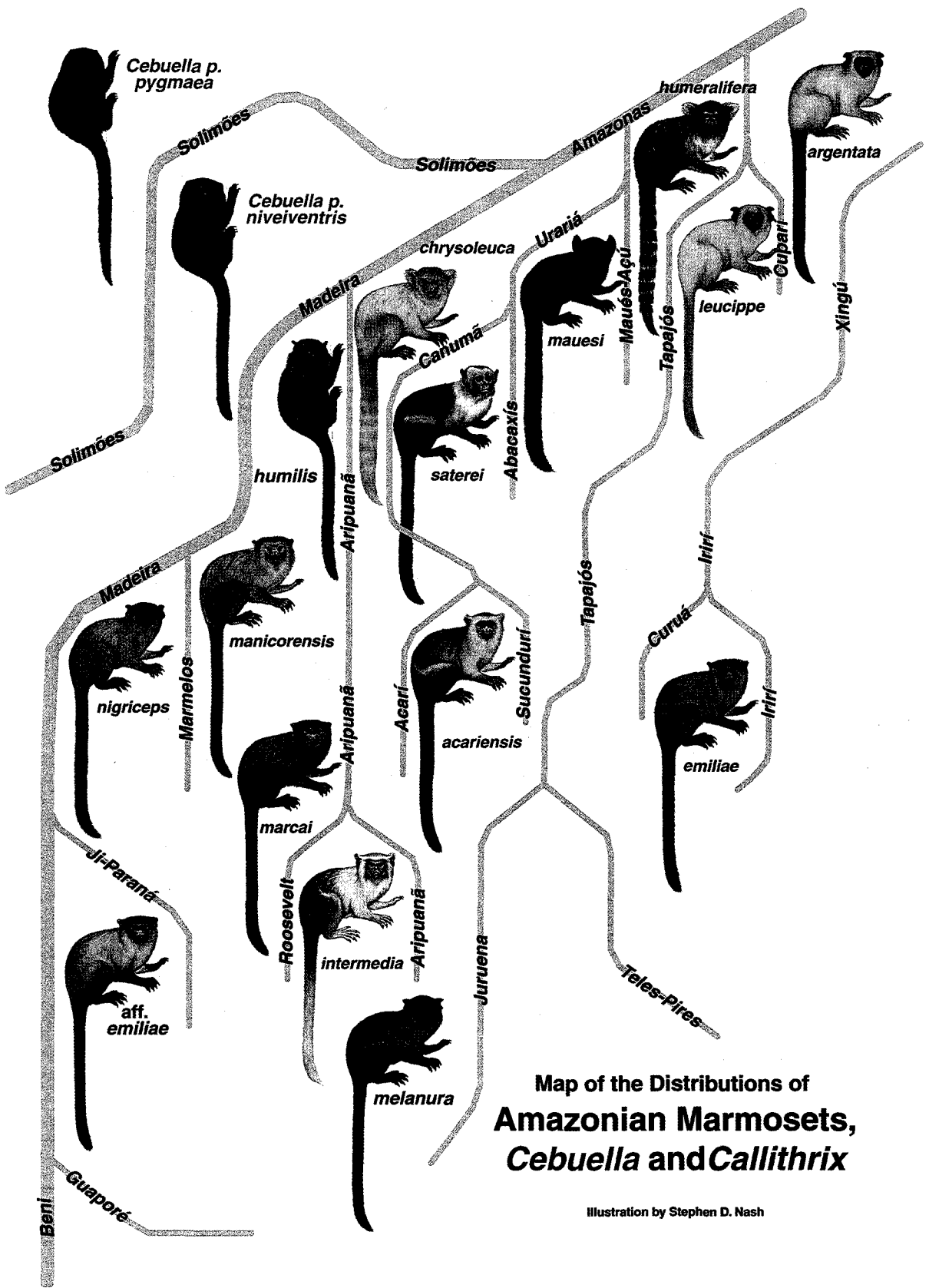


Figure 3. A schematic representation of the distributions of the Amazonian marmosets, *Callithrix*, delimited by rivers. Illustration by Stephen D. Nash.



