Göttinger Zentrum
für Biodiversitätsforschung und Ökologie
-Göttingen Centre for Biodiversity and Ecology-

TAXONOMY, PHYLOGENY AND DISTRIBUTION OF TAMARINS (GENUS *SAGUINUS*, HOFFMANNSEGG 1807)

Dissertation zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität zu Göttingen

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Göttingen, Dezember 2010

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Tag der mündlichen Prüfung:

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1 General Introduction

Primates are one of the most diverse orders within the mammals. About 634 taxa are known to science so far (Mittermeier et al., 2009). But the taxonomy of primates is still a field of great discussion and dynamics. Many new species have been described in recent years, others lumped together, because new molecular methods offer new possibilities to review morphological similar taxa on a molecular basis and new species concepts prevailed (Groves, 2004).

As well as the number of newly described primate species increased, the number of primates appearing on the IUCN red list of threatened species increased over the past years. 47.8 % were listed as threatened in the 2008 red list (Mittermeier et al., 2009) Some even face extinction within the near future. The need for space of the exploding human population causes a rapid loss of habitat for primates and other animals and plants as well. In many regions of the tropics hunting for meat is a major threat for the declining numbers of primates (Strier, 2007). So the research on primate diversity more and more gets important in conservation issues.

To contribute to the illumination of primate taxonomy and diversity five research projects have been established by the German Primate Centre (DPZ) and the University of Göttingen, funded by the "WGL Pakt für Wissenschaft und Innovation". The projects are concerned with five primate genera, which are poorly understood in their taxonomy and biogeography: crested gibbons (genus *Nomascus*) and leaf monkeys (genus *Presbytis*) in south-eastern Asia, baboons (genus *Papio*) in southern Africa, sifakas (genus *Propithecus*) in Madagascar and tamarins (genus *Saguinus*) in the Amazon basin of South America. The obtained results of these projects contribute to draw broader picture of primate diversity and evolution and should be directly conducive to conservation matters. This particular study will be concerned with the tamarins (genus *Saguinus*).

1.1 The tamarins of the genus *Saguinus* (Hoffmannsegg 1807)

Tamarins (*Saguinus*) belong to the Callitrichinae, small bodied New World primates, and are one of the most diverse groups of primates. They are distributed throughout northern and western Amazonia up to north-western Colombia and Panama. Tamarins inhabit tropical forests, open woodlands, and secondary growth. The diet of tamarins consists of fruit, sap, nectar, insects and small vertebrates (Garber, 1993). They are diurnal and arboreal. Head and body length is 175-310 mm, tail length is 250-440 mm, and body mass is usually 225-600 grams (Hershkovitz, 1977). Due to their small body size they lost one set of molars. Their nails look like claws, which enable them to climb on trunks and trees like squirrels (Thorndike, 1968). They regularly produce twins, which are raised in a cooperative breeding system, where predominantly the males carry the infants (Rothe and Darms, 1993). The social organization varies from one male- one female to multimale-multifemale groups. Normally each group holds only one reproductive female (Epple et al., 1993; Digby et al. 2007). Group size normally ranges from 2 to 13 (Digby et al., 2007).

1.2 Overview of the current status of tamarin taxonomy

The tamarins (*Saguinus*) belong to the subfamily Callitrichinae, which comprises six other genera: the Goeldi`s monkey (*Callimico*), dwarf marmosets (*Cebuella* and *Callibella*), Atlantic marmosets (*Callithrix*), Amazonian marmosets (*Mico*), and lion tamarins (*Leontopithecus*) (Rylands and Mittermeier, 2009). The positions of *Leontopithecus* and *Saguinus* within the subfamily have not been fully resolved so far; different studies reveal different arrangements, with either *Saguinus* or *Leontopithecus* as basal callitrichines (see Cortés-Ortiz, 2009).

The taxonomy of the genus *Saguinus* is mainly based on the work of Hershkovitz (1977). All of the following studies concerned with tamarin systematics are resting upon his taxonomy based on morphological data, mainly the color of the pelage. He identified 33 different taxa (Tab. 1.1), which are grouped into 10 species and three sections and six species groups (Fig. 1.1).

Table 1.1 The traditional taxonomy of Hershkovitz (1977) compared to a recent taxonomy of Groves (2005). Species divisions are marked with lines.

Hershkovitz 1977	Groves 2005
S. nigricollis **group**	
S. nigricollis nigricollis	*S. nigricollis nigricollis*
	S. nigricollis hernandezi
S. nigricollis graellsi	*S. graellsi*
S. fuscicollis fuscicollis	*S. fuscicollis fuscicollis*
S. fuscicollis fuscus	*S. fuscicollis fuscus*
S. fuscicollis avilapiresi	*S. fuscicollis avilapiresi*
S. fuscicollis cruzlimai	*S. fuscicollis cruzlimai*
S. fuscicollis illigeri	*S. fuscicollis illigeri*
S. fuscicollis leucogenys	*S. fuscicollis leucogenys*
S. fuscicollis nigrifrons	*S. fuscicollis nigrifrons*
S. fuscicollis lagonotus	*S. fuscicollis lagonotus*
S. fuscicollis weddelli	*S. fuscicollis weddelli*
S. fuscicollis primitivus	*S. fuscicollis primitivus*
S. fuscicollis melanoleucus	*S. melanoleucus*
S. fuscicollis crandalli	
S. fuscicollis acrensis	
S. fuscicollis tripartitus	*S. tripartitus*
S. mystax **group**	
S. mystax mystax	*S. mystax mystax*
S. mystax pluto	*S. mystax pluto*
S. mystax pileatus	*S. pileatus*
S. labiatus labiatus	*S. labiatus labiatus*
S. labiatus thomasi	*S. labiatus thomasi*
	S. labiatus rufiventer
S. imperator imperator	*S. imperator imperator*
S. imperator subgrisescens	*S. imperator subgrisescens*
S. inustus **group**	
S. inustus	*S. inustus*
S. midas **group**	
S. midas midas	*S. midas*
S. midas niger	*S. niger*
S. bicolor **group**	
S. bicolor bicolor	*S. bicolor*
S. bicolor ochraceus	*S. martinsi ochraceus*
S. bicolor martinsi	*S. martinsi martinsi*
S. oedipus **group**	
S. leucopus	*S. leucopus*
S. oedipus oedipus	*S. oedipus*
S. oedipus geoffroyi	*S. geoffroyi*

Hershkovitz (1977) divides the genus *Saguinus* into a hairy-faced, mottle-faced and bare-faced section. Within the hairy-faced tamarins he describes three distinct species groups.

The *S. nigricollis* species group includes the black-mantle tamarin *S. nigricollis* with three subspecies and the saddle-back tamarin *S. fuscicollis* with 14 subspecies. The *S. mystax* species group consists of the mustached tamarin *S. mystax* with three subspecies, the red-bellied tamarin *S. labiatus* (two subspecies) and the emperor tamarin *S. imperator* also with two subspecies (Hershkovitz, 1977, 1979). The two subspecies of *S. midas* form the third species group of hairy-faced tamarins. The mottled-face tamarin *S. inustus* was placed in its own section and all remaining species are listed as the bare-faced tamarins containing two species-groups: The *S. bicolor* group, consisting of a single species, the pied tamarin *S. bicolor* (with three subspecies) and the *S. oedipus* group with two species, the cotton-top tamarin *S. oedipus* (two subspecies) and the white-footed tamarin *S. leucopus* (For distribution areas of the species groups see Fig. 1.2).

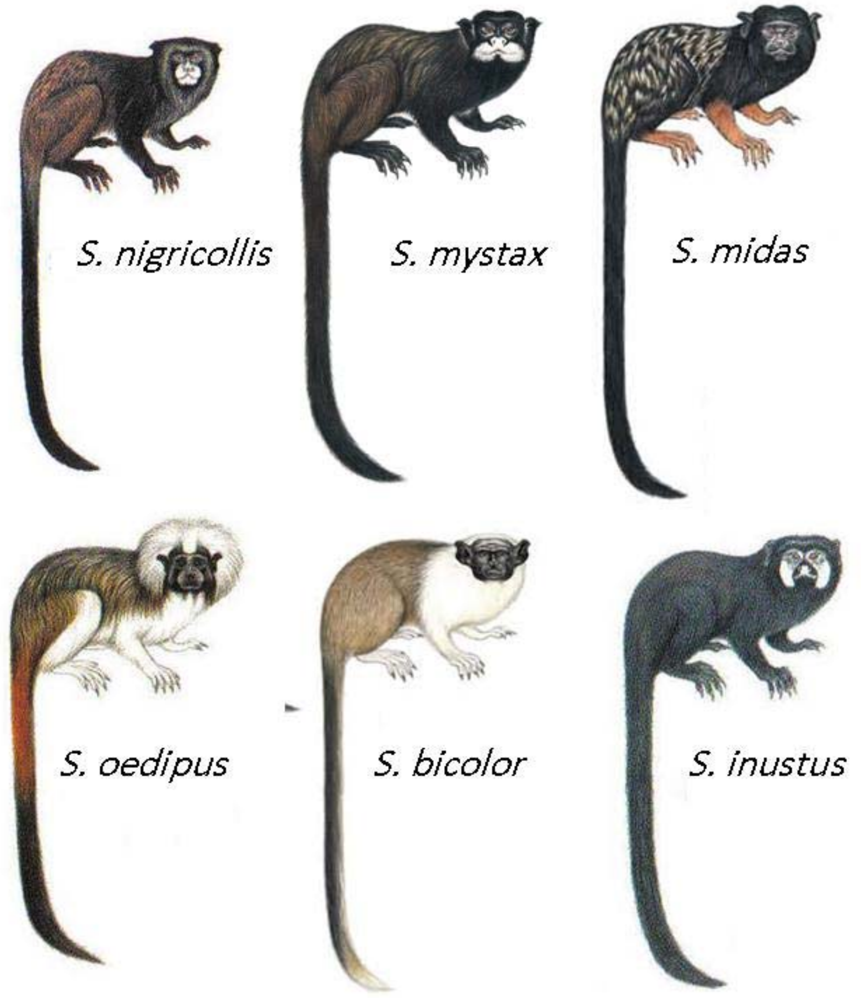

Fig 1.1 The nominate species of the six *Saguinus* species groups. Stephen D. Nash © Conservation International.

This classical tamarin taxonomy was reviewed few times and changed on some aspects (Coimbra-Filho, 1990; Rylands et al. 1993; Rylands et al., 2000; Groves, 2001). Table 1.1 gives an overview of the traditional tamarin taxonomy and the differences to newly dated taxonomy (Groves, 2005).

The interspecific taxonomic relationships of *Saguinus* are quite unsolved. Craniometrical and dental analyses supported the division in the three sections only partially (Natori and Hanihara 1988). The few genetic studies that have been done to clarify the interspecific relationship of *Saguinus* do not support a split into hairy-faced and bare-faced tamarins. Sequence data of the β_2-microglobulin nuclear gene showed that the bare-faced tamarins did not form a single clade (Canavez et al., 1999). Tagliaro et al. (2005) also argue against a monophyletic bare-faced group, on the basis of the ND1 mitochondrial gene. The bare-faced character seems to have evolved more than once within the tamarins (Cropp et al., 1999; Canavez et al., 1999; Tagliaro et al., 2005). Genetic analysis on the basis of mitochondrial DNA supports a division of *Saguinus* into two clades, no matter of hairy- or bare-faced: a small-bodied and a large-bodied clade. The small-bodied clade consists only of the *S. nigricollis* group while all other species form the large-bodied clade (Cropp et al., 1999).

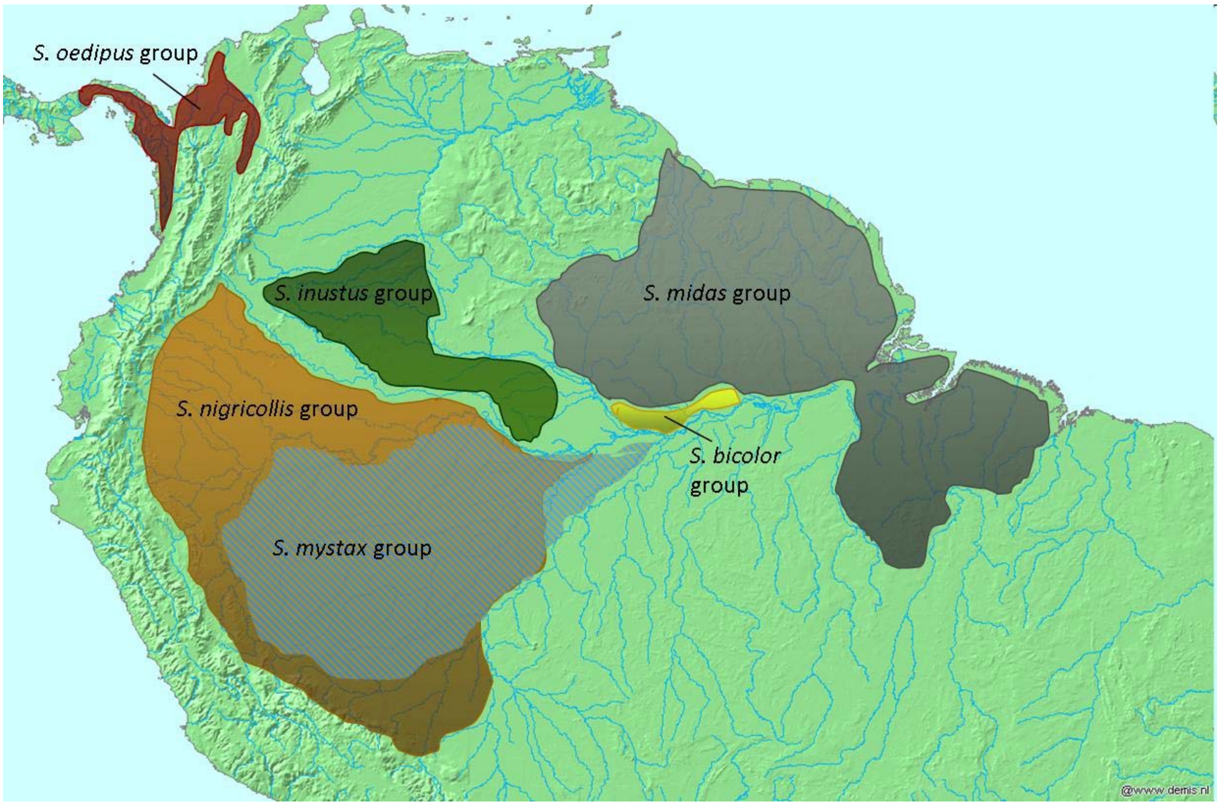

Fig. 1.2. Distribution areas of the six *Saguinus* species groups.

The tamarins of the *S. midas*-group of northeastern Amazonia are quite large, compared to the relatively small tamarins of the *S. nigricollis*-group. Hershkovitz (1977) lists them as two subspecies (*S. m. midas* and *S. m. niger*) of a single species *S. midas*. The taxonomy of *S. midas* as a single species with two subspecies was adopted by Mittermeier (1988) and Coimbra-Filho (1990). But Rylands et al. (2000) and Groves (2001) list them as two distinct species *S. midas* and *S. niger*. Vallinoto et al. (2006) found them distinct on the basis of mtDNA analyses and showed that different populations of *S. niger* have been isolated for a long time as well.

They share some dental and cranial characters, like the reduction of the entoconid on M_1 and the loss of the inferior petrosal sinus foramen, with the so called bare-faced tamarins of the *S. oedipus*- and the *S. bicolor*-group, and exclusively with the *S. bicolor*-group a derived form of the premetacristid on P_2 (Natori, 1988). The *S. bicolor*-group traditionally consisted of one species, the pied tamarin *S. bicolor* with three subspecies (*S. b. bicolor, S. b. martinsi, S. b. ochraceus*) (Hershkovitz, 1977; Coimbra-Filho, 1990; Rylands et al., 1993). In more recent taxonomies, Rylands et al (2000) and Groves (2001) divide them into two species *S. bicolor* and *S. martinsi* (with two subspecies *S. m. martinsi* and *S. m. ochraceus*). Groves (2001) assumes that *S. m. ochraceus* may be indeed a stabilized hybrid form between *S. bicolor* and *S. martinsi*, due to observations on captive hybridization experiments.

The other tamarins, which share the bare-faced character with the *S. bicolor*-group, are grouped into the *S. oedipus*- and the *S. inustus* species groups (Hershkovitz, 1977). The latter consists only of one species, the mottled-face tamarin *S. inustus*. This species is not very well known to science. In morphological regard it seems that this species shares only the derived dental enamel structure with other species groups, in this case with the hairy-faced and *S. bicolor* species groups (Natori and Hanihara, 1988). The only molecular study including *S. inustus* delivered no clear results, because only a small amount of sequence data could be generated for this species (Cropp et al., 1999).

The *S. oedipus* group has the northernmost distribution of all callitrichine primates (Fig. 1.1). Within the *S. oedipus*-group Hershkovitz (1977) originally described two species: *S. leucopus* and *S. oedipus* with two subspecies *S. o. oedipus* and *S. o. geoffroyi*. But most authors have listed the three taxa as three distinct species (Rylands et al., 1993; Rylands et al., 2000;

Groves, 2001). Morphologically they can be distinguished from all hairy-face tamarins by the narrow maxilla (Natori and Hanihara, 1988).

In contrast to the previously described species groups, the representatives of *S. mystax* group are distributed south of the Amazon river. They occur in sympatry with different representatives of the *S. nigricollis*-group almost throughout their range and commonly form stabile mixed-species troops with them (Heymann and Buchanan-Smith, 2000). This species-group is characterized by some specific morphological attributes, like the length of the incisive relative to the width (Natori, 1988). Craniometrical data places them near to the *S. nigricollis*-group (Natori and Hanihara, 1988). In contrast to this, first genetic approaches indicate a closer relationship to the other *Saguinus*-species than to the *S. nigricollis*-group (Cropp et al., 1999; Tagliaro et al., 2005). Traditionally the *S. mystax*-group consists of the mustached tamarin *S. mystax* with three subspecies, the white-lipped or red-bellied tamarin *S. labiatus* with two subspecies and the emperor tamarin *S. imperator* with also two subspecies (Hershkovitz, 1977, 1979). Within *S. mystax* the distinction between the different subspecies is not very clear. Two of the three traditionally featured subspecies, *S. m. pluto* and *S. m. pileatus*, are only known from few museum specimens, with partly uncertain localities. So, the taxonomic status of these tamarins is as cryptic as their distribution areas. Groves (2001) argues to give full-species rank to the red-capped tamarin *S. pileatus*, which is featured as a subspecies of *S. mystax* by other authors (Hershkovitz, 1977; Rylands et al., 1993). Traditionally two subspecies of the white-lipped tamarin *S. labiatus* are recognized (Hershkovitz, 1977; Coimbra-Filho, 1990; Rylands et al., 1993). But some evidence is given by examination of the color pattern of the crown spots of museum specimens, that there may be another subspecies *S. l. rufiventer* (Groves, 2001; Rylands et al., 2000). The subspecies *S. l. thomasi* was long time only known from the type locality, which is rather distant to the distribution areas of the other white-lipped tamarins. It was also described north of the Amazon river, but remains cryptic (Hershkovitz, 1977; Silva 1988). Within the *S. mystax*-group the two subspecies of the emperor tamarin *S. imperator* seem only to be distantly related to the other taxa, regarding craniometrical data (Hanihara and Natori, 1989).