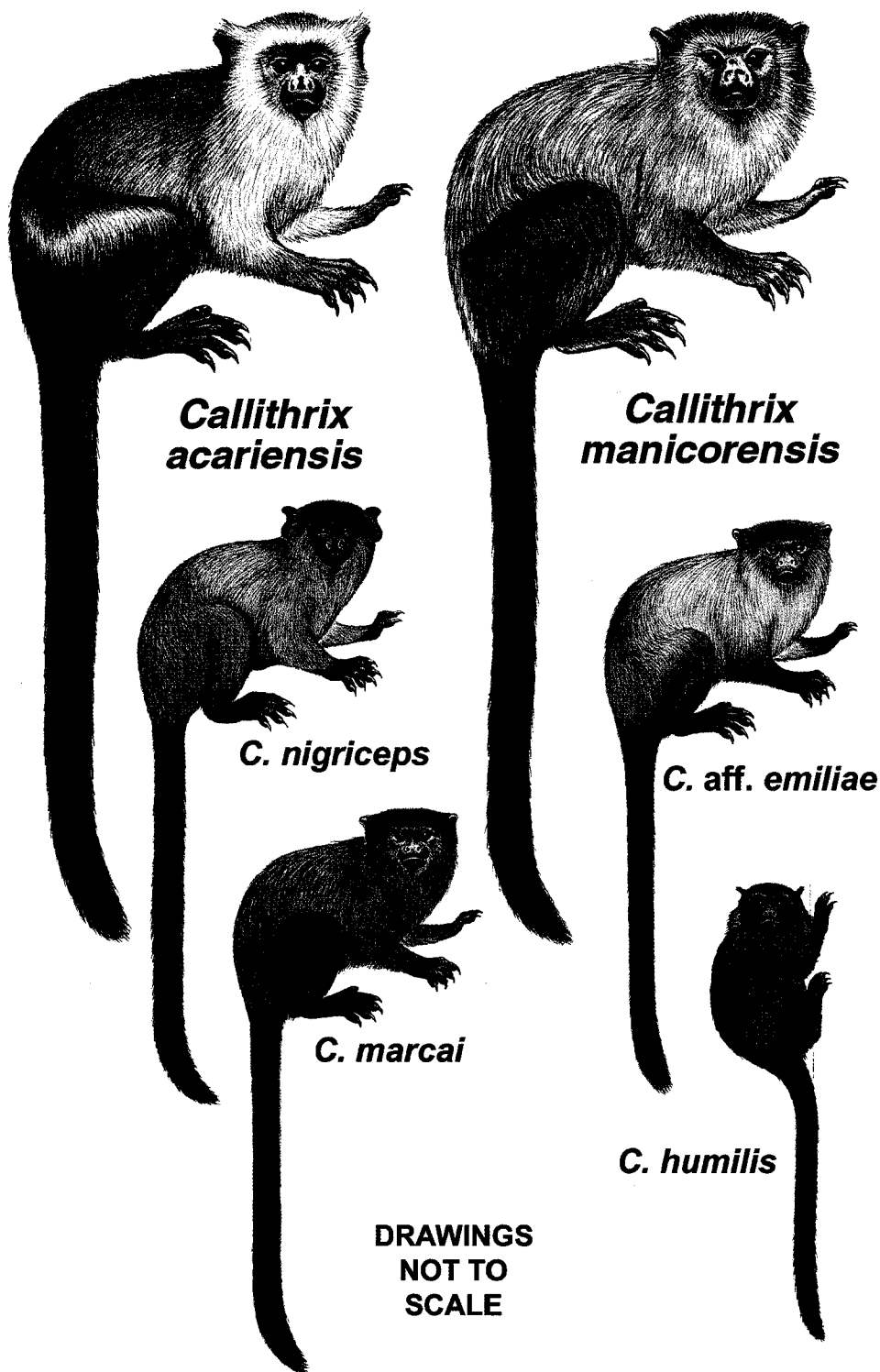


Figure 4. The Rio Acari marmoset, *Callithrix acariensis* and the Rio Manicoré marmoset, *Callithrix manicorensis* compared with *Callithrix nigriceps* Ferrari and Lopes, 1992, *Callithrix* aff. *emiliae* (to be described as a new species), *Callithrix marcai* Alperin, 1993 (possibly parapatric in the south of the range of *C. manicorensis*) and *Callithrix humilis* Van Roosmalen, Van Roosmalen, Mittermeier and Fonseca, 1998 (sympatric with *C. manicorensis*). Drawings not to scale. Illustration by Stephen D. Nash.



Figure 5. Adult male *Callithrix manicorensis* (INPA 2512) from the Rio Mariepauá (left), and a young female (INPA 3930) (right). Photographs by Marc G.M. van Roosmalen.