The species group on which will be given the main emphasis in this study is the *S. nigricollis*-group, which is the most widely distributed and most diverse group (Fig. 1.3). Hershkovitz (1977) grouped the 17 taxa into two species, the black-mantled tamarin *S. nigricollis* (bizonal color pattern) and the saddle-back tamarin *S. fuscicollis* (trizonal color pattern). He describes 14 taxa of saddle-back tamarins, different in color pattern and distribution, which he all considers as subspecies of one wide-ranging species *S. fuscicollis*. He explains the separation, as well as the radiation of the different subspecies, with the theory of metachromism based on the coat coloration (Hershkovitz, 1968, 1977). This theory of reconstructing mammal radiations by means of bleaching and saturation of coat coloration was already highly disputed decades ago (Lawlor, 1969).

The different subspecies of *S. fuscicollis* are, in most cases, clearly separated by river channels, where they often occur within sight and calling distance to each other on opposite banks of a river (Hodun, 1981) (Fig. 1.3). Whereas relative agreement between the different authors exists with regard to the number of different taxa within the saddle-back tamarins, the status of the different taxa as species or subspecies is fairly controversial. Some taxa have only been described from very few museum specimens, with an often doubtful location, like *S. f. primitivus* and *S. f. cruzlimai.* Their distribution areas were placed by Hershkovitz only due to some uncertain information of the type localities. For many interfluvial areas is not even known which type of tamarins exists. One of these areas is the large area between Rio Napo and Rio Putumayo in Peru, for example. Recently a new taxon, *S. f. mura,* was described in Brazil (Röhe et al., 2009).

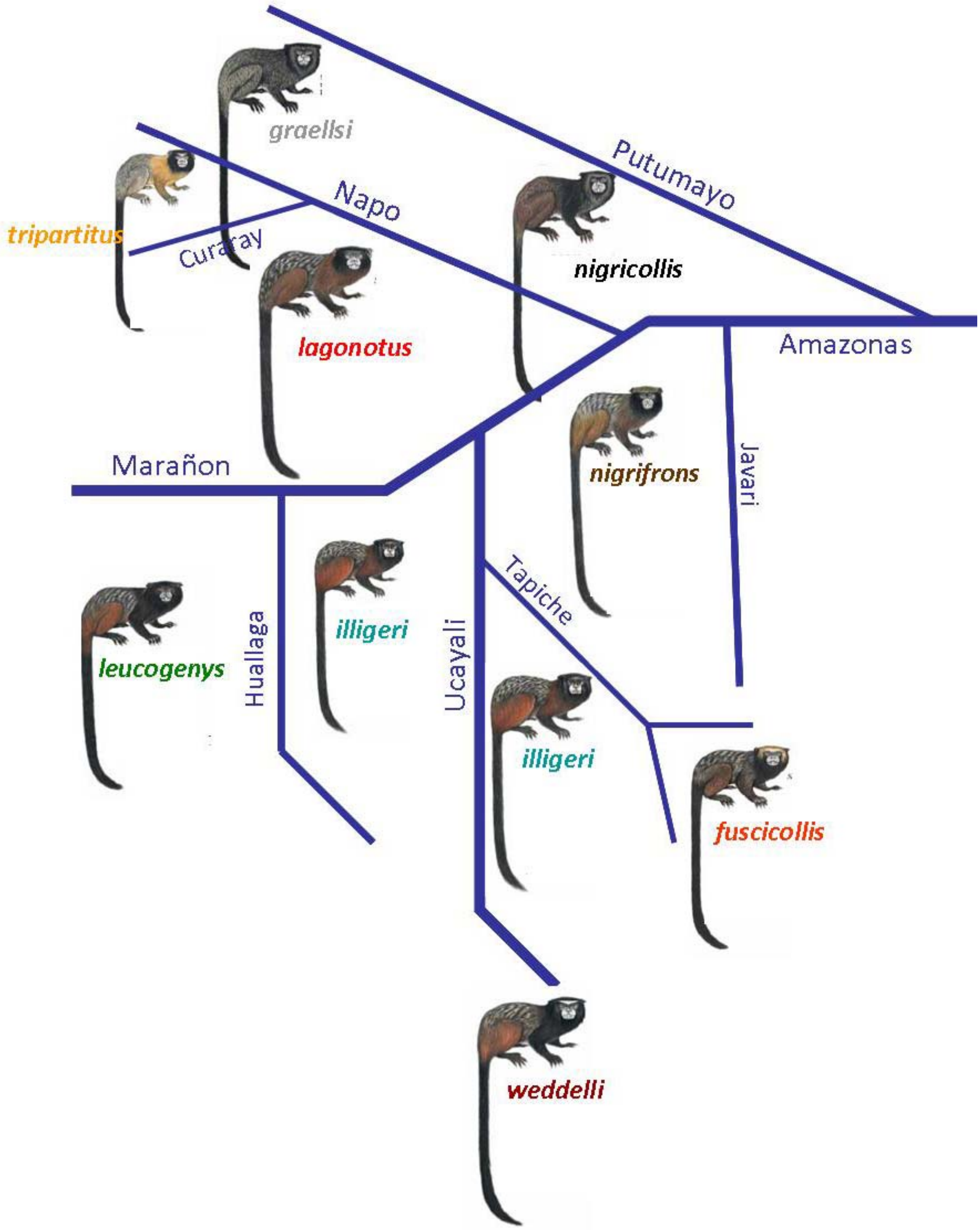

Fig. 1.3 The Peruvian taxa of the *S. nigricollis* group and their schematic distribution between the major rivers. Tamarin drawings by Stephen D. Nash © Conservation International.

The golden-mantle tamarin *S. tripartitus*, considered as a subspecies of *S. fuscicollis* by Hershkovitz, was given full species rank by other authors referring on the grounds of supposed sympatry with *S. f. lagonotus* (Thorington, 1988). From the morphological and ecological point of view, the differences between *S. tripartitus* and *S. f. lagonotus* seem not to be bigger than differences between other *S. fuscicollis* subspecies, which means that, if *S. tripar-*

titus is considered as a distinct species, other, perhaps all others, subspecies of *S. fuscicollis* should be treated as species as well (Heymann, 2000).

Another point of discussion within the *S. fuscicollis* complex is the taxonomic status of *S. f. melanoleucus* which is distributed between the Rio Jurua and Rio Tarauacu in Brazil. First Coimbra-Filho (1990) listed *S. melanoleucus* as a distinct species, with *S. f. acrensis* and *S. f. crandalli* as subspecies of *S. melanoleucus*. In the headwater region of the Rio Jurua there is hybridization with *S. f. fuscicollis*, which lead to intermediate geno- and phenotypes on the left bank of the headwater stream (Peres, 1996). Peres also assume that the *acrensis* pelage as well as the *crandalli* pelage could represent only color morphs of *S. melanoleucus*, which, thus, would be a monotypic species. The *crandalli* morph could also be the result of hybridization between *S. melanoleucus* and *S. f. fuscicollis* (Rylands et al., 2000).

The black-mantle tamarin is listed by Hershkovitz (1977) as one species *S. nigricollis* with the three subspecies *S. n. nigricollis, S. n. hernandezi, and S. n. graellsi*. The status of the latter is subject to discussions whether it should be elevated to full-species rank. This assumption is based on the assertion of *S. graellsi* living sympatric with *S. nigricollis* in southern Colombia (region of Puerto Leguizamo) (Hernandez-Camacho and Cooper, 1976). *S. graellsi* is featured as a distinct species by Rylands et al (2000) and Groves (2001). In genetic studies *S. f. fuscus* showed more similarities with *S. nigricollis* than with other *S. fuscicollis* subspecies (Cropp et al., 1999). Moore and Cheverud (1992) suggested the elevation of this subspecies to full species rank, due to differences in facial morphology.

In summary, the status of *S. fuscicollis* and *S. nigricollis* as monophyletic species can be considered as quite questionable.

1.3 Geographic origin and dispersal of *Saguinus*

The radiation and dispersal of the Callitrichinae throughout the Amazon basin remains a point of great discussion and is still quite enigmatic. In this perspective *Saguinus* appears to be one of the most complicated genera (Natori and Hanihara, 1988). Little fossil evidence is available for callitrichine primates (Cropp et al., 1999), The few fossils assigned to the Callitrichinae are highly disputed, such as the often highlighted *Lagonimico* from the La Venta formation in Colombia (Middle Miocene), described as “giant tamarin” by Kay (1994), but considered as pithecine by Rosenberger (2002). Another fossil from La Venta, *Mohanamico*, is considered as callimiconin callitrichine (Rosenberger et al., 1990). So theories for the biogeographic history of *Saguinus* mainly depend on morphological, molecular and behavioral evidence. To understand the biogeography of this genus, different approaches, amongst which is the use of molecular and divergence age estimates, should be combined with available morphological and behavioral information and with general models of speciation processes in Amazonia and the geological history of the region.

So far, there are basically two different theories for the phylogeographic origin of the genus *Saguinus*, and how they dispersed across their current range. One is given by Hershkovitz (1977), the other by Ferrari (1993). Both are based on the assumption of Pleistocene refugias as origins of dispersal. The problem with these two models is that they argue in complete contrary directions. Hershkovitz` theory of the *Saguinus* radiation is based on the current distribution of *Saguinus* and the theory of metachromism. He regards a larger body size as a derived character. According to that, the ancestral tamarin was a rather small animal with an agouti pelage. Due to this, he considers the recent *S. nigricollis* as the most basal of the living tamarins, with the agouti-colored *S. n. graellsi* as “a relict near to the ancestral type” (Hershkovitz, 1977). So Hershkovitz postulates the range of the ancestral tamarin within the range of the *S. nigricollis*- species-group in the western Amazon basin. The origin of tamarin dispersal would start in southern Peru and northern Bolivia along the eastern Andean base. From these refugia Hershkovitz suggests two dispersal routes, one northwards leading to the *S. inustus* and *S. oedipus* species groups and another northeastern route, leading to the *S. bicolor* and *S. midas* species groups.

Ferrari (1993) contradicts Hershkovitz` theory by recurring to the phyletic dwarfism hypothesis (Ford, 1980) to explain the callitrichid radiation. The key words in this hypothesis are body size and ecology. He describes the ancestral callitrichid as a relative large monkey, not very specialized in its ecology and especially feeding habits. Thus, the small-bodied forms (i.e. *Cebuella*) would be the most derived, and not the most primitive as Hershkovitz argues. Also, the gum-feeding specialization in marmosets is seen as a highly derived character. Within *Saguinus* he describes the small-bodied and highly gum-feeding *S. fuscicollis* as the most derived form and the larger-bodied and probably least gummivorus *S. midas* as the most primitive one (Ferrari, 1993). So Ferrari claims that the initial radiation of the tamarins is represented by the *S. midas/S. bicolor* group. The second tamarin radiation could have been the mustached tamarins and the third the saddle-back tamarins. Small size, increased gummivory and specialized foraging behavior of the saddle-back tamarins (*S. fuscicollis*) could be explained by the selective pressure exerted by members of the mustached group, living sympatric with *S. fuscicollis* in most parts of their range. Ferrari proposes the origin of the genus within the current distribution of *S. midas* in the northeast of the Amazon basin. From this region north of the Amazon river and east of the Rio Negro he suggests different dispersal route in all directions, with one southwestern route which lead to the *S. mystax* and *S. nigricollis* species groups.

Only few studies have been published to contribute to this discussion. However, the radiation of *Saguinus* is still far from solved and also the mechanisms of speciation processes remaining quite ambiguous. Only few molecular genetic studies in the past were concerned with the taxonomy and biogeography of the tamarins. Most of them were based on museum specimens and captive animals, which often cannot be assigned to a certain geographic origin. This is the first comprehensive molecular assessment of tamarin taxonomy and phylogeny using geo-referenced samples from wild tamarin populations.

1.4 Specific questions

The current study was designed to re-examine tamarin evolution, taxonomy and biogeography. We conducted two comprehensive field expeditions throughout the Peruvian Amazon of in total 10 month. For the genetic analysis we collected over 100 samples of wild tamarins from 29 locations covering 12 of 13 taxa, described for the country. Furthermore we collected data on distribution areas, barriers or possible sympatry. Interviews with local hunter using pictures and drawings provided reliable information on the local primate fauna.

Phylogenetic relationships within the genus *Saguinus*:

- What is the position of *Saguinus* within the Callitrichinae?
- How are the phylogenetic relationships among the different tamarin species groups?
- Was the ancestral tamarin more similar to the *S. nigricollis*- or to the *S. midas*-species group or has another model to be developed?
- Which taxonomic implications can be made on the genus and subgenus level?

Phylogeny of the *S.nigricollis* species group:

- Are *S. fuscicollis* and *S. nigricollis* monophyletic species?
- Can we delimitate species within the diverse *S. nigricollis* group?
- What is the taxonomic status of the proposed species *S. graellsi, S. tripartitus* and *S. melanoleucus,* and their relationship to the remaining tamarin taxa?
- Can we detect any sympatry between taxa of the *S. nigricollis* group?

Biogeography of *Saguinus*:

- What is the geographic origin and direction of the *Saguinus* radiation?
- Can we find eventual coincidence with palaeogeographic history of the Amazon basin?
- Did they cross the main Amazon channel or was it more or less a parallel dispersal north and south of the Amazon?

These questions are addressed in the current study. The structure of the study comprises three main parts.

The first part (**chapter 2**) starts with a broader perspective, illuminating the phylogenetic relationships within the Callitrichinae on a broader scale, including nearly all genera and species groups. Special emphasis in this part is given the position of the different *Saguinus* species groups within the callitrichine tree and the comparison of divergence ages between marmosets and tamarins. Therefore the sequence data of whole mitochondrial genomes is used.

The second part (**chapter 3**) goes more into detail by focusing on the *S. nigricollis* species group, which is the most diverse group and with its numerous taxa, subspecies and/or color variants and cryptic distribution areas surely one of the most complicate genera of New World primates. More variable markers and samples from 100 wild tamarins with known origin are used to define clusters and lineages, which provide a basis for species delimitations and a taxonomic revision of the species group.

The third part (**chapter 4**) is bringing together the information on distribution areas, distribution limits and sympatry of certain taxa of the *S. nigricollis* group, obtained during our extant field surveys in the Peruvian Amazon, with the evidence of other field researchers and the current knowledge provided by museum specimens and historic reports. This provides a comprehensive overview over the distribution areas and possible sympatry of at least four tamarin taxa, which are crucial to understand the taxonomy of this species group.

2 Complete mitochondrial genome data reveal the phylogeny of callitrichine primates and a late Miocene divergence of tamarin species groups

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For submission to: PLos ONE

Abstract

The Callitrichinae are small bodied New World primates, including two of the most diverse groups of primates, the marmosets (genera *Callithrix* and *Mico*) and tamarins (genus *Saguinus*). The phylogenetic relationships within this subfamily and in particular the phylogeny of the six species groups of tamarins (*Saguinus*) and their position within the Callitrichinae is disputed. To further address these issues, we sequenced 13 complete mitochondrial genomes of all callitrichine genera and nearly all *Saguinus* species groups. Based on our phylogenetic reconstructions, *Saguinus* branched off first among Callitrichinae, followed by *Leontopithecus.* Among the remaining genera, *Callimico* is basal, and *Mico* and *Cebuella* form sister genera to the exclusion of *Callithrix*. The genus *Saguinus* is further divided into various, unexpectedly old lineages. We found much older and deeper phylogenetic splits between different species groups of *Saguinus* than between the different genera of marmosets. The *S. nigricollis* group split off from other tamarins in the late Miocene around 9.5 mya. The other species groups diverged between 7.5 and 5.3 mya. The taxonomic nomenclature of the different species groups should be revised and we recommend an elevation to generic level for at least the *S. nigricollis* species group. We bring our data together with the current standard of knowledge of Amazonian geology. Our data support a West-Amazonian origin and an eastward dispersal of the genus *Saguinus*.

2.1 Introduction

The Callitrichinae are small bodied New World primates, including two of the most diverse groups of primates, the marmosets (genera *Callithrix* and *Mico*) and tamarins (genus *Saguinus*). Rosenberger (1981) grouped them as subfamily Callitrichinae into the family Cebidae together with the subfamily Cebinae. Rylands and Mittermeier (2009) keep them as family Callitrichidae, rather than subfamily Callitrichinae. Seven genera are currently recognized: Goeldi`s monkey (*Callimico*), dwarf marmosets (*Cebuella* and *Callibella*), Atlantic marmosets (*Callithrix*), Amazonian marmosets (*Mico*), tamarins (*Saguinus*) and lion tamarins (*Leontopithecus*) (Rylands and Mittermeier, 2009).

In the last two decades analyses of DNA sequence data have significantly increased our understanding of the phylogenetic relationships among New World primates. The phylogenetic relationships within the Callitrichinae on the genus level have been addressed in several studies and are quite good resolved on generic level. Only the position of *Leontopithecus* and *Saguinus* has not been fully resolved so far; different studies reveal different arrangements, with either *Saguinus* or *Leontopithecus* as basal callitrichines (see Cortés-Ortiz, 2009). Meanwhile the long-time debate about the position of the monotypic genus *Callimico* is almost completed. Molecular data strongly support a sister relationship between *Callimico* and the marmosets (Cronin and Sarich, 1975; Seuanez et al., 1989; Schneider et al., 1993; Pastorini et al., 1998; Chaves et al., 1999; Canavez et al., 1999).

The internal phylogeny of the marmosets is less clearly resolved. A number of studies showed a closer phylogenetic relationship between *Cebuella* and the Amazonian marmosets than between Amazonian and Atlantic marmosets, and therefore support an own genus for the Amazonian marmosets (*Mico*) (Rosenberger, 1981; Barroso et al., 1997; Porter et al., 1997; Tagliaro et al., 1997). Groves (2001, 2005) lists *Cebuella, Callibella* and *Mico* as subgenera of *Callithrix*.