Figure 6. The adolescent male *C. manicorensis*, the paratype which is still alive, displaying its genitals. Photograph by Marc G.M. van Roosmalen.

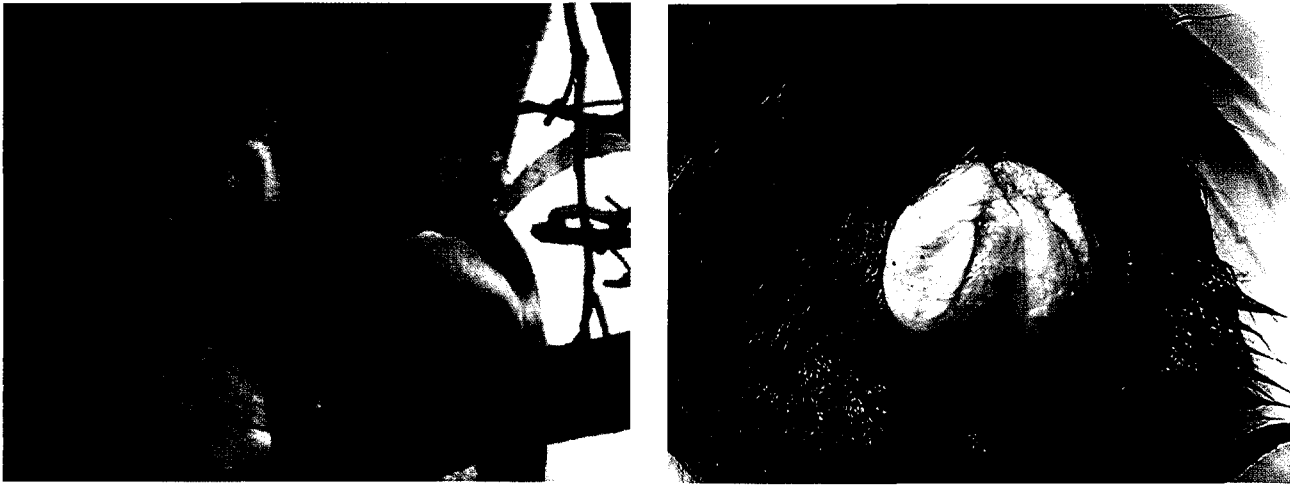


Figure 7. Adult female *Callithrix acariensis* (holotype INPA 3931), showing the distinct thigh stripe (left), and the hypertrophied pudenda (right). Photographs by Marc G.M. van Roosmalen.



Figure 8. Adult female *Callithrix acariensis* (holotype INPA 3931). Photograph by Marc G.M. van Roosmalen.

Table 4. Cranial and dental measurements of the holotype (adult female) of *Callithrix acariensis* (INPA 3931) and the adult female of *Callithrix manicorensis* (INPA 3930) (mm).

Skull and mandible	INPA 3930	INPA 3931
Greatest Skull Length (SL)	45.7	47.6
Zygomatic Width (ZW)	31.3	30.8
Biorbital Width (BW)	26.2	27.3
Postorbital Width (PW)	22.4	23.3
Nasion-Basion	32.2	33.1
Basion-Prosthion	33.7	34.8
Biauricular Breadth	26.3	26.5
Height of Canine (CH)	4.3	5.2
Length of Mandible (ML)	28.8	31.1
Across First Molars (AM)	11.7°	13.5
Across Canines	8.4	8.9
Dental Field (P2-M2)	11.1°	10.8
Premaxillary Height	8.3	8.3
Intradentale Superior to Premaxilla-Maxilla Junction at Alveolus (IS-P)	5.0	4.6
Intradentale Superior to Nasion (IS-NA)	15.1	15.8
Intradentale Superior to Posterior Nasal Spine (IS-PNS)	15.6	16.3
Bregma to Nasion (BR-NA)	23.6	23.3
Nasion to Fronto-Malar Junction at Orbit (NA-FM)	14.1	14.2
Fronto-Malar Junction to Pterion	6.7	8.3
Fronto-Malar Junction to Zygomaxillare Superior (FM-ZS)	4.9	6.8
Fronto-Malar Junction to Maxillary Tuberosity (FM-MT)	13.2	9.9
Zygomaxillare Inferior to Premaxilla-Maxilla Junction at Alveolus (ZI-PM)	12.0	12.1
Zygomaxillare Inferior to Zygomaxillare Superior (ZI-ZS)	7.5	6.8
Zygomaxillare Inferior to Maxillary Tuberosity (ZI-MT)	5.6	6.0
<b>Anterior Teeth</b>		
I <sup>1</sup> Length		2.1
I <sup>1</sup> Breadth		1.6
I <sup>1</sup> Height		2.7
I <sup>2</sup> Length		1.9
I <sup>2</sup> Breadth		1.6
i <sub>1</sub> Length		1.4
i <sub>1</sub> Breadth		1.6
i <sub>1</sub> Height		3.0
i <sub>2</sub> Length	1.3	1.4
i <sub>2</sub> Breadth	2.2	2.2
C <sub>1</sub> Length	2.5	2.7
C <sub>1</sub> Breadth	1.5	1.7
<b>Cheek Teeth</b>		
P <sub>2</sub> Length		2.4
P <sub>2</sub> Breadth		1.7
P <sub>4</sub> Length		1.9
P <sub>4</sub> Breadth		1.8
M <sub>1</sub> Length		2.5
M <sub>1</sub> Width		2.0
M <sub>2</sub> Length		2.0
M <sub>2</sub> Width		1.7