In contrast to the marmosets, the phylogeny of the tamarins has been widely neglected. The current classification of the genus *Saguinus* is mainly based on studies of Hershkovitz (1977) who identified 33 taxa, grouped into six species groups. He divided the species groups into three sections: hairy-faced (*S. nigricollis* group, *S. mystax* group), mottle-faced *(S. inustus*) and bare-faced tamarins (*S. midas* group, *S. oedipus* group, *S. bicolor* group). The few genetic studies that have been conducted to clarify the interspecific relationship of *Saguinus* do not support a split into hairy-faced and bare-faced tamarins (Cropp et al., 1999; Canavez et al., 1999; Tagliaro et al., 2005). They rather support a division of *Saguinus* into two clades, no matter of hairy- or bare-faced: a small-bodied and a large-bodied clade. The small-bodied clade consists only of the *S. nigricollis* group, while all other species form the large-bodied clade (Cropp et al., 1999). With 16-17 taxa the *S. nigricollis* species group is the most diverse of all *Saguinus* species groups (Hershkovitz, 1977).

Hershkovitz (1977) proposed the small-bodied *S. nigricollis* group as the most primitive one and concluded that the origin of the entire genus lies in western Amazonia within the current distribution area of this species group. In contrast, Ferrari (1993) regarded them as most derived and recognized the relatively large-bodied *S. midas* group as the ancestral tamarins with a north-eastern Amazonian origin.

Most of the described species groups of *Saguinus* are confirmed in their monophyly, but their phylogenetic relationships are not fully resolved and also serious estimations of divergence ages are still lacking. This study includes all genera and subgenera of the Callitrichinae (except for the dwarf marmoset *Callibella*), and nearly all species groups of *Saguinus* (except *S. inustus*). The use of whole mitochondrial genomes provides a solid basis for phylogenetic reconstructions and in particular (1) illuminating the phylogenetic relationships between different *Saguinus* species groups, (2) examining their phylogenetic age, also in comparison with the different genera of marmosets and (3) reconstructing the biogeographic history of the genus *Saguinus*.

2.2 Methods

2.2.1 Laboratory methods

Blood, tissue or fecal samples from representatives of six callitrichine genera as well as from several outgroup taxa were obtained from specimens kept in zoos or breeding facilities, or collected in the field (Table 2.1). Sample collection was conducted according to relevant German and international guidelines, including countries where we collected samples. Faecal samples were collected in a non-invasive way without disturbing, threatening or harming the animals. Blood samples were taken by veterinarians for diagnostic reasons to check the health status of the respective individuals, and tissue samples were obtained only from deceased specimens. Total genomic DNA was extracted with the DNeasy Blood & Tissue or QIAamp DNA Stool Mini kits from Qiagen. Extractions followed standard protocols as recommended by the supplier, with the exception that the DNA was diluted in HPLC quality water and stored at -20 °C before further processing.

To reduce the likelihood of amplifying nuclear pseudogenes (numts), complete mitochondrial genomes from 13 species (*Saguinus fuscicollis weddelli, Saguinus nigricollis nigricollis, Saguinus midas, Saguinus bicolor, Saguinus oedipus, Saguinus labiatus labiatus, Mico argentatus, Callithrix geoffroyi, Callimico goeldii, Cebuella pygmaea, Leontopithecus rosalia, Aotus azarae, Saimiri boliviensis*) were generated following an approach in which overlapping fragments were amplified via long-range PCR (Sterner et al., 2006). For high-quality material, we performed two long-range PCRs, each with a length of ~10 kb long, while for DNA extracted from feces, up to four ~5 kb long fragments were generated.

All long-range PCRs were performed with the SuperTaq Plus polymerase from Ambion following protocols of the supplier. Long-range PCR amplicons were separated on 1% agarose gels, excised from the gel, purified with the Qiagen Gel Extraction kit and used as template for nested PCRs. PCR conditions for all nested PCR amplifications (for primers and amplified fragments see Supplementary Table 2.1 and Supplementary Figure 2.1) were identical and comprised a pre-denaturation step at 94°C for 2 min, followed by 30 cycles each with denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min. At the end, a final extension step at 72°C for 5 min was added. Nested PCR products (800 - 1,200 bp in length) were cleaned with the Qiagen PCR Purification kit and sequenced on an ABI 3130xl sequencer using the BigDye^{TM}Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). The results of the PCR amplifications were checked by running an aliquot on a 1 % agarose gel, stained with ethidium bromide. PCR products were purified using Montage^{TM}PCR Centrifugal Filter Devices (Millipore) following the delivered instructions. For one of the fragments in *Callimico goeldii*, the PCR product was cloned into a pGEM vector (pGEM^{TM}-T Easy Vector System I, Promega), averaging 24 clones. Both strands of the PCR products were sequenced, using the respective primer pair used for amplification and standard vector primers M13F and M13R for the cloned products. To prevent cross-species contamination, we followed standard methods as described in Roos et al. (2008). Direct sequencing revealed no multiple peaks or inconsistent positions in overlapping fragments and, thus, numts are most likely not included in our data set. Moreover, all protein-coding regions were correctly transcribed. Newly generated sequences will be deposited at GenBank and available under accession numbers XX-XX (Table 2.1).

Table 2.1 Origin of the samples and GenBank accession numbers of complete mitochondrial genome sequences from studied callitrichine and other primates.

Taxon	Origin/GenBank accession no.
Callimico goeldii	Mulhouse Zoo, France
Callithrix geoffroyi	Dresden Zoo, Germany
Mico argentatus	Romagne Zoo, France
Cebuella pygmaea	Mulhouse Zoo, France
Saguinus oedipus	German Primate Center
Saguinus bicolor	Mulhouse Zoo, France
Saguinus midas	Mulhouse Zoo, France
Saguinus labiatus labiatus	Pando Dept., Bolivia
Saguinus nigricollis nigricollis	Peru, Rio Apayacu
Saguinus fuscicollis weddelli	Peru, Rio Tambopata
Leontopithecus rosalia	Krefeld Zoo, Germany
Aotus azarae	Romagne Zoo, France
Saimiri boliviensis	Nuremberg Zoo, Germany
Cebus albifrons	AJ309866
Ateles belzebuth	FJ785422
Callicebus donacophilus	FJ85423
Papio hamadryas	NC_001992
Theropithecus gelada	FJ785426
Macaca fascicularis	FJ906803
Pan troglodytes	CHPMTB
Homo sapiens	AF346963

2.2.2 Phylogenetic analysis

Sequences were assembled and manually edited using GENEIOUS 4.8 (Drummond et al., 2009). For statistical analysis, additional sequences deposited in GenBank were included in our data set. These comprised the platyrrhines *Cebus albifrons, Ateles belzebuth* and *Callicebus donacophilus* and the catarrhines *Macaca fascicularis, Papio hamadryas, Theropithecus gelada, Pan troglodytes* and *Homo sapiens* which were used as outgroup. The final alignment comprised 21 complete mitochondrial genome sequences. Alignments were conducted with ClustalW as implemented in GENEIOUS 4.8 and corrected by eye. Poorly aligned positions and indels were eliminated using GBLOCKS 0.91b (Castresana, 2000). Therefore, a relaxed selection of blocks was used (Talavera and Castresana, 2007). After the elimination of these sites and also of the control region, the original alignment with a length of 16,752 bp was reduced to 14,263 bp (data set *mt 1*). All analyses were conducted also with a second data set, including only protein coding genes of the heavy strand (data set *mt 2*). The final alignment length of this data set was 10,810 bp. Phylogenetic trees were calculated for all data sets with a maximum-likelihood (ML) and a Bayesian approach. For both analyses, the GTR+I+G model was selected as the optimal nucleotide substitution model with jMODELTEST 0.1.1 (Posada, 2008) using the Akaike Information Criterion (AIC). ML trees were constructed with GARLI 0.96.win23 (Zwickl, 2006). 500 pseudoreplications were conducted to estimate ML bootstrap percentages. A 50%-consensus tree was calculated in PAUP 4.0b10 (Swofford, 2002). Bayesian analyses were carried out in MrBAYES v3.1.2 (Hulsenbeck et al., 2001; Ronquist and Hulsenbeck, 2003). In each analysis, four Monte Carlo Markov chains (MCMC) with a default temperature of 0.2 and a chain length of 10,000,000 generations were performed. Trees and parameters were sampled every 1,000 generations. 2,500 of the sampled trees were discarded as burn-in. Obtained phylogenetic trees were visualized and edited using FigTree v1.3.1 (Rambaut, 2006).

2.2.3 *Estimations of divergence ages*

Calculations of divergence ages were carried out using a Bayesian approach implemented in the programme BEAST (Drummond and Rambaut, 2007). We used a GTR model with a gamma distributed rate variation between sites and invariant sites as optimal nucleotide substitution model. The model was chosen by jMODELTEST 2.2 using Akaike Information Criterion (AIC) (Posada, 2008). For the analysis, we used a relaxed lognormal clock model of lineage variation, and for branching rates, a Yule process as prior was assumed. Since the estimation of phylogenetic relationships was not the main aim of this analysis, we used an a-priori fixed tree topology as obtained from phylogenetic reconstructions and published data for taxa other than *Saguinus* (Osterholz et al., 2009). Analyses were run for 25,000,000 generations, with tree and parameter sampling every 1,000 generations, of which 10% were discarded as burn-in. The adequacy of a 10% burning and convergence of all parameters were assessed by visual inspection of the trace of the parameters across generations using the software TRACER v1.3 (Rambaut and Drummond, 2005). Subsequently, the sampling distributions of multiple independent replicates were combined using the software LogCombiner v1.4.6 and then summarized and visualized using the software TreeAnnotator v1.4.6. Both programs are part of the Beast package (Drummond, 2007). For the Bayesian MCMC method we used a relaxed lognormal clock model of lineage variation and for branching rates we assumed a Yule Process as prior.

Four calibration points based on well-documented fossils were used. The split between *Pan* and *Homo* was dated about 6-7 mya ago (Brunet et al., 2002, Vignaud et al., 2002) and between *Theropithecus* and *Papio* at about 3.5-4.5 mya ago (Leakey, 1993; Delson et al., 2000). For the initial split between platyrrhines and catarrhines Poux et al. (2006) give a range from about 34-40 mya. Within platyrrhines, the fossil record is rather scarce. For a fourth calibration point within the platyrrhine lineage we used the split between *Saimiri* and *Cebus*, which was dated by Lavergne et al. (2010) at about 16 mya, with a upper limit of 20 mya, marked by *Dolichocebus* (Szalay & Delson, 1979) and a lower limit of about 12 mya, resulting from *Neosaimiri,* dated by Stirton (1951).

Instead of hardbound calibration points, we used published dates as a normal distribution prior for the respective node. For C1 (*Homo-Pan*) this translates into a normal distribution with a mean of 6.5 mya and a standard deviation (SD) of 0.31, for C2 (*Theropithecus-Papio*) into a mean of 4 mya and a SD of 0.31, for C3 (Catarrhini-Platyrrhini) into a mean of 37 mya and a SD of 1.8 and for C4 (*Saimiri-Cebus*) a mean of 16 mya and a SD of 2.0.

2.3 Results

We successfully sequenced mitochondrial genomes from 13 cebid primates (*Saguinus fuscicollis, S. nigricollis, S. midas, S. labiatus, S. oedipus, S. bicolor, Callithrix geoffroyi, Mico argentatus, Cebuella pygmaea, Callimico goeldii, Leontopithecus rosalia, Saimiri boliviensis, Aotus azarae*). All of them show the typical organization of mammalian mitochondrial genomes, including 13 protein-coding genes, two rRNA genes, 22 tRNAs and the control region.

2.3.1 Phylogenetic reconstructions

Maximum-likelihood and Bayesian reconstructions reveal identical tree topologies (Fig. 2.1). All splits within the Callitrichinae are very well supported by strong bootstrap values and posterior probabilities for all nodes in both data sets. The only exception is the split between *Leontopithecus* and the marmoset/*Callimico* clade, which is only weekly supported by ML bootstrap values (Fig. 2.1). Within the platyrrhines *Callicebus* as representative of the Pitheciidae family branches off first, followed by *Ateles* as representative of the Atelidae family. Among Cebidae, *Cebus* and *Saimiri* cluster together to the exclusion of *Aotus*. The Callitrichinae form a monophyletic group with a basal position of *Saguinus* and *Leontopithecus* branching off as second lineage. *Callimico, Cebuella, Callithrix* and *Mico* show a close relationship. Within the marmosets, *Cebuella* is related with the Amazonian marmosets of the genus *Mico*. Within *Saguinus* we find a well supported deep initial split between the small-bodied *S. fuscicollis* and *S. nigricollis* on the one hand and all other larger-bodied representatives of the *S. mystax-, S. bicolor-, S. midas*- and *S. oedipus* group on the other hand. Within the large-bodied clade we find a separation of South Amazonian (*S. mystax* group) and North Amazonian (*S. bicolor-, S. midas*- and *S. oedipus* group) lineages. *S. labiatus* (representing the *S. mystax* group) branches off first, while *S. midas* and *S. bicolor* form a clade to the exclusion of *S. oedipus*.

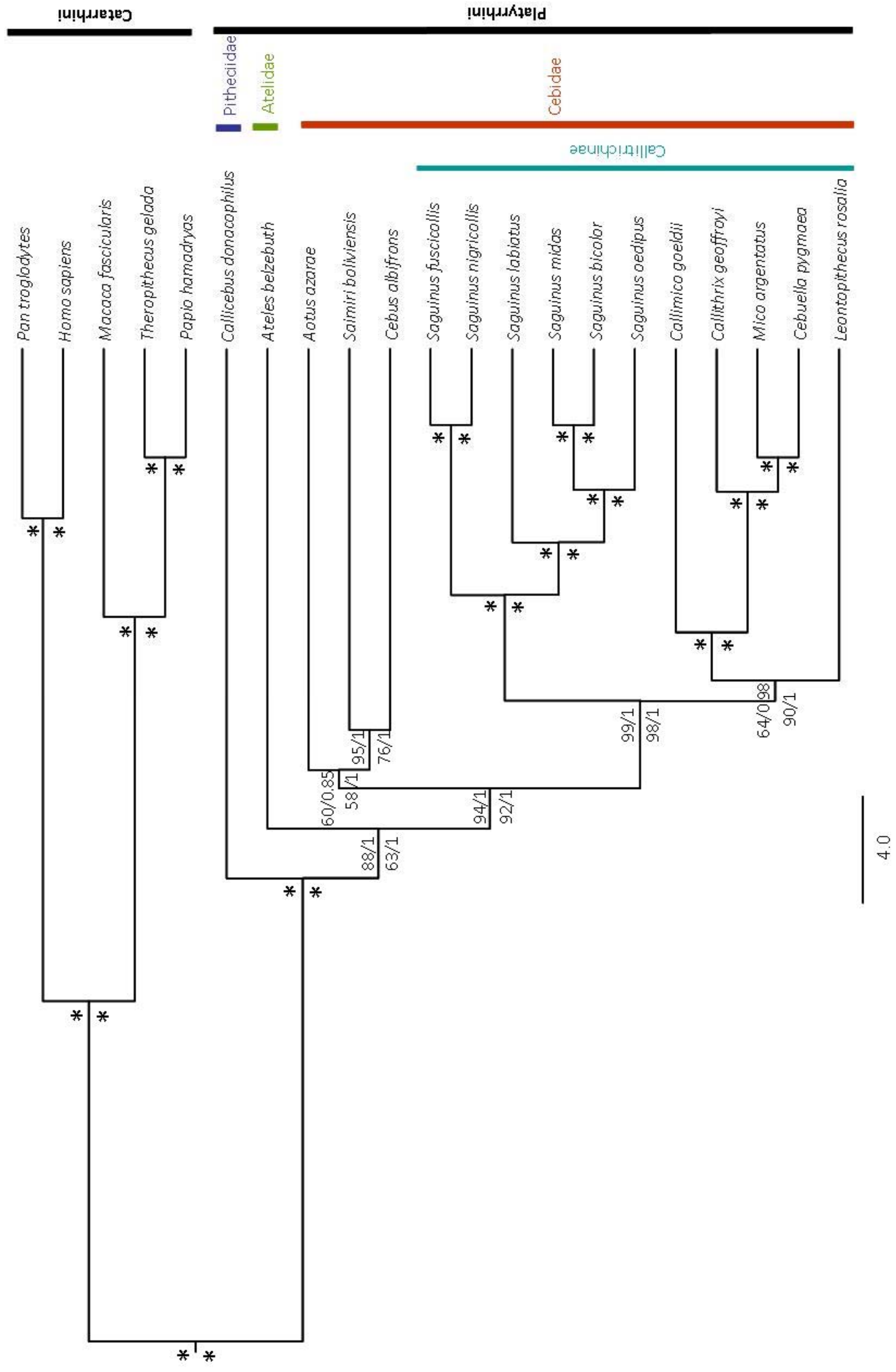

Fig. 2.1 Phylogram based on the 21 mitochondrial genomes, as revealed by Bayesian analysis. Bootstrap values for ML are indicated together with posterior probabilities for the Bayesian approach (ML/Bayesian) for data set *mt 1* above branches and for data set *mt 2* below branches (*: 100/1).

2.3.2 Estimates of divergence ages

According to divergence age estimations (Fig. 2.2, Table 2.2), the split between platyrrhines and catarrhines is dated at 37.4 mya (C1) (for 95% credibility intervals see Table 2.2), the split between *Homo* and *Pan* at 6.39 mya (C2) and between *Theropithecus* and *Papio* at 4.0 mya (C3). Among platyrrhines, the initial split separating *Callicebus* from the other platyrrhines occurred 20.24 mya (N3). *Cebus* + *Saimiri* split 14.4 mya (C4). Within the Callitrichinae *Saguinus* branched off first, around 13.29 mya (N7). The initial division within *Saguinus* occurred 9.2 mya (N12). This division separated all members of the *S. nigricollis* group from the large-bodied tamarins. *S. nigricollis* and *S. fuscicollis*, here represented by *S. f. weddelli* diverged 2.86 mya (N13). Among the large-bodied tamarins, the mustached tamarin species group (*S. labiatus*) split from *S. oedipus, S. midas* and *S. bicolor* shortly after the initial *Saguinus* division 7.3 mya (N14). The separation between *S. oedipus* and the *S. midas/S. bicolor* clade occurred 5.32 mya (N15) and the split between the latter two around 2.87 mya (N16).

Table 2.2 Bayesian divergence age estimates in mya*.

Node	mean	95% credibility interval
C1 Catarrhini – Platyrrhini	37	34.14 – 40.63
N1 Hominoidea (*Homo* + *Pan*) – Cercopithecoidea (*Macaca* + *Papio*	24.59	19.55 – 30.12
C2 *Homo – Pan*	6.39	5.79 – 6.98
N2 *Macaca-Theropithecus + Papio*	10.11	7.6 – 12.82
C3 *Theropithecus – Papio*	4.0	3.54 – 4.66
N3 Pitheciidae – Cebidae + Atelidae	19.97	16.87 – 23.52
N4 Atelidae – Cebidae	18.11	15.29 – 21.1
N5 Callitrichinae – *Cebus + Saimiri + Aotus*	16.58	14.08 – 19.32
N6 *Aotus – Cebus + Saimiri*	15.9	13.45 – 18.59
C4 *Saimiri – Cebus*	14.4	11.97 – 16.88
N7 *Saguinus– Leontopithecus + Callimico + Callithrix + Mico +*	13.29	11.14 – 15.65
N8 *Leontopithecus – Callimico + Callithrix + Mico + Cebuella*	12.49	10.35 – 14.76
N9 *Callimico – Callithrix + Mico + Cebuella*	10.63	8.58 – 12.87
N10 *Callithrix – Mico + Cebuella*	5.3	3.81 – 7.01
N11 *Mico – Cebuella*	4.0	2.72 – 5.53
N12 *Saguinus*	9.2	7.44 – 11.17
N13 *S. nigricollis – S. fuscicollis*	2.86	1.81 – 4.03
N14 *S. labiatus – S. oedipus + S. midas + S. bicolor*	7.3	5.73 - 9
N15 *S. oedipus - S. midas + S. bicolor*	5.32	3.99 – 6.76
N16 *S. midas - S. bicolor*	2.87	1.95 – 3.89

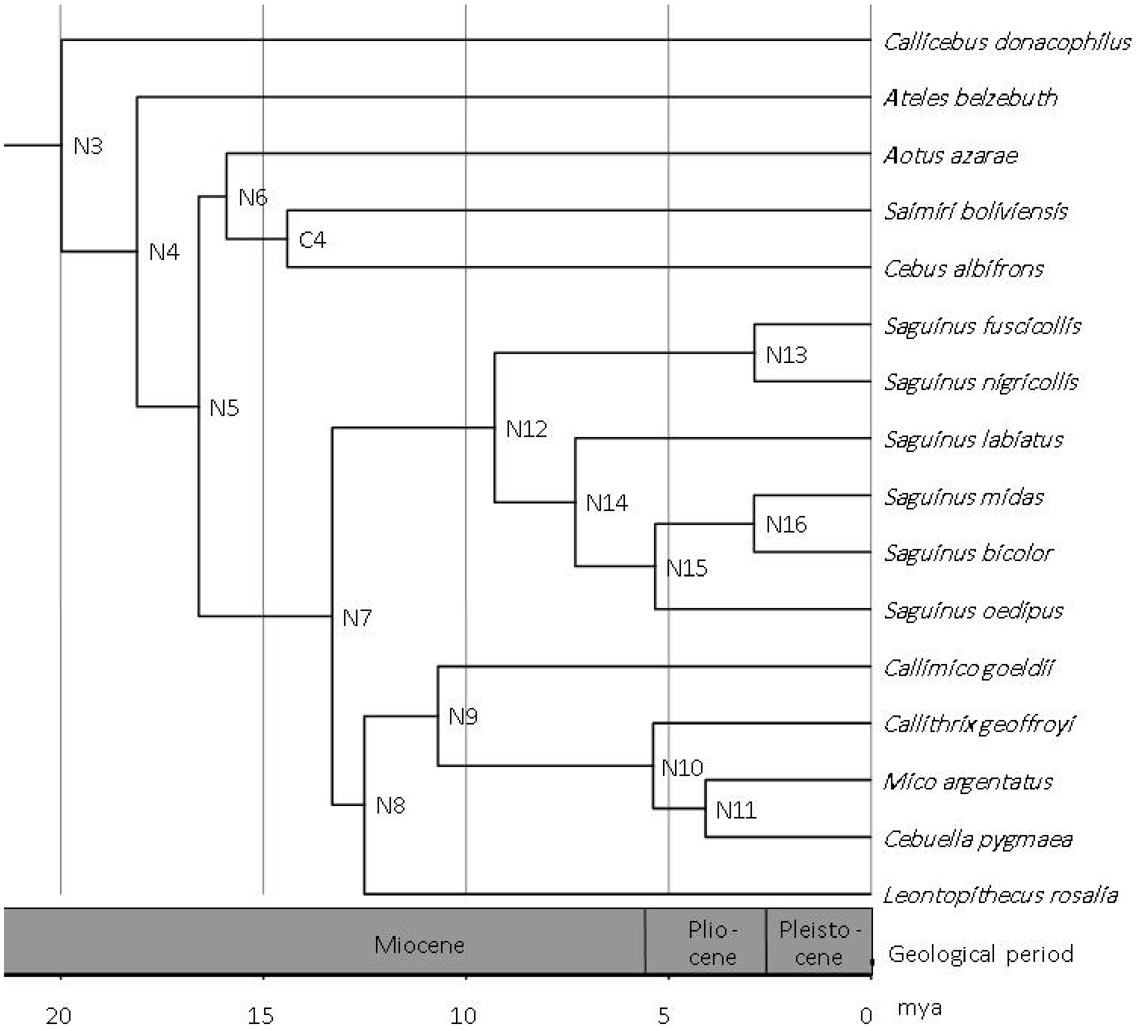

Fig. 2.2 Ultrametric tree showing divergence ages among platyrrhine primates. Nodes of interest are arbitrarily numbered (N2-15). C4 refers to a node used for calibration purposes. Nodes N1 and C1-3 are not shown in the tree. Full details of age estimates are presented in Table 2.2.

2.4 Discussion

We sequenced complete mitochondrial genomes from at least one representative of each species group of *Saguinus.* For the *S. mystax* species group, we included *S. labiatus labiatus*, since previous studies clearly showed that all members of this species group*, S. mystax, S. labiatus* and *S. imperator* form a monophyletic group (Cropp et al., 1999; Araripe et al., 2008; Matauschek et al., in press). The *S. oedipus* species group, including *S. oedipus*, *S. geoffroyi* and *S. leucopus* is represented by *S. oedipus*. Furthermore we included with *S. bicolor* and *S. midas* as representatives of the correspondent species groups. From the highly diverse *S. nigricollis* species group the mitochondrial genomes from the two species proposed by

Hershkovitz (1977) *S. fuscicollis weddelli* and *S. nigricollis nigricollis* were sequenced. To complete the picture of the position of these *Saguinus* species groups one mitochondrial genome of each callitrichine genus (except *Callibella*) were generated.

2.4.1 Phylogenetic relationships within the Callitrichinae

In contrast to previous studies, we found a basal position of *Saguinus*, instead of, A sister grouping of *Saguinus* and *Leontopithecus*, as suggested by several authors is not supported by our data. Cronin and Sarich (1975) argue for a basal position of *Leontopithecus* based on immunological evidence. An analysis of ALU insertions rather supports a basal position of *Saguinus* (Osterholz et al. 2009), which is also indicated by nuclear sequence data (Canavez et al., 1999; Chaves et al., 1999; Opazo et al., 2006; Borges et al., 2008). All other depicted relationships among callitrichine genera, e.g. the close relationship of *Callimico* to the marmosets are in agreement with earlier studies (Cronin and Sarich, 1975; Seuanez et al., 1989; Schneider et al., 1993; Pastorini et al., 1998; Chaves et al., 1999; Canavez et al., 1999).

Our data show a close relationship of the Amazonian marmosets (*Mico*) with the dwarf marmoset (*Cebuella*) to the exclusion of the Atlantic marmosets (*Callithrix*). This phylogenetic arrangement is widely accepted today and also confirmed on the basis of chromosome studies and numbers (Barroso et al., 1997; Canavez et al., 1999; Porter et al., 1997; Tagliaro et al., 1997). The black-crowned dwarf marmoset was described as new species within the genus *Callithrix* by van Roosmalen et al. (1998) and was placed later on in its own genus *Callibella* by van Roosmalen and van Roosmalen (2003). The exact phylogenetic position of *Callibella* is not addressed in this study, as samples from these animals are not available.

2.4.2 Phylogenetic relationships within the genus Saguinus

The different species groups of the genus *Saguinus* were never included as distinct entities in phylogenetic studies of callitrichine phylogeny. Our data show that the genus *Saguinus* is divided into different, unexpectedly old lineages.

The two main lineages within the tamarins separated already during the late Miocene, around 9.2 mya. They differ considerably from each other. The lineage leading to the *S. nigri-*

collis group consists of small-bodied tamarins. They underwent a rapid radiation around 2-3 mya which lead to the current diversity of 17 described taxa (Matauschek et al., in press).

Within the large-bodied tamarins the first major split occurred between the mustached tamarins (*S. mystax* species group) south of the Amazon river and the North-Amazonian tamarins already during the outgoing Miocene around 7.3 mya. The monophyly of the mustached tamarins (*S. mystax, S. imperator, S. labiatus*), which was described already by Hershkovitz (1977), could be confirmed by several studies (Cropp, 1999; Araripe et al., 2008; Matauschek et al., in press). It is also the only species group which lives sympatrically with another tamarin species group, the S. *nigricollis* group, throughout its range and even forms stable mixed-species association with them (Heymann, 1997; Heymann and Buchanan-Smith 2000).

The next lineage leads to the *S. oedipus* group, the northern most representatives of the Callitrichinae. *S. oedipus* and *S. geoffroyi*, which are considered as subspecies by Hershkovitz (1977), are assigned to this group. The position of *S. leucopus*, which geographically represents the link between the *S. oedipus* group and the Amazonian tamarins, is not clear yet. Hershkovitz (1977) positioned it in the *S .oedipus* species group. In a molecular study by Araripe et al. (2008), it formed a distinct lineage. Given that samples from this species are difficult to obtain and that it is very difficult to generate whole mitochondrial genomes out of museum material, this species could not be included in this study. The inclusion of *S. leucopus* in future studies is desirable. The same applies to the mottle-faced tamarin *S. inustus*, which is even less known than *S. leucopus* and which was placed into its own species group by Hershkovitz (1977).

The result of our phylogenetic reconstructions is a close relationship between *S. midas* and *S. bicolor.* This is in concordance with the findings of Cropp et al. (1999) and Araripe et al. (2008). Rylands et al. (1993) even proposed that *S. midas* and *S. bicolor* should form a distinct group. The two species diverged around 2.9 mya. Rylands et al. (1993) approve a grouping of these taxa into one group. They share the morphology of the P_2 premetacristid exclusively and other dental features, like the loss of a separate inferior petrosal sinus foramen and the derived states of entoconid reduction on M_1 with the *S. oedipus* group (Groves 2001).

2.4.3 *Taxonomic implications for the different* Saguinus *species groups*

In a time-based phylogenetic classification, as proposed by Goodman et al. (1998), taxa with a divergence time of more than 6 mya should be treated as separate genera. Although an only time-dependent concept of generic nomenclature is proposed, we think that also other factors, like morphology, ecology or behavior should be taken into account to confirm whether a group of animals really forms a distinct evolutionary lineage or belongs to one and the same radiation. Considering that divergence time estimations are highly dependent from the choice of calibration points and generally show high variances, we believe that the relations between the divergence ages between different genera or groups should be more considered than the absolute values. For instance, we find much deeper splits within the genus *Saguinus* than within the marmosets (the *S. nigricollis* group splitting of at 9.5 mya). Divergence ages for other New World primate genera are similar to the splits within the Callitrichinae (*Cacajao-Chiropotes*: 6-7 mya; *Lagothrix-Brachyteles*: 10-11 mya; *Cebus-Saimiri*: 16-19 mya) (Opazo et al., 2006).

In summary it can be concluded, that the different species groups of *Saguinus* are in most cases considerably older than the different marmoset genera. They can also be identified as distinct monophyletic entities by molecular and morphological indications (Tab. 2.3). Therefore a rearrangement of their taxonomic designation should be considered. Keeping the four genera of marmosets, like Rylands et al. (2000), then, in consequence, the different species groups of tamarins have to be elevated genera as well. Groves (2001, 2005) lists *Cebuella, Callibella* and *Mico* as subgenera of *Callithrix*, which is in concordance with the concept of Goodman et al. (1998) and confirmed by our data, showing divergence ages between the different marmoset lineages between 4 and 5.3 mya. Thus, we rather support the taxonomic arrangement of Groves (2001) with one genus *Callithrix* comprising the subgenera *Callithrix, Mico, Cebuella* and *Callibella*.

In *Saguinus* at least the differences between the *S. nigricollis* lineage and the remaining tamarins and their divergence time of about 9.5 mya, in our opinion, should be reflected in the taxonomic nomenclature. They also differ considerably morphologically and in many aspects of their ecology and behavior (e.g. foraging strategies and diet; microhabitat use and locomotion; olfactory communication; [Garber, 1988, 1991, 1993; Heymann and Buchannan-

Smith, 2000; Heymann, 2001]). Considering the divergence time and their ecological, behavioral and morphological distinctiveness, we clearly support a generic status of the *S. nigricollis* species group. A generic status of the *S. mystax* group with an age of 7.3 mya can be discussed, but maybe more comparative data on behavior and ecology should be added to further determine their distinctiveness from the other large-bodied tamarins. The *S. oedipus* and *S. midas/bicolor* group lie with 5.3 mya slightly below this 6 mya-threshold. Considering the low resolution, which such divergence age estimations naturally underlie, we would support a subgeneric status for the latter three species groups (Tab. 2.3).

Table 2.3 Tamarin species groups with characteristic morphological features, estimated divergence ages and the proposed genus or subgenus name, respectively.

Species group	Morphological characters	Divergence time [mya]	Proposed genus/subgenus name
S. nigricollis	Lower body-size	9,2	Genus *Leontocebus*, Wagner 1840
S. mystax	I_2 only little wider than long	7,3	Subgenus *Mystax*, Gray 1870
S. oedipus	Narrow maxilla	5,32	Subgenus *Oedipus*, Lesson 1870
S. midas/bicolor	Derived form of premetacristid on P_2	5,32	Subgenus *Saguinus*, Hoffmannsegg 1807

2.4.4 Biogeographic implications

Molecular phylogenetic studies on various Amazonian organisms indicate major diversifications during the late Miocene or Pliocene with the uplift of the Eastern Cordilleras of the northern and central Andes (Gregory-Wodzicki, 2000; Garzione et al., 2008). The division of the Callitrichinae in the major lineages occurred in the middle Miocene, the initial split within the *Saguinus* lineage in the late Miocene.

During that time, ~10 mya, western Amazonia was mainly influenced by the formation of the Acre mega-wetland, a large fluvio-tidal landscape covering most of the area in central and western Amazonia and the establishment of the main transcontinental drainage system (Figueiredo et al., 2009). Thus, the great Acre wetland could account for the first initial split within *Saguinus*, which separated the ancestors of the *S. nigricollis* species group from the *Saguinus* stem, which led to the larger-bodied tamarins. Within the large-bodied tamarin lineage our data indicate a western origin with the mustached lineage branching off first with a subsequent northward dispersal around 7 mya forming the *S. oedipus* group in today's Colombia and Panama and a consecutive movement north of the Amazon main course to the east leading to the *S. midas* and *S. bicolor* species groups. This split into a South-Amazonian and a North-Amazonian lineage is roughly conform with the onset of the Amazon drainage system and the course of the Amazon as we see it today, which is dated around seven to 10 mya (Figueiredo et al., 2009). Thus, our data clearly support an eastward dispersal with a West-Amazonian origin as suggested by Hershkovitz (1977) rather than an eastern origin of *Saguinus* with a westward dispersal as proposed by Ferrari (1993).

The radiation within the *S. nigricollis* group occurred much later, ~ 3 mya (Matauschek et al., in press). This radiation may coincide with the decline of the Acre wetland and the following formation of the Amazonian river system (Wesselingh et al., 2010). The expansion of tropical rainforest across the former wetland area enabled a subsequent dispersal of the ancestral *S. nigricollis* stock. The emerging rivers formed barriers to the dispersing tamarins and enhanced their diversification and brought them back into contact with the *S. mystax* group.

If we compare this hypothesis with other Neotropical primates, we detect parallels with the biogeography and dispersal patterns on a similar timescale in squirrel monkeys (*Saimiri)* (Lavergne et al., 2010). The disappearance of the great Acre mega-wetland and the subsequent formation of the Amazonian fluvial system could have enhanced speciation in both groups.

Amazonia's extremely rich biodiversity, which we see today, surely evolved not during by a single, short-term event (Wesselingh et al., 2010). As recent molecular studies on different groups of organisms suggest, the diversification of life in Amazonia was a more continuous process that took place throughout the Cenozoic. For some groups, a sort of acceleration of this process is indicated during the Miocene (see Rull, 2008). Of course, we should be aware that correlating geological and putative speciation events might be oversimplified, as the documentation for both is often incomplete and that molecular methods can only reflect the situation found in modern biota (Lundberg et al., 1998; Wesselingh et al., 2010). Nevertheless, combining molecular studies on the evolution of wide-spread and diverse groups of animals, such as the tamarins, can surely be a useful tool to uncover the biogeographic history of one of the most species-rich trerrestrial ecosystems of the world.

Acknowledgements

We thank the field assistant Manuel Shahuano Tello for excellent work during the field surveys, Stephan Behl and Peter Gottleuber for their help and assistance in the field, and Christina Oberdieck and Marion Seiler for help and support in the lab. We are further grateful to Pierre Moisson and the staff of Mulhouse Zoo, France, Jan Vermeer from Valle des Singes, Romagne and Sandra Suarez for providing samples from Bolivia. We thank INRENA in Lima for authorizing this study (106-2007-INRENA-IFFS-DCB) and to the dean of the Forestry Department of the Universidad Nacional de la Amazonía Peruana in Iquitos for administrative support. This study was financially supported by the German Primate Center, the Biodiversitäts-Pakt of the Wissenschaftsgemeinschaft Gottfried-Wilhelm Leibniz, and the Margot Marsh Biodiversity Foundation.

3 Mitochondrial Phylogeny of Tamarins (*Saguinus*, Hoffmannsegg 1807) with Taxonomic and Biogeographic Implications for the *S. nigricollis* Species Group

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American Journal of Physical Anthropology

Received: 17 June 2010 / Accepted: 27 September 2010

Abstract

Tamarins of the genus *Saguinus*, subfamily Callitrichinae, represent one of the most diverse primate radiations. So far, about 35 taxa have been described, but detailed information about their taxonomy and phylogeny is still lacking. To further elucidate the phylogenetic relationships and the biogeographic history within the genus, and to contribute to a more reliable classification of its taxa, we sequenced the complete mitochondrial cytochrome *b* gene and the hypervariable region I of the D-loop. Therefore, we mainly used fecal samples from wild tamarins collected during two expeditions to the Peruvian Amazon, an area of high tamarin diversity. Our data suggest that the numerous taxa of the *S. nigricollis* species group are derived from a common ancestor that separated from the other representatives of the genus ~10 mya. Most taxa of the *S. nigricollis* group form monophyletic clusters, which mainly originated in a single rapid radiation ~2.9 mya. *S. fuscicollis* and *S. nigricollis* appear as polyphyletic taxa, but we could identify various clusters, which are mainly consistent with differences in coat coloration. We could confirm most of the existing taxa as distinct entities and suggest species status for *fuscicollis, illigeri, lagonotus, leucogenys, nigricollis, nigrifrons*, *tripartitus* and *weddelli*. Our genetic data do not support a separate status for *melanoleucus* and *graellsi*, but due to differences in fur coloration, we give them subspecies status. The species group most likely originated in western Amazonia and diversified during the decline of the Acre wetland and the formation of the Amazonian river system.