° = missing

The pairwise divergences between the Atlantic forest *Callithrix* (the *Callithrix jacchus* clade), or ouistiti's, and the Amazonian *Callithrix* (the *Callithrix argentata* clade) range from 12-13%, while divergence between Atlantic forest *Callithrix* and *Cebuella* range from 14-15%. Divergence between all marmosets, pygmy marmoset, and dwarf marmoset and the out-group, the lion tamarins *Leontopithecus* was over 20%. These genetic distances suggest that, contrary to suggestions by previous molecular studies (e.g., Tagliaro *et al.*, 1997; Barroso *et al.*, 1997) to subsume *Cebuella* within the *Callithrix* genus, the Amazonian *Callithrix* species and the Atlantic forest *Callithrix* species should be separated into distinct genera. Given a 3-7% divergence for species within either clade, and a 10-11% divergence between clades, it seems phylogenetically more parsimonious to group the two clades into separate genera in recognition of their evolutionary distinctness. If we want to use taxonomic classifications to reflect actual evolutionary distinctness, we should keep the Atlantic forest *Callithrix* or ouistiti's in the genus *Callithrix* Erxleben, 1777, and place the Amazonian *Callithrix* or Amazonian marmosets in the genus *Mico* Lesson, 1840. Groves (in press) lists the Amazonian marmosets as belonging to the subgenus *Mico* Lesson, 1840.

Using PAUP, a maximum parsimony analysis with 100 bootstrap replications yielded the phylogenetic tree for the mitochondrial control region seen in Figure 11. 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. The relationship of the Atlantic forest marmosets, or ouistiti's (the *Callithrix jacchus* clade), as diverging before the radiation of Amazonian marmosets (also known as the *Callithrix argentata* clade) including the pygmy marmosets (*Cebuella pygmaea*), remains in con-

cordance with the phylogenetic trees presented by Tagliaro *et al.* (1997). *Callithrix manicorensis* joins most closely with *Callithrix argentata* with significant bootstrap values.

The  $\beta_2$ -microglobulin intron 2 phylogenetic tree yielded much less percent variation (0.4-1.0 %) among taxa, and was insufficient for clear resolution. However, it did support the grouping of *Callithrix manicorensis* within the Amazonian *Callithrix* clade with a significant bootstrap value.

## Discussion

The finding that *Callithrix acariensis* is more closely related to the *C. mauesi/humeralifera* clade concurs with these species' shared geographical location between the Rios Tapajós and Madeira/Aripuanã, an area that is largely contiguous and undivided by major rivers. Given the morphological similarity between *C. acariensis* and *C. melanura* and the recently described *C. saterei*, and the fact that they all share the same geographic region between the Rio Tapajós and the Aripuanã/lower Madeira, it seems plausible that marmoset species in this region form a separate subclade within the Amazonian marmoset subgenus. It, therefore, seems likely that starting with the archetypic *C. melanura* with its huge distribution in the headwaters of the Rios Madeira, Mamoré and Guaporé, and Aripuanã and Juruena, lacking major river barriers, this subclade speciated toward the north via *C. intermedia* confined to the Rios Roosevelt and Aripuanã interfluvium, *C. acariensis*, confined to the interfluvium of the Rios Acari and Sucundurí, *C. humeralifera* in the interfluvium of the Rios Tapajós, Amazonas and Maués-Açú, finally speciating into *C. chrysouleuca*, occupying the Rios Amazonas, Madeira, Canumã, Aripuanã and Acari interfluvium from north to south, while *C. saterei* branched off into the area between the Rios Canumã and Abacaxís, and *C. mauesi* between the



Figure 9. Skull of adult female *Callithrix manicorensis* (INPA 3930). Photograph by Stephen D. Nash.