Key words: *Saguinus*, cytochrome *b*, D-loop, evolution, Peru

3.1 Introduction

Tamarins are small-bodied Neotropical primates, which together with marmosets (*Callithrix* and *Mico*), pygmy marmosets (*Cebuella* and *Callibella*), Goeldi`s monkey (*Callimico*) and lion tamarins (*Leontopithecus*) constitute the New World monkey subfamily Callitrichinae (Groves, 2001; Osterholz et al., 2009). Despite their wide distribution and their relatively high abundance throughout Amazonia and Central America, still little is known about the actual diversity and phylogenetic relationships of the genus *Saguinus*. This is exemplified by the recent discovery of a new tamarin form Brazil (Röhe et al., 2009). Today, about 35 tamarin taxa are recognized, thus making tamarins one of the most diverse primate genera.

Tamarin taxa can be distinguished by a variety of morphological characters, mainly differences in pelage coloration (Groves, 2001). Hershkovitz (1977) identified 33 taxa, which were grouped into 10 species and three species groups (hairy-faced, mottle-faced, bare-faced tamarins). The few genetic studies that have been conducted to clarify the interspecific relationship of *Saguinus* do not support this split (Cropp et al., 1999; Canavez et al., 1999; Tagliaro et al., 2005). Instead, these studies support a division of the genus into small-bodied and large-bodied clades; both of which include individuals with hairy and bare faces. The small-bodied clade consists only of the *S. nigricollis* group. All other species form the large-bodied clade (Cropp et al., 1999).

With 16 to 17 taxa, the *S. nigricollis* species group is the most diverse of all *Saguinus* species groups (Hershkovitz, 1977). It is distributed throughout western Amazonia, where it seems to have differentiated within the large interfluvial areas between the major tributaries of the Amazon River (Fig. 3.1). The highest diversity of this species group is found in Peru (Herhskovitz, 1977). According to Hershkovitz (1977), the *S. nigricollis* group includes the black-mantled tamarin *S. nigricollis* with three subspecies and the saddle-back tamarin *S. fuscicollis* with 14 subspecies, including the golden-mantled tamarin *S. f. tripartitus*. This classical tamarin taxonomy was reviewed and changed several times (Coimbra-Filho, 1990; Rylands et al., 1993; Rylands et al., 2000; Groves, 2001). Some of these subspecies may represent only color morphs or hybrid populations (Peres et al., 1996). Proposed sympatry between *S. fuscicollis* and *S. nigricollis* is highly disputed (e.g. Heymann, 1997) and species or subspecies descriptions are often based on single museum specimens. Molecular studies indicate that some subspecies of *S. fuscicollis* are more closely related to *S. nigricollis,* so that the status of these two species as monophyletic entities remains doubtful (Cropp et al., 1999). The taxon with the greatest variety of subspecies and color patterns is the saddle-back tamarin *S. fuscicollis* (Cheverud and Moore, 1990). Hershkovitz (1977) mentions 14 subspecies, which differ from each other in color pattern and distribution. The taxonomic status of several of these taxa is fairly controversial. Some subspecies, *S. f. tripartitus*, *S. f. graellsi* and *S. f. melanoleucus,* have been given full species rank, so that there are now up to five different species within this species group (Hernandez-Camacho and Cooper, 1976; Thorington, 1988; Coimbra-Filho, 1990; Groves, 2001). We follow here the classification of Groves (2001) and recognize five species within the *S. nigricollis* group.

Most recent studies on *Saguinus* taxonomy are based on the comparison of morphological characters like pelage coloration (e.g. Hershkovitz, 1977) or on morphometrical analyses of the skull or dentition (Natori, 1988; Natori and Hanihara, 1988). Molecular approaches have been applied only to a limited sample of tamarin taxa (Jacobs et al., 1995; Peres et al., 1996; Cropp et al., 1999; Canavez et al., 1999; Tagliaro et al., 2005; Vallinoto et al., 2006). Nearly all of these studies rely solely on museum material or captive animals for which the exact geographic origin is often unknown.

Due to these uncertainties, we set up a molecular genetic study to examine (i) the status of *S. fuscicollis* and *S. nigricollis* as monophyletic species, (ii) the taxonomic status of the proposed species *S. graellsi, S. tripartitus* and *S. melanoleucus,* and (iii) their relationship to the remaining Peruvian tamarin taxa.

3.2 Methods

3.2.1 Sampling sites and sample collection

To obtain fecal samples from wild tamarin populations, two field trips to Peruvian Amazonia were conducted between June and October 2007 and 2008. Samples were collected at 29 locations (Fig. 3.1, Supplementary Table 1). Sampling locations were selected to include all major interfluvial areas in the Peruvian Amazon according to the distribution pattern as proposed by Hershkovitz (1977) and Aquino and Encarnación (1994). The main focus was on the members of the *S. nigricollis* species group, but whenever possible, we additionally sampled the sympatric taxa *S. mystax mystax* (AGC, PIJ, RBR, EBQB) and *S. imperator subgrisescens*

(LAM). Taxa were identified by their putative distributions (Hershkovitz, 1977; Aquino and Encarnación, 1994) and fur coloration as described in Hershkovitz (1977). At each sampling site, we tried to obtain samples from several tamarin groups. Coordinates of each sampling location were recorded with a Garmin GPSMAP 76CSx (Supplementary Table 1). Only fresh droppings were collected. Additionally, samples of animals kept as pets by local villagers were taken, but only if information of exact geographic origin was traceable. We further included fecal samples from the Estación Biológica Quebrada Blanco (EBQB), the Estación Biológica Cahuana (CA) and the Estación Biológica Tahuamanu, Bolivia (BOL), which were kindly provided by other researchers. All samples were stored in plastic tubes (5 ml) filled with ethanol (90%). Complementary to the fecal samples, a muscle sample of *S. melanoleucus melanoleucus* taken from a specimen preserved in ethanol and stored at the Zoological State Collection Munich (ZSM) was included. Although the origin of this specimen was not available, the individual could be clearly identified as *S. m. melanoleucus* based on its overall whitish coloration as described in Hershkovitz (1977). Blood samples from *S. oedipus* and *Callithrix geoffroyi* were obtained from the German Primate Center (DPZ) and Dresden zoo, Germany, respectively. In total, we analyzed 101 tamarin individuals.

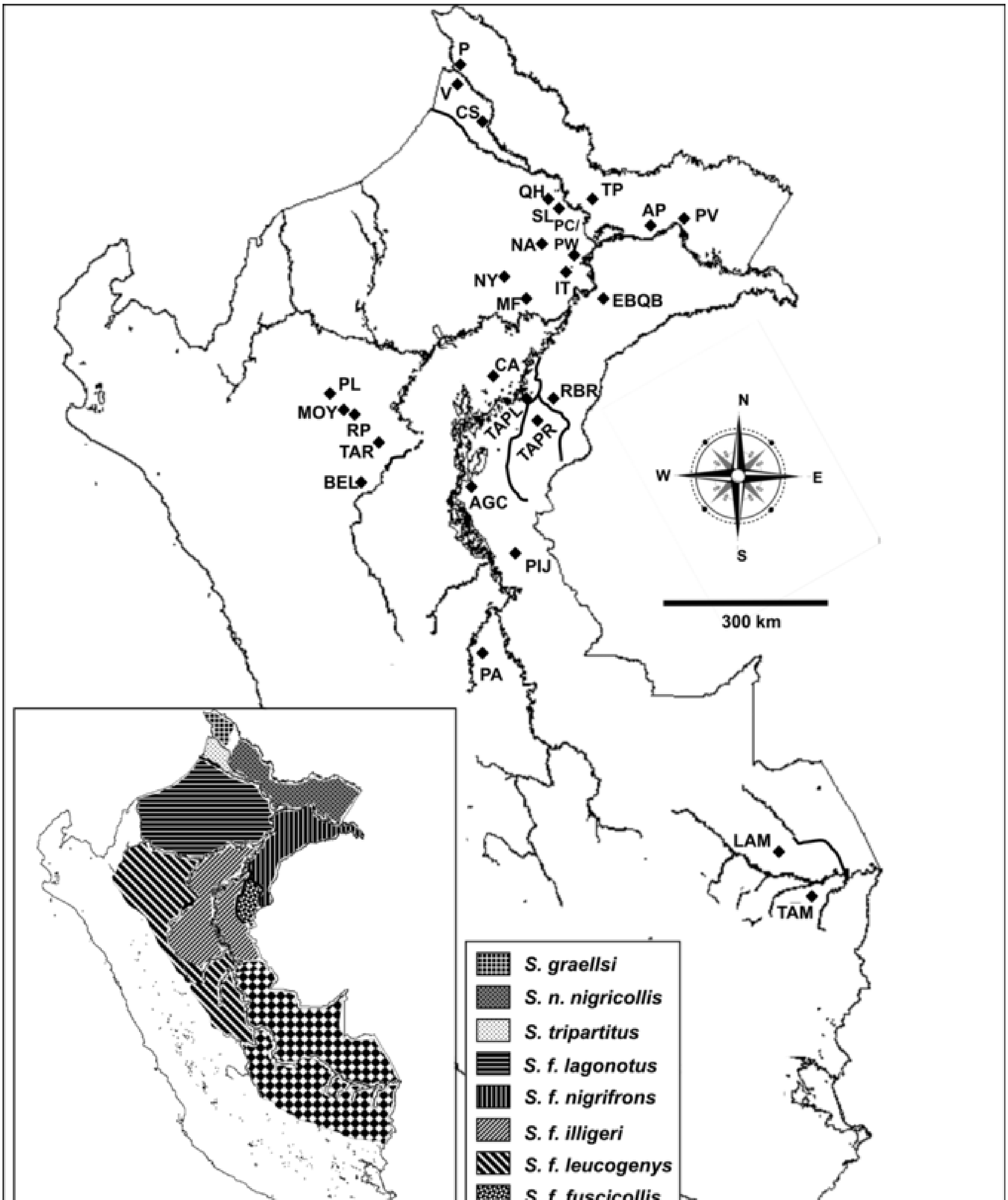

Fig. 3.1 Map of Peru with all sampling locations of the two field surveys and distribution map of the *S. nigricollis* group. (Abbreviations: **P**: Pantoja, Rio Napo; **V**: Vencedores, Rio Napo; **CS**: Campo Serio, Rio Napo; **QH**: Quebrada Huiririma, Rio Napo; **SL**: Sergento Lores, Rio Napo; **TP**: Tutapishcu, Rio Napo; **AP**: Rio Apayacu, Amazonas; **PV**: Pevas, Amazonas; **NA**: Diamante Azul, Rio Nanay; **PC**: Padre Cocha, Rio Nanay; **PW**: Pilpintuwasi, Rio Nanay; **IT**: Rio Itaya; **NY**: Nueva York, Rio Tigre; **MF**: Miraflores, Rio Marañon; **EBQB**: Estaciŏn Biologica Quebrada Blanco; **CA**: Cahuana; **RBR**: Rio Blanco, right bank; **TAPL**: Rio Tapiche,left bank; **TAPR**: Rio Tapiche, right bank; **AGC**: Aguas Calientes, Rio Ucayali; **PIJ**: Pijuyal, Rio Ucayali; **PL**: Pueblo Libre; **MOY**: Moyobamba; **RP**: Rumi Pata; **TAR**: Tarapoto; **BEL**: Bellavista; **PA**: Biological Station Panguana, Rio Pachitea; **LAM**: Biological Station Los Amigos, Rio Madre de Dios; **TAM**: Rio Tambopata).

3.2.2 *Laboratory methods*

For the DNA extraction from fecal and museum samples, the QIAGEN QIAamp Stool Kit and QIAGEN QIAamp Tissue Kit was used. Extractions followed standard protocols as recommended by the supplier, with the exception that the DNA was diluted in HPLC quality water and stored at -20 °C before further processing. From the mitochondrial genome, we sequenced the complete cytochrome *b* gene (cyt *b*) and a 546 bp fragment of the D-loop, including the HVI region. Amplifications were carried out in 30µl reactions containing a final concentration of 0.33 µM of each primer, 3 mM MgCl2, 0.166 mM dNTPs, 1 x amplification buffer and 1U Taq DNA polymerase (Biotherm, Genecraft). Primer sequences are listed in Table 3.1. For all amplifications, wax-mediated hot-start PCRs were performed. The program for all amplifications comprised 40 cycles, each with a 1 min denaturation step at 92°C, 1 min annealing (for annealing temperatures see Table 3.1) and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. The results of the PCR amplifications were checked by running an aliquot on a 1 % agarose gel, stained with ethidium bromide. PCR products were purified using Montage^{TM}PCR Centrifugal Filter Devices (Millipore) following the delivered instructions. PCR products were sequenced in both directions with respective amplification primers on an ABI PRISM^{TM}3130xL Genetic Analyzer (Applied Biosystems) using the BigDye^{TM}Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). To prevent cross-taxon contamination, we followed standard methods as described in Roos et al. (2008). The amplification of nuclear pseudogenes (numts) was reduced by using mainly faecal and museum material in which nuclear DNA is highly degraded (Hofreiter et al., 2003; Thalmann et al., 2004), by applying *Saguinus*-specific primers which were designed in our laboratory, and by direct sequencing of PCR products. Sequencing revealed no multiple peaks or inconsistent positions in overlapping fragments and no premature stop codons were detected in the cyt *b* sequences.Newly generated sequences were deposited at GenBank and are available under accession numbers HM367879-HM368078 (Supplementary Table 3.1).

Table 3.1 Primers used in this study. Given are locus, primer sequence, reference and annealing temperature (AT) in °C.

Locus	Primer sequence (5`-3`)	Reference	AT
cyt *b*	AATGATATGAAAAACCATCGTTGTA	This study	58
	TTTCAGCTTTGGGTGTTGATG	This study	58
cyt *b* + HVI	CCYTAA ACA CCC CTC CCC	This study	60
	CCATCGTGATGTCTTATTTAAG	This study	60
	ATTGATATGAAAARYCATCGTTG	This study	60
	GGAATGTTATGCTTTGTTGTTTG	This study	60
HVI	AACATTAAAGTACTTTACAAGTACATA	Huck et al. 2007	60
	CTGGCAAAACACAGTCAGGCG	Huck et al. 2007	60
	CTA CCATCAACACCCAAAGC	This study	60
	CCTCCCGGCAATGCTGAT	This study	60

3.2.3 Statistical methods

Sequences were assembled and manually edited using SEQUENCHER 4.7 (Gene Codes Corporation, Ann Arbor, MI, USA). As outgroup, we used an orthologous sequence from *C. geoffroyi*. Alignments were conducted with ClustalW as implemented in GENEIOUS 4.8 (Rozen and Skaletsky, 2000) and corrected by eye. For further studies, identical tamarin haplotypes were removed. Indel positions in the HVI alignment were eliminated using GBLOCKS 0.91b (Castresana, 2000). Therefore, a relaxed selection of blocks was applied. For phylogenetic tree reconstructions, the cyt *b* and HVI datasets were concatenated. Trees were calculated with maximum-parsimony (MP), maximum-likelihood (ML) and Bayesian approaches. MP reconstructions were performed in PAUP 4.0b10 with 10,000 bootstrap replications (Swofford, 2002). Therefore, all characters were treated as unordered and equally weighted throughout. A heuristic search was performed with the maximum number of trees set to 100. For both datasets, the GTR+I+G model was selected as the optimal nucleotide substitution model with jMODELTEST 0.1.1 (Posada, 2008) using the Akaike Information Criterion (AIC). ML trees were constructed with GARLI 0.96.win23 (Zwickl, 2006). 500 pseudoreplications were conducted to estimate ML bootstrap percentages and a 50%-consensus tree was calculated in PAUP. Bayesian analyses were carried out in MrBAYES v3.1.2 (Hulsenbeck et al., 2001; Ronquist and Hulsenbeck, 2003). The dataset was partitioned into cyt *b* and HVI regions. In the analysis, four Monte Carlo Markov chains (MCMC) with a default temperature of 0.2 and a chain length of 10,000,000 generations were carried out. Trees and parameters were sampled every 1,000 generations. 2,500 of the sampled trees were discarded as burn-in. Obtained phylogenetic trees were visualized and edited using FigTree v1.3.1 (Rambaut, 2006).

Calculations of divergence ages were carried out using a Bayesian approach implemented in the programme BEAST (Drummond and Rambaut, 2007). Due to the high mutation rate of the HV1 region, divergence age estimations were based only on the cyt *b* alignment. In the final dataset, we included 20 tamarin sequences, which represent at least one haplotype per major clade. For calibration purposes, additional sequences of other platyrrhines (*C. geoffroyi*, *Cebus albifrons, Saimiri boliviensis, Aotus nancymaae, Callicebus torquatus, Cacajao calvus, Pithecia monachus, Alouatta seniculus*) and outgroup taxa (*Macaca mulatta, Papio hamadryas, Pan troglodytes, Homo sapiens*) were taken from GenBank (Supplementary Table 1). Thus, the final alignment comprised 32 sequences. Due to a triplet deletion in *Cebus, Pithecia* and *Cacajao,* the alignment length for the BEAST calculations was reduced to 1,137 bp. We used a HKY model with a gamma distributed rate variation between sites and invariant sites as optimal nucleotide substitution model. The model was chosen by jMODELTEST 2.2 using the Bayesian information criterion (BIC) (Posada, 2008). For the analysis, we used a relaxed lognormal clock model of lineage variation, and for branching rates, a Yule process as prior was assumed. Since the estimation of phylogenetic relationships was not the main aim of this analysis, we used an a-priori fixed tree topology as obtained from phylogenetic reconstructions (Fig. 3.2) and published data for taxa other than *Saguinus* (Osterholz et al., 2009). Analyses were run for 25,000,000 generations, with tree and parameter sampling occurring every 1,000 generations, of which 10% were discarded as burn-in. The adequacy of a 10% burnin and convergence of all parameters was assessed by visual inspection of the trace of the parameters across generations using the software TRACER v1.3 (Rambaut and Drummond, 2005). Subsequently, the sampling distributions of multiple independent replicates were combined using the software LogCombiner v1.4.6, and then summarized and visualized using the software TreeAnnotator v1.4.6. Both programs are part of the BEAST package (Drummond and Rambaut, 2007).