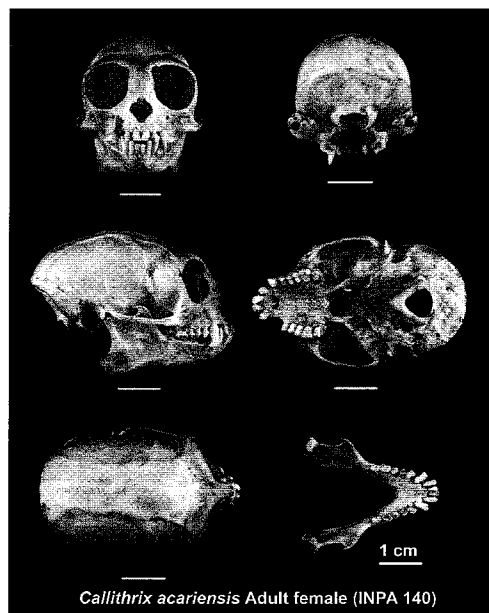


Figure 10. Skull of adult female *Callithrix acariensis* (INPA 3931). Photograph by Stephen D. Nash.

Paraná Urariá and Rio Maués-Açú. In this context it is interesting to note the strikingly white hip/thigh stripe consistent in all these species (rather inconspicuous only in *C. mauesi*), and the partially hairy auricles in especially juvenile *C. intermedia* and *C. acariensis*, culminating in the tufted ear *C. humeralifera*, *C. mauesi*, and *C. chrysoleuca*. Both characteristics are absent in all other existing Amazonian marmosets.

The grouping of *C. manicorensis* with *C. argentata* seems to be a paradox because the two species' geographic ranges are on opposite ends of the Amazonian marmosets' distribution. Neighboring species of *C. manicorensis* such as *C. marcai*, *C. nigriceps* and *C. aff. emiliae* should be analyzed before any conclusions may be drawn from this result. However, these results do indicate a division of the Amazonian marmosets that includes *C. melanura*, *C. intermedia*, *C. acariensis*, *C. saterei*, *C. mauesi*, *C. humeralifera*, and *C. chrysoleuca* in one clade, and *C. aff. emiliae*, *C. nigriceps*, *C. manicorensis*, *C. marcai*, *C. emiliae*, *C. leucippe* and *C. argentata* in the other clade. *C. marcai* and *C. nigriceps* are morphologically similar to *C. manicorensis* (Van Roosmalen *et al.*, 1998). The status of *C. emiliae* is less easy to predict, although its geographical

distribution suggests it is more closely related to the *C. argentata/manicorensis* subclade. Genetic analysis of all species should be done in the future to help resolve this question. The present data indicate that one subclade (*C. argentata/manicorensis*) is divided geographically by the other subclade (*C. acariensis/mauesi/chrysoleuca*). A plausible scenario would be the speciation from an ancestral *C. melanura*-like archetype going both northwest and filling the interfluvium of the Rios Madeira, Aripuanã and Roosevelt with *C. aff. emiliae* in the Rios Madeira and Ji-Paraná interfluvium, *C. nigriceps* in the Rios Madeira and Marmelos interfluvium, *C. marcai* in the Rios Marmelos and Roosevelt interfluvium, and finally *C. manicorensis* 'trapped' in the interfluvium between the unsurpassable Rio Madeira and the lower Rio Aripuanã, and northeast passing the upper Rios Juruena and Tapajós filling the Rios Xingú and Tapajós interfluvium with *C. emiliae* in the Rios Curuá and Irití interfluvium, *C. argentata* in the Rios Xingú, Amazonas and Cuparí interfluvium, and *C. leucippe* in the Rios Tapajós and Cuparí interfluvium. It is rather likely that new species will be found in the near future east of the Rios Juruena and Tapajós.