As calibration points, we selected four well-documented fossils. First, the split between *Pan* and *Homo* is dated 6-7 million years ago (mya) (Brunet et al., 2002; Vignaud et al., 2002; Steiper and Young, 2006) and between *Macaca* and *Papio* 7-8 mya (Delson et al., 2000; Steiper and Young, 2006). For the initial split between platyrrhines and catarrhines, Poux et al. (2006) give a range of 34-40 mya. As calibration point among platyrrhines, we used the split between *Saimiri* and *Cebus*, which was dated by Lavergne et al. (2010) at ~16 mya. They set the upper limit to 20 mya, marked by *Dolichocebus*, which most likely is the crown *Saimiri* (Szalay and Delson, 1979), and the lower limit to 12 mya, resulting from *Neosaimiri,* dated by Stirton (1951). Instead of hardbounded calibration points, we used the published dates as a normal distribution prior for the respective node. For C1 (Catarrhini - Platyrrhini), this translates into a normal distribution with a mean of 37 mya and a standard deviation (SD) of 3.0 mya, for C2 (*Homo/Pan*) into a mean of 6.5 mya and a SD of 0.5 mya, for C3 (*Macaca* - *Papio*) into a mean of 7.5 mya and a SD of 0.5 mya and for C4 (*Saimiri* - *Cebus*) into a mean of 16 mya and a SD of 4.0 mya.

3.3 Results

We successfully sequenced the complete cyt *b* gene and the HVI region from 99 *Saguinus* individuals from 29 localities in the Peruvian Amazon and Bolivia, and each one individual from captivity (*S. oedipus*) and museum collections (*S. m. melanoleucus*). The combined alignment had a length of 1,487 bp, of which 483 sites were variable and 345 parsimony-informative. Among all studied individuals, we observed 66 haplotypes in the HVI region, 58 in the cyt *b* gene and 72 in the combined dataset.

All phylogenetic reconstructions as obtained from various algorithms revealed identical tree topologies and similar support values. Accordingly, *Saguinus* initially diverged into a clade consisting of *S. oedipus* and the mustached tamarin group members, *S. mystax, S. imperator* and *S. labiatus*, and a clade including all the members of the S. *nigricollis* group (Fig. 3.2). Although the sister grouping of the mustached tamarin group and *S. oedipus* is only weakly supported, at least species group monophylies are well supported. Within the mustached tamarin group, *S. imperator* branched off first, before finally *S. mystax* diverged from *S. labiatus*.

Within the *S. nigricollis* group, we detected four well-supported major clades, but their phylogenetic relationships are only weakly supported and therefore remain unresolved. Within clade 1, we found two subclades, of which one included all *S. f. illigeri* individuals from the Estación Biológica Cahuana (CA) and from the left bank of the Rio Tapiche (TAPL), and the other, all *S. f. illigeri* from Aguas Calientes/Contamana (AGC), east of the Ucayali river, and all haplotypes of *S. f. leucogenys* collected in the Departamento San Martìn (TAR, MOY, RP, BEL, PL) in the northern part of the proposed distribution area of this subspecies (Hershkovitz, 1977). Clade 2 could be divided into four well-supported subclades, which refer to individuals identified as *S. f. weddelli* + *S. m. melanoleucus, S. f. fuscicollis, S. f. nigrifrons* and *S. f. leucogenys* from Panguana (PA), south of the Rio Pachitea. All reconstructions suggested a close relationship between *S. f. fuscicollis* and southern *S. f. leucogenys*, and between the *S. m. melanoleucus* specimen and *S. f. weddelli*. Clade 3 consisted of *S. n. nigricollis* and *S. graellsi* haplotypes. Interestingly, *S. graellsi* formed a monophyletic clade nested within *S. n. nigricollis,* which appeared as paraphyletic taxon. Clade 4 comprised all individuals of *S. f. lagonotus* and *S. tripartitus*, which both formed reciprocal monophyletic clades.

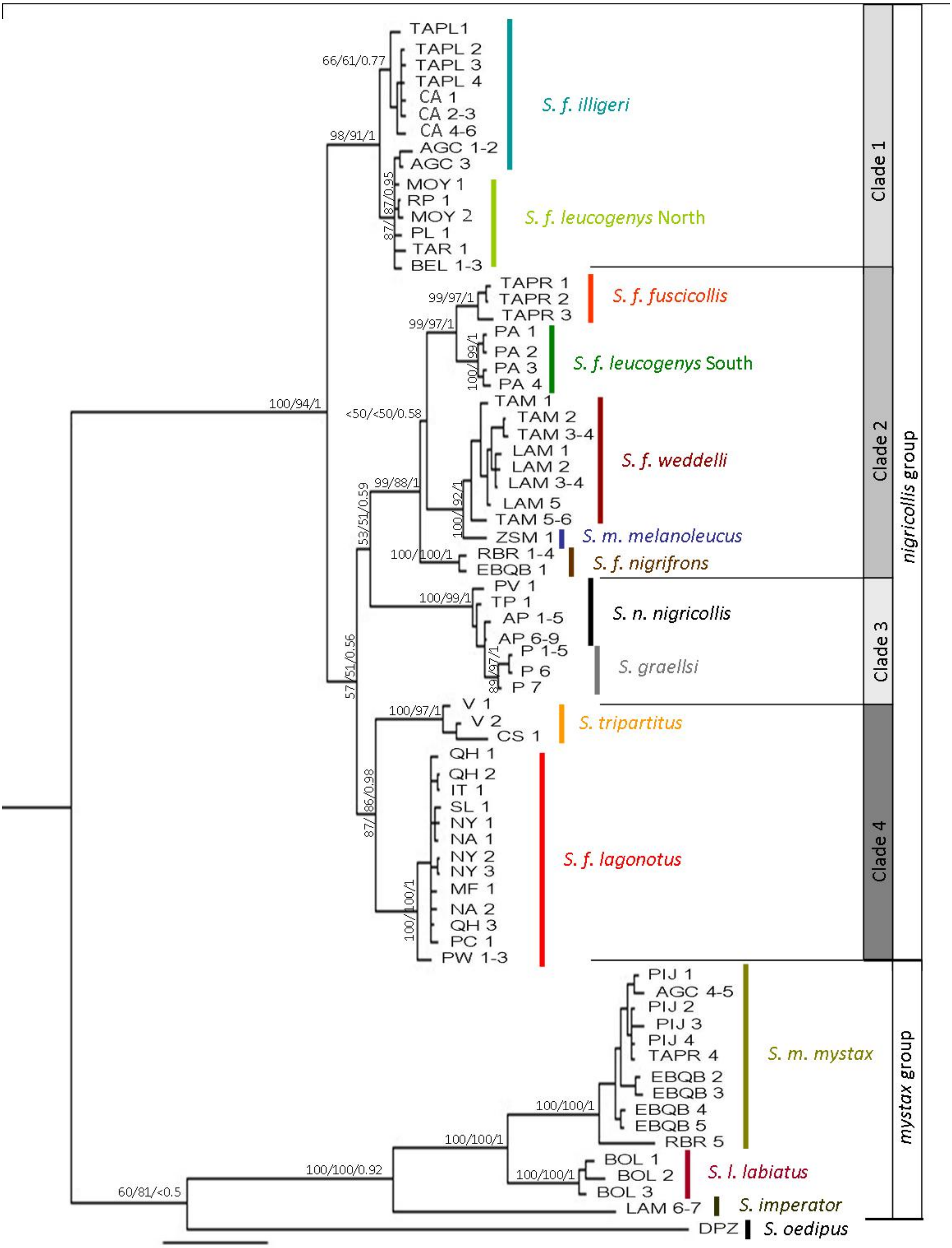

Fig. 3.2 Phylogram of the 72 haplotypes in the combined dataset (cyt *b* and HVI) as revealed by Bayesian analysis. Outgroup rooted with *C. geoffroyi*. Bootstrap values for MP and ML are indicated above branches together with posterior probabilities for the Bayesian approach (MP/ML/Bayesian).

According to divergence age estimations (Fig. 3.3, Table 2.2), the split between platyrrhines and catarrhines was dated at 42.51 mya (C1) (for 95% credibility intervals see Table 2.2), the split between *Homo* and *Pan* at 6.55 mya (C2) and between *Macaca* and *Papio* at 7.67 mya (C3). Among platyrrhines, the initial split separating Pitheciidae including *Callicebus, Pithecia* and *Cacajao* from the other platyrrhines occurred 21.1 mya (N2). Afterwards, Atelidae represented by *Alouatta,* branched off from Cebidae 19.87 mya (N5). Among the latter, *Saimiri, Cebus* and *Aotus* diverged from Callithrichinae 18.36 mya (N6). *Aotus* separated from the *Cebus* + *Saimiri* clade 17.62 mya (N7), and latter two split 14.46 mya (C4). The split between *Saguinus* and *Callithrix* was dated 14.23 mya (N8), while the initial division within *Saguinus* occurred 10.07 mya (N9). This division separated all members of the *S. nigricollis* group from the larger-bodied tamarins. Among the latter, the moustached tamarin species group split from *S. oedipus* shortly after the initial *Saguinus* division 9.07 mya (N10). *S. imperator* diverged from *S. labiatus* and *S. mystax* 6.03 mya (N11), while the split between latter two was dated at 1.15 mya (N12). The initial differentiation of major clades within the *S. nigricollis* group (clades 1-4) was dated 2.91 mya (N13). Further splitting events within the species group into recognized taxa occurred 2.58-0.07 mya (N14-20). For detailed information see Table 3.2.

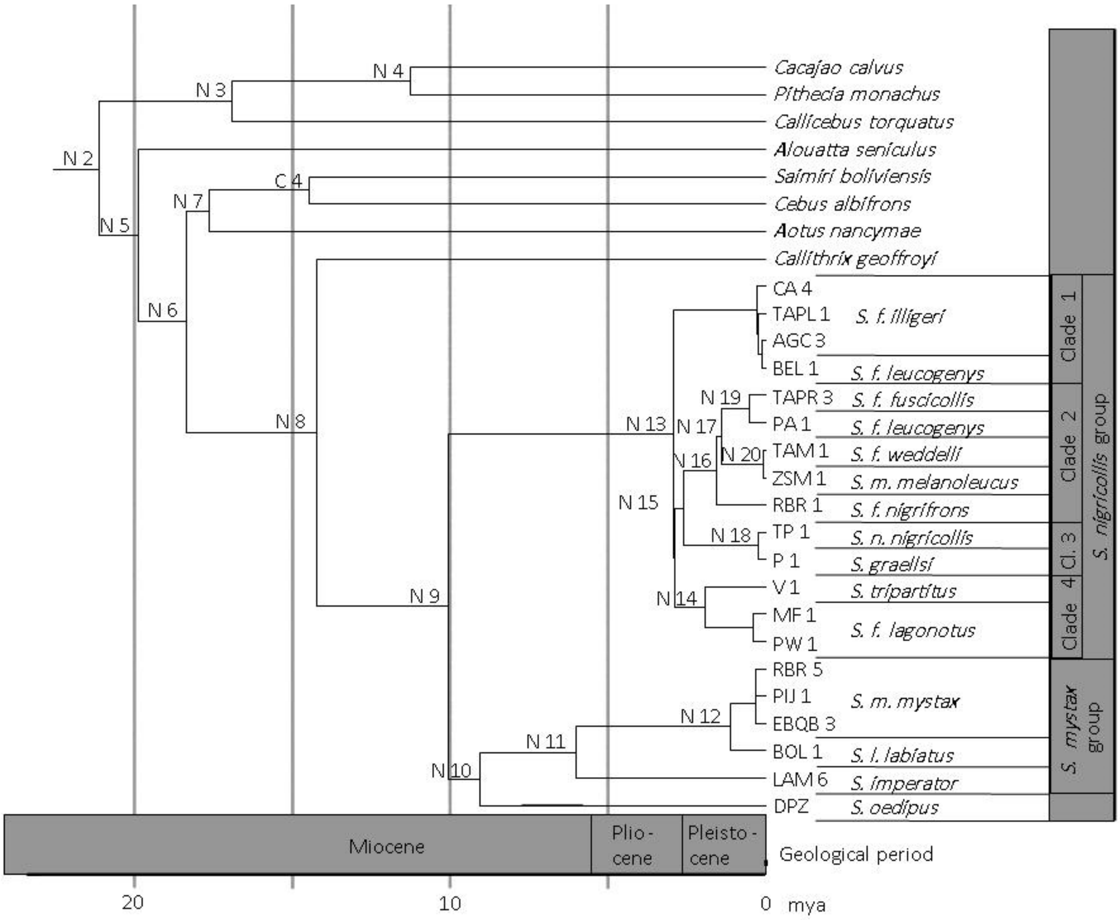

Fig. 3.3 Ultrametric tree showing divergence ages among platyrrhine primates based on complete cyt *b* sequence data. For individual codes see Figs. 1, 2 and Supplementary Table 1. Nodes of interest are arbitrarily numbered (N2-20). C4 refers to a node used for calibration purposes. Nodes N1 and C1-3 are not shown in the tree. Full details of age estimates are presented in Table 2.

Table 3.2 Bayesian divergence age estimates in mya*.

Node	**mean**	**95% credibility interval**
C1 Catarrhini – Platyrrhini	42.51	37.37 – 47.92
N1 Hominoidea (*Homo* + *Pan*) – Cercopithecoidea (*Macaca* + *Papio*)	25.45	22.66 – 27.14
C2 *Homo* – *Pan*	6.55	5.93 – 7.10
C3 *Macaca* – *Papio*	7.67	6.63 – 8.66
N2 Pitheciidae – Cebidae + Atelidae	21.10	16.64 – 27.07
N3 *Callicebus* – *Pithecia* + *Cacajao*	16.89	11.81 – 22.75
N4 *Pithecia* – *Cacajao*	11.23	5.22 – 13.16
N5 Atelidae – Cebidae	19.87	15.69 – 24.64
N6 Callitrichinae – *Cebus* + *Saimiri* + *Aotus*	18.36	15.12 – 23.77
N7 *Aotus* – *Cebus* + *Saimiri*	17.62	13.83 – 22.00
C4 *Saimiri* – *Cebus*	14.46	12.43 – 19.28
N8 *Callithrix* – *Saguinus*	14.23	10.67 – 17.96
N9 *Saguinus*	10.07	7.06 – 12.40
N10 *S. oedipus* – *S. mystax* group	9.07	6.29 – 11.11
N11 *S. imperator* – *S. mystax* + *S. labiatus*	6.03	2.91 – 6.61
N12 *S. labiatus* – *S. mystax*	1.15	1.01 – 3.01
N13 *S. nigricollis* group	2.91	1.82 – 3.64
N14 *S. tripartitus* – *S. f. lagonotus*	1.92	1.04 – 2.57
N15 *S. graellsi* + *S. n. nigricollis* – *S. f. fuscicollis* + *S. f. leucogenys* (South) + *S. f. weddelli* + *S. m. melanoleucus* + *S. f. nigrifrons*	2.58	1.38 – 2.86
N16 *S. f. nigrifrons* – *S. f. weddelli* + *S.melanoleucus* + *S. f. fuscicollis* + *S. f. leucogenys* (South)	1.55	0.70 – 1.72
N17 *S. f. weddelli* + *S. m. melanoleucus* – *S. f. fuscicollis* + *S. f. leucogenys* (South)	1.40	0.60 – 1.52
N18 *S. n. nigricollis* – *S. graellsi*	0.26	0.07 – 0.48
N19 *S. f. fuscicollis* – *S. f. leucogenys* (South)	0.52	0.16 – 0.68
N20 *S. f. weddelli* – *S. m. melanoleucus*	0.07	0.02 – 0.28

*see also Fig. 3.3. Nodes C1-C3 and N1 are not shown in Fig. 3.3.

3.4 Discussion

This study is so far the most complete assessment of the highly diverse *S. nigricollis* species complex using almost exclusively samples from wild tamarin populations with confirmed sampling localities. Our results allow a clear identification of distinct clusters within *Saguinus* that mainly coincide with described taxa.

The phylogenetic relationships between the three species belonging to the mustached tamarin (or *S. mystax*) species group are well resolved and in agreement with Araripe et al. (2008). As in our study, Araripe et al. (2008), using mitochondrial 16S rRNA sequence data, revealed a basal position of *S. imperator* and a sister grouping of *S. labiatus* and *S. mystax*. While the branching pattern in the latter species group is well resolved, relationships among taxa within the highly diverse small-bodied tamarin clade, the *S. nigricollis* species group, are disputed. Concordant with Cropp et al. (1999), our data show that most taxa of the *S. nigricollis* group form monophyletic groups, but not all branching patterns among the different lineages with in the species group are well resolved. Thus, most taxa, which differ mainly in coat coloration, seem to have evolved during a single rapid radiation, most likely ~2.9 mya.

3.4.1 The status of S. tripartitus

The golden-mantled tamarin, *S. tripartitus*, considered as a subspecies of *S. fuscicollis* by Hershkovitz (1977), was given full species rank by Thorington (1988) due to supposed sympatry with *S. f. lagonotus* on the right bank of the Rio Napo. However, neither Aquino and Encarnación (1994) nor Heymann et al. (2002) found evidence for a sympatric occurrence of both taxa and the Rio Curaray might be a natural border between them (Heymann et al., 2002; Rylands et al., in press). Moreover, it was assumed that only tamarin taxa are able to live sympatrically, which differ sufficiently in body-size and ecology, which is not the case between the taxa of the *S. nigricollis* group (Heymann, 1997; Heymann and Buchanan-Smith, 2000). During our field survey, we found no evidence of sympatry along the Rio Napo. Morphologically and ecologically, *S. tripartitus* and *S. f. lagonotus* do not seem to differ more than other *S. fuscicollis* subspecies (Hershkovitz, 1977; Heymann, 2000). In contrast to Cropp et al. (1999), our phylogenetic data clearly support a close relationship between *S. tripartitus* and *S. f. lagonotus*.

3.4.2 The status of S. melanoleucus

Another disputed issue is the taxonomic status of *S. melanoleucus*, which is distributed between the Rio Jurua and Rio Tarauacu in Brazil. Coimbra-Filho (1990) listed *S. melanoleucus* as a distinct species, with *acrensis* and *crandalli* as subspecies. There is no uninterrupted chain of intermediate forms, which leads from *S. fuscicollis* to the entirely white *S. melanoleucus* in the sense of Hershkovitz' theory of metachromism (Rylands et al., 1993; Groves, 2001). Hybridization with *S. f. fuscicollis* occurs in the headwater region of the Rio Jurua, which lead to intermediate phenotypes on the left bank of the headwater stream (Peres et al., 1996). The *acrensis* and *crandalli* pelage might represent only color morphs of *S. melanoleucus*, which thus would be a monotypic species (Groves, 2001). The *crandalli* morph could also be the result of hybridization between *S. melanoleucus* and *S. f. fuscicollis* (Rylands et al., 2000). In our data, the single *S. melanoleucus* individual represents a sister lineage to the *S. f. weddelli* clade, with an estimated divergence age of only 0.07 mya. A close relationship between both taxa was also suggested by Tagliaro et al. (2005). Hence, in view of the close genetic affiliation to *S. f. weddelli*, the specific status of *S. melanoleucus* remains doubtful.