Patterns of marmoset radiation emerging from the findings here presented suggest that the river barrier hypothesis (Wallace, 1852) could be used to account for much of the primate diversity in the Amazon basin. Unlike the small rodents in Da Silva and Patton's (1993) study, primates as a rule cannot swim for very long distances and particularly marmosets, tamarins and titi monkeys drown at the spot when fallen into the water. Therefore, their only chance for substantial cross-river migration involves passive migration, in which tracts of land (with periodically flooded forest) 'jump' from one side of the river to the other, in the process of ox-bow lake formation of meandering rivers. Although ox-bow lakes are common in the upper reaches of the Rios Purús and Juruá in western Amazonia (Peres *et al.*, 1997), the greater slope of rivers coming from the Brazilian Shield in eastern Amazonia, starting with the Rio Madeira and its upper course tributaries and moving east, precludes such meandering and, with it, passive cross-river migration of breeding primate populations. Therefore, it is possible that rivers in central and eastern Amazonia are effective barriers that have led to the genetic diversity despite their relatively recent divergences, 1-3 million years ago, according to molecular clocks based on IRBP intron sequence divergences (Barroso *et al.*, 1997). Besides the evidence for hybridization found in this study and Tagliaro *et al.* (1997) between *C. humeralifera* and *C. mauesi*, there have been no reports of naturally occurring hybridizations between other Amazonian marmosets, suggesting that its various taxa represent actual species, or distinct evolutionary entities, a conclusion further corroborated by the extreme intolerance of parapatric species toward each other (pers. obs. in captivity). Tamarins, on the other hand, are not exclusively allopatric, as they are separated by meandering rivers which are less effective as reproductive barriers, and their populations are characterized by geographic gradations of pelage color variations. The greater slope in the marmoset's distribution, from the Brazilian Shield in the south toward the Ama-

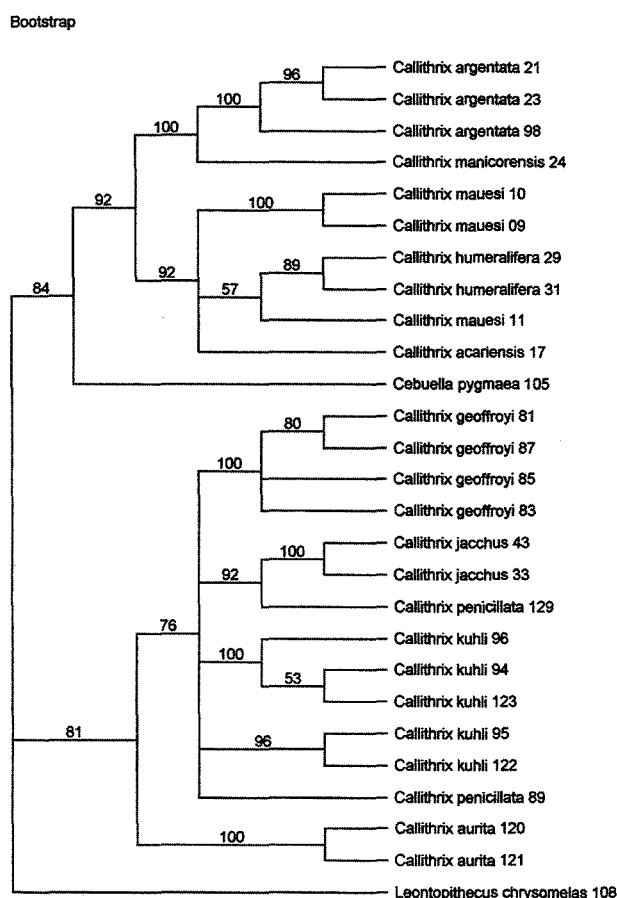


Figure 11. Callitrichid gene tree based on 902 base pairs (bp) of the mitochondrial control region and 635 bp of intron 2 of the nuclear  $\beta$ 2-microglobulin gene, analyzed using maximum parsimony. Bootstrap values are given above branches. *Callithrix manicorensis* clearly belongs to the *C. argentata* clade, *Callithrix acariensis* is most closely related to *C. mauesi* and *C. humeralifera*, the tufted ear *Callithrix* clade.

zon River, leading to south-northward, fast-flowing, straight rivers may thus have contributed to the species diversity of the marmosets, whereas many of the tamarin taxa continue to interbreed at irregular intervals, and are thus unable to fully differentiate into reproductively-isolated species. For this reason, the current classification of many of the tamarin taxa into subspecies (Rylands *et al.*, 1993) and marmosets into distinct species is probably truly reflective of the evolutionary history of these primates.