3.4.3 The status of S. fuscicollis subspecies

There is an incongruity between the described subspecies *S. f. leucogenys* and its distribution area with our observations in the field and the genetic data. We observed intergradation between the dark brown-mantled *S. f. illigeri* type and the more or less black-mantled *S. f. leucogenys* type. Especially at the locations in San Martìn in the area of Moyobamba (MOY, RP) and further north in the Rio Mayo valley (PL), tamarins showed more the *S. f. illigeri* phenotype, like animals we found along the Rio Ucayali (AGC) and at the Rio Tapiche (TAPL), and observed by Heymann (1990) at the Rio Pacaya (CA). Hershkovitz (1977) made a similar observation on museum specimens from Moyobamba, which were indistinguishable from *S. f. illigeri* individuals. But according to their origin, which lies inside the range of *S. f. leucogenys,* he assigned them to *S. f. leucogenys*. These observations on the phenotype reflect very well our mitochondrial data, where all individuals sampled in the northern part of the proposed range of *S. f. leucogenys* (BEL, MOY, TAR, PL) form a well supported clade with all *S. f. illigeri* individuals, while all animals from the only southern sampling point (PA), south of the Rio Pachitea, clearly fall into the clade with all eastern/south-eastern taxa, *S. f. weddelli,* *S. f. fuscicollis, S. f. nigrifrons* and *S. melanoleucus*. These southern individuals also show the very dark-mantled phenotype, as described for *S. f. leucogenys*. Morphological and mitochondrial evidence suggests a range limit for *S. f. leucogenys* far more southwards than described by Hershkovitz (1977) and an inclusion of the northern populations of *S. f. leucogenys* into *S. f. illigeri*. For the determination of the exact border between these two taxa, if there is one, further research is needed.

3.4.4 The status of S. n. nigricollis and S. graellsi

The black-mantled tamarin, *S. nigricollis,* is listed by Hershkovitz (1977) as species with the three subspecies, *S. n. nigricollis, S. n. hernandezi* and *S. n. graellsi*. The status of the latter is subject to discussion whether it should be elevated to full-species rank or not. This assumption is based on the putative sympatry of *S. graellsi* with *S. n. nigricollis* in southern Colombia (region of Puerto Leguizamo) (Hernandez-Camacho and Cooper, 1976). This claim, however, may have been due to a mislabeled museum specimen (Hershkovitz, 1977). Nevertheless, *graellsi* is featured as a distinct species by Rylands et al. (2000) and Groves (2001).

In our study, *S. graellsi* forms a monophyletic clade, but is nested within the *S. n. nigricollis* clade. Thus, *S. n. nigricollis* appears paraphyletic. Moreover, the clade consisting of all *S. n. nigricollis* and *S. graellsi* individuals (clade 3) is nested in *S. fuscicollis*. Also during the field survey, we could not detect any clear barrier or geographical delimitation between the two taxa. The north-western part of the interfluvium between the Rios Putumayo and Napo-Amazonas seems to be the range of *S. graellsi*, the south-eastern part the range of *S. n. nigricollis*. Since there is no major geographic barrier in this interfluvium, it is more likely that a zone of intergradation exists, perhaps with intermediate phenotypes, instead of a clear barrier. Despite the unresolved genetic relationships and the relatively recent divergence time (0.26 mya), the coloration of *graellsi* is well-defined (Hershkovitz 1977) and can be verified in the field as well as in museum specimens. Taking this into account, we favor subspecific status for *graellsi* as previously suggested by Hershkovitz (1977) and Rylands and Mittermeier (2009).

Questionable is also the status of *S. f. fuscus*. Moore and Cheverud (1992) suggested the elevation of this subspecies to full species rank due to differences in facial morphology. In previous genetic studies, *S. f. fuscus* showed more similarities with *S. nigricollis* than with other *S. fuscicollis* subspecies (Cropp et al., 1999). For further research, the inclusion of *S. f. fuscus* and *S. n. hernandezi* would be desirable to fully resolve the complex phylogeny and taxonomy of the *S. nigricollis* complex in northern Peru, Colombia and Ecuador.

3.4.5 Species concepts and general taxonomic implications

Our data show that all taxa of the *S. nigricollis* group derived from a common ancestor, which separated early from the lineage leading to all larger bodied tamarins. Despite this early separation from other tamarins, the taxa of the *S. nigricollis* group evolved relatively recently during a rapid radiation ~2.9 mya.

Based on our data, *S. fuscicollis* can be considered as polyphyletic and the taxonomic status of the numerous subspecies needs to be reconsidered. The taxa *nigricollis, graellsi, tripartitus* and *melanoleucus*, lately featured as distinct species, are included in the *S. fuscicollis* clade and evolved during the same radiation. Sympatry between *S. nigricollis, S. fuscicollis* and *S. tripartitus* has yet to be proven and is lacking solid evidence. If we accept the taxonomic status of *S. tripartitus, S. melanoleucus* and *S. nigricollis/graellsi* as distinct species, the status of other taxa, especially the different subspecies of *S. fuscicollis*, has to be revised as well, as already suggested by several authors (e.g. Heymann, 2000; Rylands and Mittermeier, 2009). Depending on the applied species concept, one can discuss the taxonomy of the *S. nigricollis* species group in two directions. Either all taxa are collapsed as subspecies of a single, widespread and diverse species or they are all upgraded to full species.

From a more conservative point of view, we could consider the taxa of the *S. nigricollis* group, included in this study, as members of one widespread super-species. Accordingly, *nigricollis, graellsi, tripartitus, lagonotus, nigrifrons, fuscicollis, weddelli* and *melanoleucus* would be included as local subspecies into the super-species *S. nigricollis*. For the northern *leucogenys/illigeri* clade and southern *leucogenys* clade, further research will be necessary to resolve their taxonomic status.

Recently, another concept of species delimitation prevailed in primate taxonomy. The application of the so-called phylogenetic species concept, as it was suggested for example by Groves (2001), which defines a species as ”*the smallest cluster of individual organisms within there is a parental pattern of ancestry and descent and that is diagnosably distinct from other such clusters by a unique combination of fixed character states*“ (Cracraft, 1983). The consequence is a dramatic increase in primate species numbers. Nevertheless, at present it is widely used and applied in most recent studies on primate taxonomy. In platyrrhine primates, almost all former subspecies of titi monkeys (*Callicebus*) (van Roosmalen, 2002) and marmosets (*Callithrix* and *Mico*) (see Groves, 2001) have been elevated to full species. So far, it has been applied to the tamarins only on a limited scale.

Most of the *S. nigricollis* species group members emerged during a time in which speciation events occurred also in other platyrrhines. For example, Lavergne et al. (2010) found divergence times between species of squirrel monkeys (*Saimiri*) 1.7-4.3 mya, and Cortés-Ortiz et al. (2003) estimated the split between some howler monkey (*Alouatta*) species at 2.3-4.0 mya. Within *Saguinus*, *S. labiatus* and *S. mystax* diverged 1.15 mya, which is significantly more recent than the splits between most of the taxa of the *S. nigricollis* group, although both taxa are widely recognized as distinct species. Among the *S. nigricollis* group, terminal clades can be directly assigned to a taxon with distinct coat coloration. The only exception hereof is *S. f. leucogenys* and *S. n. nigricollis.* By applying the phylogenetic species concept on the herein examined tamarin taxa, we can identify *lagonotus, tripartitus, nigrifrons, weddelli* and *fuscicollis* morphologically and genetically as distinct and well-defined entities and, thus, we would recommend full species status for these taxa. For *graellsi*, a subspecific rank should be reconsidered as previously suggested by Hershkovitz (1977). For final conclusions concerning *melanoleucus*, which is genetically closely related to *S. f. weddelli*, despite its striking distinctiveness in fur coloration, more individuals and also individuals from the intermediate morphs, *crandalli* and *acrensis*, should be examined. Due to its close genetic affiliation, we do not support the specific status as introduced by Rylands et al. (1993), but considering its distinctive coloration, we recommend *melanoleucus* as subspecies of *S. weddelli*. More complicated is the situation for *S. f. illigeri* and *S. f. leucogenys*, where we can observe an incongruity between the genetic data and the described data on fur coloration and distribution. To further examine the distinctiveness, especially of the southern *leucogenys* clade, data from more locations from the southern parts of the distribution area and in particular from the area between our northern sampling points in San Martìn and the north bank of the Rio Pachitea would be necessary. Furthermore, a detailed revision of the available museum material and the application of additional genetic markers (nuclear loci) to detect possible hybridization are recommended. Provisionally, we include tamarins of the northern parts of the *leucogenys* range into *S. illigeri* and keep the name *S. leucogenys* for the southern populations. For the determination of exact distribution areas, further research and sampling is required. A summary of the taxonomic implication derived from our data is given in Table 3.3.

Table 3.3 Classification of tamarins examined in this study, following Hershkovitz (1977) and Groves (2001) compared with the herein proposed classification*.

Hershkovitz 1977	Groves 2001	Proposed classification
S. nigricollis nigricollis	*S. nigricollis nigricollis*	*S. nigricollis nigricollis*
S. nigricollis graellsi	*S. graellsi*	*S. nigricollis graellsi*+
S. fuscicollis fuscicollis	*S. fuscicollis fuscicollis*	*S. fuscicollis*
S. fuscicollis illigeri	*S. fuscicollis illigeri*	*S. illigeri* (including northern *leucogenys*)
S. fuscicollis leucogenys	*S. fuscicollis leucogenys*	*S. leucogenys*
S. fuscicollis nigrifrons	*S. fuscicollis nigrifrons*	*S. nigrifrons*
S. fuscicollis lagonotus	*S. fuscicollis lagonotus*	*S. lagonotus*
S. fuscicollis weddelli	*S. fuscicollis weddelli*	*S. weddelli weddelli*
S. fuscicollis melanoleucus	*S. melanoleucus melanoleucus*	*S. weddelli melanoleucus*+
S. fuscicollis tripartitus	*S. tripartitus*	*S. tripartitus*

*Species splittings are marked with double lines. +Differences in fur coloration

3.4.6 Biogeographic implications

Molecular phylogenetic studies on various Amazonian organisms indicate major diversifications during the late Miocene or Pliocene when the Eastern Cordillieras of the northern and central Andes uplifted (Gregory-Wodzicki, 2000; Garzione et al., 2008). During that time, ~11 mya, western Amazonia was mainly influenced by the formation of the Acre mega-wetland, a large fluvio-tidal landscape covering most of the area in central and western Amazonia and the establishment of the main transcontinental drainage system (Figueiredo et al., 2009). Thus, the great Acre wetland could have separated the ancestors of the *S. nigricollis* species group from the *Saguinus* stem, which led to the larger-bodied tamarins. The radiation within the *S. nigricollis* group occurred much later, according to our dataset ~ 3 mya, which could coincident with the decline of the Acre wetland and the following formation of the Amazonian river system (Wesselingh et al., 2010). The expansion of tropical rainforest across the former wetland area enabled a subsequent dispersal of the ancestral *S. nigricollis* stock. The emerging rivers formed barriers to the dispersing tamarins and enhanced their diversification.

If we compare this hypothesis with other Neotropical primates, we detect parallels with the biogeography and dispersal pattern in *Saimiri.* Lavergne et al. (2010) proposed a western Amazonian origin of the genus and initial speciation during the Plio/Pleistocene. The disappearance of the great Acre mega-wetland and the subsequent formation of the Amazonian fluvial system could have enhanced speciation in both groups. However, since the phylogenetic relationships among the different clades within the *S. nigricollis* group are not significantly resolved we cannot determine a direction of dispersal.

Amazonia's extremely rich biodiversity, which we see today, surely evolved not during by a single, short-term event (Wesselingh et al., 2010). As recent molecular studies on different groups of organisms suggest, the diversification of life in Amazonia was a more continuous process, which took place throughout the Cenozoic. For some groups, a sort of acceleration of this process is indicated during the Miocene (see Rull, 2008). Unfortunately, molecular methods can only reflect the situation, we find in modern biota (Wesselingh et al., 2010). We should be aware that correlating geological and putative speciation events might be oversimplified, as the documentation for both is often incomplete (Lundberg et al., 1998).

The study shows that mitochondrial markers are useful to identify distinct lineages in tamarins. Future research is needed, especially the examination of the Brazilian, Ecuadorian and Colombian taxa, to complete the picture of the taxonomy of one of the most diverse genera of New World primates.

Acknowledgements

We thank the field assistant Manuel Shahuano Tello for excellent work during the field surveys, Stephan Behl and Peter Gottleuber for their help and assistance in the field, and Christina Oberdieck for help and support in the lab. We are further grateful to Sandra Suarez for providing samples from Bolivia. Thanks also to Richard Kraft and Juliane Diller for giving us the possibility to collect samples from the Zoological State Collection in Munich and at the Biological Field Station Panguana, Peru. We thank INRENA in Lima for authorizing this study (106-2007-INRENA-IFFS-DCB) and to the dean of the Forestry Department of the Universidad Nacional de la Amazonía Peruana in Iquitos for administrative support. This study was financially supported by the German Primate Center, the Biodiversitäts-Pakt of the Wissenschaftsgemeinschaft Gottfried-Wilhelm Leibniz, and the Margot Marsh Biodiversity Foundation.

4 The Range of the Golden-mantle Tamarin *Saguinus tripartitus* (Milne Edwards, 1878): Distributions and Sympatry of Four Tamarins in Southern Colombia, Ecuador and Northern Peru

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Primates

Received: 17 June 2010 / Accepted: 5 August 2010

DOI 10.1007/s10329-010-0217-3

Abstract

A detailed understanding of the range of the golden-mantle tamarin, *Saguinus tripartitus* (Milne Edwards, 1878), in Amazonian Peru and Ecuador is of particular relevance, not only because it is poorly known but also because it was on the basis of its supposed sympatry with the saddleback tamarin (*Saguinus fuscicollis lagonotus*) that Thorington (*Am. J. Primatol*.15: 367–371, 1988) argued that it is a distinct species rather than a saddleback tamarin subspecies, as was believed by Hershkovitz (*Living New World Monkeys*, Vol. I. The University of Chicago Press, Chicago, 1977). A number of surveys have been carried out since 1988 in the supposed range of *Saguinus tripartitus*, in both Ecuador and Peru. Here we summarize and discuss these, and provide a new suggestion for the geographic range of this species; that is, between the ríos Napo and Curaray in Peru, extending east into Ecuador. We also review current evidence for the distributions of Spix's black-mantle tamarin (*S. nigricollis nigricollis*), Graells' black-mantle tamarin (*S. n. graellsi*) and the saddleback tamarin (*S. fuscicollis lagonotus*), which are also poorly known, and examine the evidence regarding sympatry between them. We conclude that despite the existence of a number of specimens with collecting localities which indicate overlap in their geographic ranges, the fact that the four tamarins are of similar size and undoubtedly very similar in their feeding habits militates strongly against the occurrence of sympatry among them.

Keywords: Callitrichidae, tamarin, *Saguinus,* Taxonomy, Distribution, Sympatry, northwestern Amazon

4.1 Introduction

The golden-mantle tamarin, *Saguinus tripartitus* (Milne Edwards, 1878), of the western Amazon, was one of the fourteen subspecies of saddleback tamarin, *Saguinus fuscicollis* (Spix, 1823), proposed in the taxonomy of Hershkovitz (1977). Thorington (1988) showed that that the information concerning its geographic distribution was scarce and confused but, following a reappraisal of the evidence, he argued that it was in fact a full species due to its sympatry with the red–mantle saddleback tamarin, *S. fuscicollis lagonotus.* Since this publication, a number of surveys have been carried out in Ecuador (Albuja, 1994; de la Torre, 1996), and Peru (Encarnación et al., 1990; Aquino and Encarnación, 1996; Heymann, 2000; Heymann et al., 2002; Aquino et al., 2005; Matauschek, in prep.; Aquino, unpubl. data). Here we summarize the information obtained in these surveys and propose new boundaries for the geographic range of this species. We also examine the evidence regarding sympatry with *S. fuscicollis*, as well as with Graells' black-mantle tamarin (*Saguinus nigricollis graellsi*) and Spix's black-mantle tamarin (*Saguinus nigricollis nigricollis*).

4.2 The Distribution of *Saguinus tripartitus*

The distribution of *Saguinus tripartitus* has been in doubt for many decades. The type of *Midas tripartitus* Milne Edwards, 1878 is a skin of a female in the Museum National d'Histoire Naturelle in Paris (No.122 (653[633A. 1877-562]), and the type locality is Río Napo, Oriente, Ecuador (Hershkovitz, 1977; Groves, 2001). Ávila-Pires (1974) proposed that the species occurs along the north bank of the Río Amazonas right up to the mouth of the Río Putumayo-Içá in Brazil. Apparently supporting this, Hernández-Camacho and Cooper (1976, p.39; see also Hernández-Camacho and Defler 1985) reported that *S. fuscicollis* is not known in the immediate region of Leticia (on the Río Amazonas, north bank, in the Colombian trapezium), but that they had examined specimens referable to *S. fuscicollis tripartitus* in Leticia, which were reportedly from the Colombian bank of the Río Amazonas; Puerto Nariño by the Quebrada Pichuna Yacú, upstream of Leticia (Fig. 4.1). Neville et al. (1976) and Freese et al. (1982) reported that they found only *S. nigricollis* during surveys along the Río Ampiyacu, north of the Río Amazonas, near to Colombia, in Peru (Fig. 4.1). Hershkovitz (1977) concluded that *S. tripartitus* occurred between the Ríos Putumayo (right bank) and Napo (left bank), west to the Andes in Peru and Ecuador, and east to beyond the confluence of the Río Napo with the Río Amazonas.