In summary, our findings suggest that the common ancestral population of *Callithrix*, the dwarf marmoset (see Van Roosmalen and Van Roosmalen, in prep.), and *Cebuella* was initially separated into an Amazonian and Atlantic clade. The Amazonian clade subsequently gave rise to the ancestral dwarf marmoset and then to *Cebuella*. The isolation of the ancestral *Cebuella* from other marmosets (after passing the upper Rio Madeira) in the geographic range of the tamarins west of the Rio Madeira, may explain its subsequent divergence from the other marmosets. The dwarf marmoset, is, however, sympatric with *Callithrix manicorensis*. There is no way of knowing whether its initial divergence involved its isolation from the ancestral *Callithrix* in its present location in the northern part of the interfluvium of the Rios Madeira and Aripuanã, and that subsequent invasion by *Callithrix* followed much more recently. This possible scenario would explain most parsimoniously their present distributions. The geographic division of the *Callithrix argentata* subclade from the *Callithrix mauesi/humeralifera* subclade recognized in this paper, suggests that eastern Amazonia was predominantly occupied by the *Callithrix argentata* subclade, and that a subsequent radiation from the south by the *Callithrix mauesi/humeralifera* subclade drove the *Callithrix argentata* subclade species from the region between the Rios Canumã and Aripuanã, and the Rio Tapajós. Alternately, the *Callithrix mauesi/humeralifera* subclade may have predated the *Callithrix argentata* subclade in eastern Amazonia, and a radiation of the *argentata* subclade from the south into two directions, northeast and northwest, enveloped the *mauesi/humeralifera* subclade. The lack of tolerance for marmoset sympatry, due to ecological niche similarities, necessitated much of this excluding radiation and extreme competition, leaving no room for sympatric existence even between clearly distinct species such as those in the *argentata* subclade and the *mauesi/humeralifera* subclade. It follows that river isolation is a necessary prerequisite for initial isolation and diversification, and that rivers tend to act as divisions between marmoset species that have evolved into separate species, acting as buffer zones that prevent potentially more successful species from invading neighboring areas and effectively out-competing other species. Rivers, therefore, do not merely give rise to new species by genetic isolation, they also serve as boundaries that help maintain species diversity throughout east central Amazonia.

### Conservation Status of the New Species

There is no reason to suspect that *Callithrix manicorensis* is threatened. However, the total area in which it has been con-

firmed to occur is c. 15,000 km<sup>2</sup> (c. 90 x 170 km), a rather small distribution. High densities of this monkey are found close to human habitations, but it seems to be rather rare in undisturbed *terra firme* rain forest far from the banks of rivers and larger creeks, where human settlements are mainly situated. The forests between the lower Rio Aripuanã and Rio Madeira are still in good, almost pristine condition, although logging has taken place along the more navigable rivers, occurring especially when the rivers are high. There are no major towns or cities in the area, except for Manicoré. Novo Aripuanã is located just north of the species' northern limit, and is on the opposite side of the Rio Aripuanã near its mouth. No Indian tribes (which usually hunt even the smallest birds and mammals) live in the area, and the local people (called *caboclos*) are widely scattered and live only in small settlements of one to several families along the major rivers, the Rio Madeira and the Rio Aripuanã, and along the lower courses of a number of minor rivers, such as the Rios Maturá, Uruá, Maripauá, Arauá, Atininga, and the right bank of the Rio Manicoré (Fig. 2). The interfluvial basins of these black and clear-water rivers are practically uninhabited.

The conservation status of *Callithrix acariensis* is unknown, but the interfluvium of the Rios Acari and Sucundurí, where it occurs, though relatively small, is practically uninhabited. The only way to reach the area is by means of a 40 km dirt road which links the town of Novo Aripuanã with the lower Rio Araras, a tributary of the Rio Acari, or by boat entering the Rio Canumã via the Rio Madeira, all the way upriver to the confluence with the Rio Sucundurí and Rio Acari. Since marmosets generally prefer edge habitats and secondary forest on high dry land (*terra firme*), *Callithrix acariensis* is assumed to be relatively rare, considering the fact that there is almost no human habitation in the interfluvium of the Rios Acari and Sucundurí. No major deforestation or logging has been planned for the area, and therefore the monkey is not considered threatened.

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