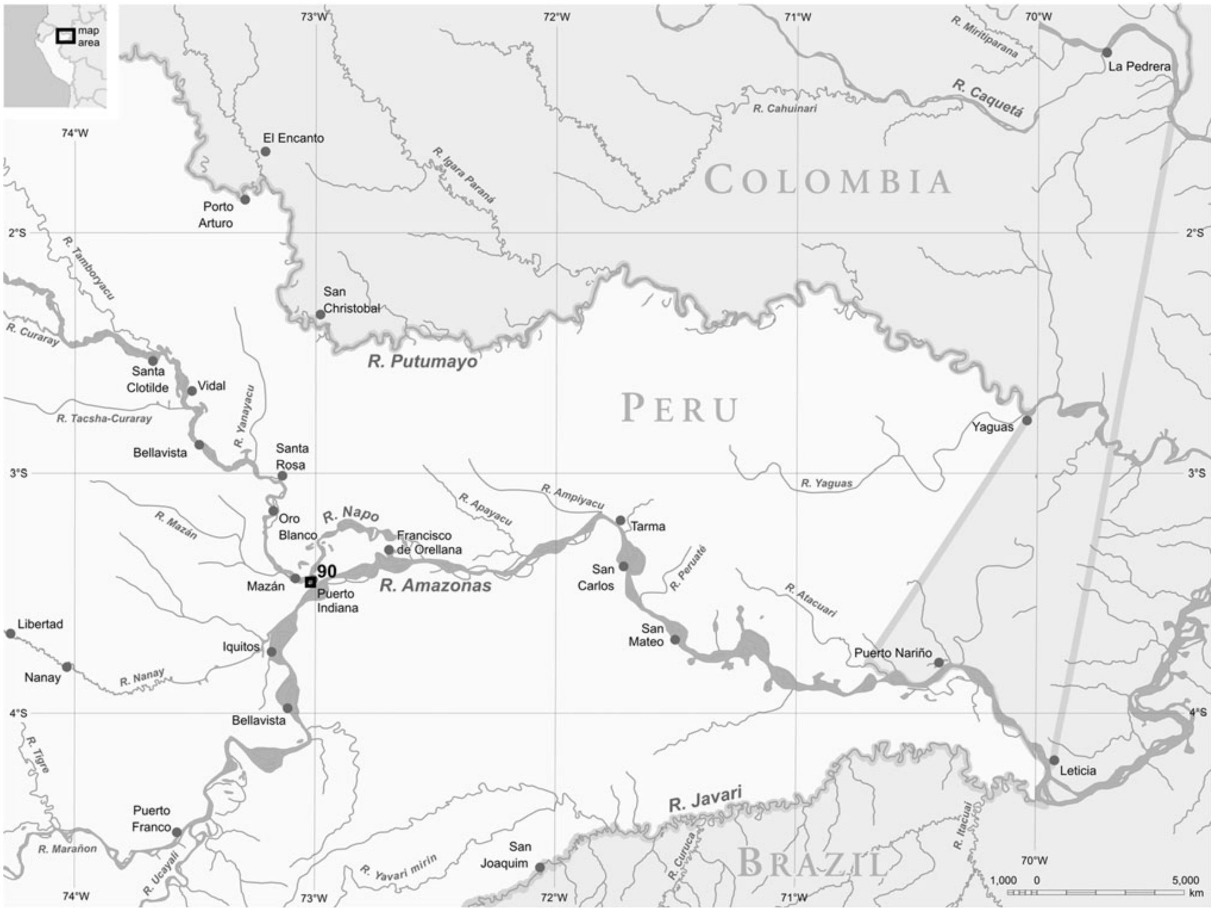

Fig. 4.1 The western Amazon, including southern Colombia, northeastern Peru and Brazil. Locality 90 is Puerto Indiana, cited as the north bank Río Marañon and a locality for *Saguinus tripartitus* and *Saguinus fuscicollis lagonotus* by Hershkovitz (1977). See Table 4.1. Map © Kellee Koenig / Conservation International.

Defler (1994, 2004) mentioned that the occurrence of *S. tripartitus* between the Ríos Putumayo and Amazonas in Colombia had still to be confirmed. In more recent surveys, Montenegro and Escobedo (2004), like Neville et al. (1976), found only *S. nigricollis* (presumed by them to be *S. n. nigricollis*) at survey sites along the ríos Ampiyacu and Apayacu (a little to the west of Ampiyacu), but recorded *S. nigricollis* and a saddleback tamarin (*S. fuscicollis*) at sites a little east on the Río Yaguas (Fig 4.1). They made no mention of *S. tripartitus*, and one could suppose that the saddleback tamarin they saw is an undescribed subspecies (*S. f. fuscus* occurs to the north of the Putumayo and *S. f. nigrifrons* to the south of the Río Amazonas [Aquino and Encarnación, 1994; Defler, 2004]). *S. tripartitus* has never been recorded in Brazil.

Hershkovitz's (1977) belief that it occurred north of the Río Napo east as far as the Río Amazonas in Peru was based on specimens collected by Carlos Olalla and his sons in 1926 and labelled Puerto Indiana, a town on the left bank of the Río Amazonas, approximately 40 km north of Iquitos. Hershkovitz (1977) placed Puerto Indiana north of the Río Marañon (Amazonas) just downstream of the mouth of the Río Napo (Fig. 4.1, Table 4.1) but, as pointed out by Thorington Jr. (1988), Puerto Indiana is in fact on the left bank of the Rio Amazonas upstream of the mouth of the Río Napo (Fig. 4.1). Puerto Indiana is also a locality for *S. f. lagonotus* (see Table 4.1), and for this reason Thorington (1988) concluded that 1) *S. tripartitus* occurs on the *right* bank of the lower Río Napo at its mouth (not the left bank as argued by Hershkovitz [1977]), and 2) that, occurring on the right bank, it is sympatric with *S. f. lagonotus*, and therefore must be considered a distinct species. Thorington Jr. (1988) also believed that a further two localities identified by Hershkovitz (1977) the mouth of the Curaray (south of the Napo, locality 80, gazetteer p.928, Fig XIII.4 on page 916 in Hershkovitz [1977]; see Table 4.1 and Fig 4.1), and San Francisco (north of the Napo, locality 66, gazetteer p.927, Fig XIII.4 on page 916 in Hershkovitz [1977]; see Table 4.1 and Fig. 4.2) confirmed sympatry between the saddleback tamarins *tripartitus* and *lagonotus* (Table 4.1).

This confusion stimulated Aquino and Encarnación (1996) to investigate the Napo basin specifically to clarify the distributions of *S. tripartitus* and *S. f. lagonotus*. The results were surprising. They were unable to find any evidence at all for the occurrence of *S. tripartitus* along either bank of the lower Río Napo. Their survey included Puerto Indiana, the localities of Francisco de Orellana and Mazán nearby, as well as the left bank tributaries of the Río Amazonas west of Puerto Indiana, the Ríos Ampiyacu, Apayacu, Peruaté, and Atacuarí, and tributaries on the left (Río Yanayacu) and right (Río Tacsha-Curaray) banks of the lower Río Napo (Fig. 4.1). In none of these areas were they able to locate *S. tripartitus* or find any evidence of its occurrence there. On the Río Mazán, a tributary entering the Río Amazonas upstream (west) of the Río Napo (Fig 4.1), they found only *S. f. lagonotus* and *S. n. graellsi*. They considered, and discarded, the possibility that *S. tripartitus* had become extinct in the area, and were forced to the conclusion that the locality attributed by the Olalla Bros. (Puerto Indiana) was not where the type had been collected. On the same expedition (1925/1926) that included Puerto Indiana, the Olalla Bros. also collected and purchased animals and specimens along and from the Río Curaray, an upstream tributary of the Río Napo (Table 4.1), where the occurrence of *S. tripartitus* was confirmed by Aquino and Encarnación (1994, 1996), and later by Heymann (2000; Heymann et al., 2002) and Aquino et al. (2005).

Table 4.1 The five localities of *Saguinus fuscicollis tripartitus* listed by Hershkovitz (1977).

Hershkovitz (1977)	Notes
66 San Francisco, Río Napo, left bank, 0º30'S, 76º22'W. *Saguinus fuscicollis tripartitus* P. Hershkovitz, February–March 1936, on left bank at 200 meters. *Saguinus nigricollis graellsi* P. Hershkovitz, February–March 1936, on right bank at 200 meters.	North of the Rio Napo. The proximity of the locality of Coca (also Francisco de Orellana) that is the type locality of *S. f. lagonotus* indicated sympatry, and comprised part of the argument of Thorington Jr. (1988) that *tripartitus* should be considered a distinct species. See Figure 2.
67a Lagarto Cocha, mouth, 0º39'S, 75º16'W *Saguinus nigricollis graellsi* Olalla Bros., January 1926. *Saguinus fuscicollis tripartitus* Olalla Bros., January 1926.	North of the Río Napo. The Lagartococha is tributary of the Río Aguarico which enters the Río Napo from the north. See Figure 2.
67b Aguarico (Río), 0º59'S, 75º11'W *Saguinus fuscicollis tripartitus* Olalla Bros., January 1924, December 1925.	North of the Río Napo. Napier (1976) lists the two specimens as follows: 1. 1934 .9.10.23. Male adult, skin, skull. "Oriente, near the R. Napo, 2000-3000 ft." January 1924. 2. 1934.9.10.24. Male adult, skin, skull, slide of hair. "Oriente, near Aguarico [0º, 76º20'W], 2000 ft." December 1925. See Figure 2.
80 Curaray (Río) (mouth), 2º22'S, 74º05'W, 140 meters. *Saguinus fuscicollis tripartitus* Olalla Bros., October-December 1925. *Saguinus fuscicollis lagonotus* Olalla Bros., May 1926 *Saguinus fuscicollis graellsi* Olalla Bros., 1925.	South of the Río Napo. The Río Curaray is a right bank tributary of the lower Río Napo. Thorington Jr. (1988) inferred that *S. f. lagonotus* and *S. f. tripartitus* were sympatric at this locality. See Figure 2.
90 Puerto Indiana, Río Marañon, north bank, 3º20'S, 72º40'W, 100 meters. *Saguinus fuscicollis tripartitus* Olalla Bros., May, June, July 1926. *Saguinus fuscicollis lagonotus* Olalla Bros., August 1926.	South of the Río Napo. As pointed out by Thorington Jr. (1988), Puerto Indiana is in fact on the left bank of the Río Marañon (Amazonas) abovethe mouth of the Río Napo. The apparent sympatry of the two subspecies comprised part of the argument of Thorington Jr. (1988) that *tripartitus* should be considered a distinct species. See Figure 1.

Numbers in the left hand column are those used by Hershkovitz (1977; pp.927–928, and map p.916). See Figures 1 and 2.

From the surveys of Aquino and Encarnación (1996), Heymann (2000; Heymann et al., 2002) Aquino et al. (2005) and Aquino (this paper), it is now evident that in Peru *S. tripartitus* occurs on the right bank of the Río Napo only west from the mouth of the Río Curaray (Fig. 2). Aquino et al. (2005) registered 14 groups in three localities in the basin of the Río Aushiri (mainstream and tributary Rio San José) (Fig. 4.2). Based on a sighting of one group on the south (right) bank of the lower Río Yuvineto (Encarnación et al., 1990), Aquino and Encarnación (1996) concluded that the distribution of *S. tripartitus* extends to the left bank of the Río Napo only along the upper reaches of the Río Santa Maria, a northern (left bank) tributary of the Napo, and from there north to the Río Putumayo on the Colombian border, and in the west as far as the right bank of the Río Güeppi and left bank of the Río Lagartococha (Fig. 4.2). However, C. Matauschek (in prep.) was unable find *S. tripartitus* on the left (north) bank of the Napo along its entire course in Peru. Interviews of settlers at different locations along the course of the Napo showed that they did not know of *S. tripartitus* north of the Napo, confirming its presence only south of the Napo.

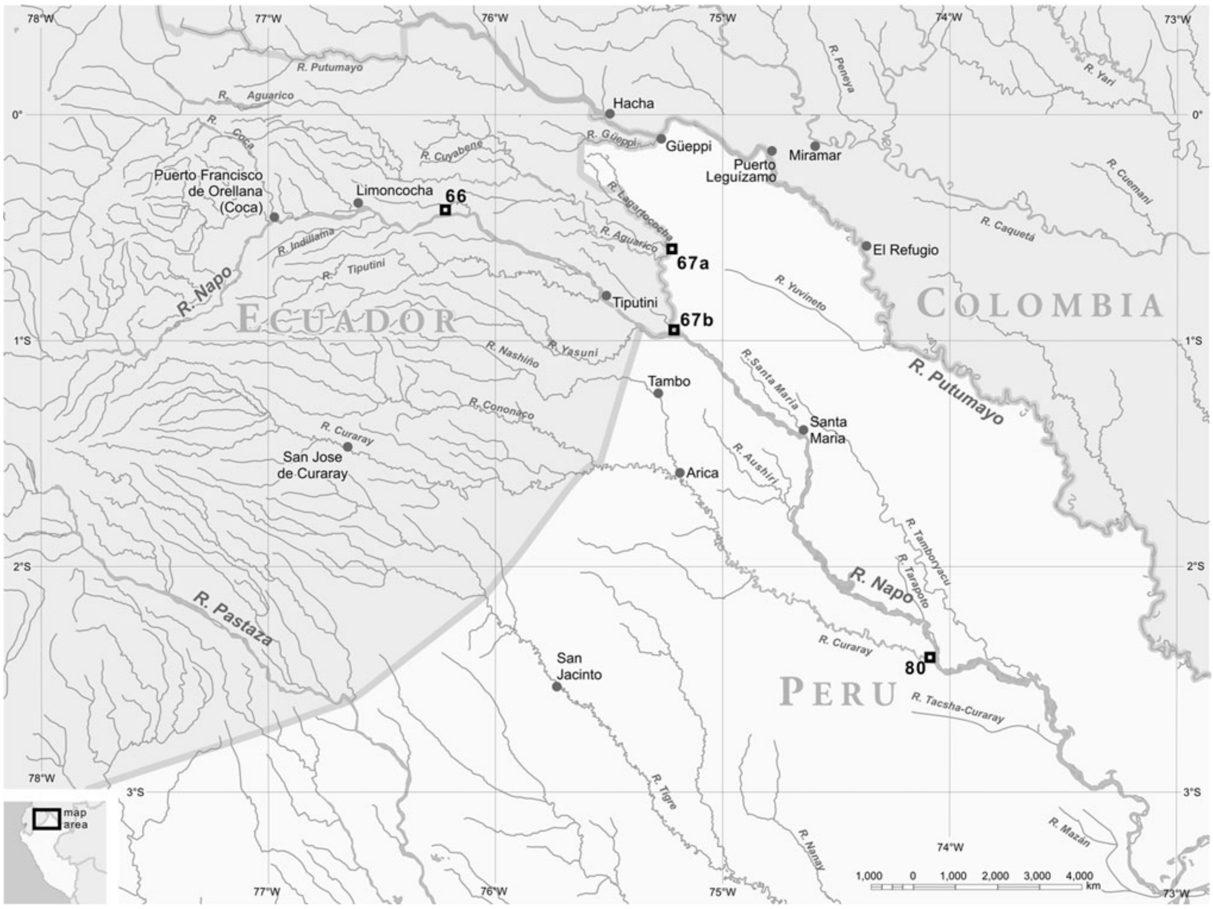

Fig. 4.2 The western Amazon, including eastern Ecuador, southern Colombia, and northeastern Peru. For an explanation of localities 66, 67a, 67b and 80, see Table 4.1. Map © Kellee Koenig / Conservation International.

The northern limit to the distribution in Peru could be the Río Putumayo, but the evidence denies its occurrence north of the Río Napo in Ecuador. There, Albuja (1994) reported that it is restricted to the left bank of the Río Curaray, north to and *not beyond* the lower reaches of the Río Napo in Ecuador, but limited to the Río Tiputini (a right bank tributary of the Napo) further west. Hershkovitz (1977), on the other hand, reported it from the left (north) bank of the Río Napo at the locality of San Francisco (two specimens in the University of Michigan Museum of Zoology, collected by Hershkovitz in 1936 [locality 66, Table 4.1, Fig 4.2]). Olalla also supposedly collected *S. tripartitus* from the mouth of the Río Aguarico, a northern (left bank) tributary of the Río Napo on the border of Ecuador and Peru (specimen in the British Museum of Natural History, see Table 4.1), as well as the mouth of the Río Lagartococha, a northern (right bank) tributary of the Río Aguarico in Ecuador (two specimens in the American Museum of Natural History). This would indicate that at least near to the Peruvian border, *S. tripartitus* extends to the north of the Río Aguarico, north of the Río Napo. However, de la Torre et al. (1995a) recorded only *S. n. graellsi* from the Río Cuyabeno (a left bank tributary of the Río Aguarico west of the Río Lagartococha). Over seven years during her research in the Cuyabeno Faunal Reserve, Stella de la Torre travelled frequently along the Río Lagartococha in Ecuador and was unable find any evidence indicating that *S. tripartitus* occurs there (on either bank). De la Torre (1996) stated that there is no evidence of its presence north of the Río Napo, nor along the Ríos Aguarico, Cuyabeno and Lagartococha (p.88). There is a possibility that the Lagarto Cocha [*sic*] locality of Olalla (January 1926) listed by Hershkovitz (1977, p.927) in fact refers to a lake of the same name south of the Río Napo. This being the case, evidence for its occurrence to the north of the Napo in Ecuador would be restricted to Hershkovitz's locality of San Francisco, and the Río Aguarico locality of Olalla, the provenance of which is given by Hershkovitz (1977) on page 927 as Aguarico (Río), and on page 658 as near Aguarico, Río Napo, and is mapped on page 916 at the mouth of the Río Aguarico. Napier (1976) listed the two Olalla specimens in the British Museum with localities (labels) which are difficult to interpret (Table 4.1). We were unable to locate Oriente, and the altitudes 2000 to 3000 feet cannot refer to the mouth of Aguarico (see notes in Table 4.1). It might be that Hershkovitz (1977) interpreted the localities from the travels of the Olalla Bros. and the possibility remains that both specimens were really collected from the south bank of the Napo, opposite the mouth of the Río Aguarico or, as would be indicated by Aquino and Encarnación (1996), from the Peruvian, right bank of the Río Aguarico.

The San Francisco locality of Hershkovitz (66, see Table 4.1, Fig. 4.2) remains a mystery. We were unable to locate San Francisco on maps of Ecuador, but the locality, as he plotted it on his map on page 916 in Hershkovitz (1977), is in or near to the Limoncocha Biological Reserve (north bank of the Napo), and near (opposite) the mouth of the Río Indillama. Hershkovitz (1977, 1982) also collected a specimen of *S. n. graellsi* from San Francisco, on the right bank of the Napo (locality 66, p.927). Despite this, we conclude (see below) that it is unlikely that this species occurs south of the Napo. De la Torre (1996) recorded *S. f. lagonotus* between the Río Indillama and the Río Napo, and has interviewed, in different places and times, six old Secoya men who lived in the area of the ríos Santa María and Putumayo, and all of them informed that they had never seen *S. tripartitus* in that area, only *S. nigricollis*.

The lack of evidence today for the occurrence of *S. tripartitus* on the right bank of the Río Lagartococha, marking the Ecuadorian frontier with Peru, indicates that its range in the interfluvium of the Ríos Napo and Putumayo in Peru is limited to the east (left bank) of the river, as indicated by Aquino and Encarnación (1996) or even further restricted as indicated above.

De la Torre (1996, 2000) and Kostrub (1997) have observed golden-mantled tamarins in the Yasuní National Park, between the ríos Yasuní and Indillama, and de la Torre (1996) confirmed the occurrence of *S. tripartitus* between the southern bank of the Río Tiputini and the northern bank of the Río Yasuní. Kostrub (1997) reported that in Ecuador most, if not all, of the range of golden-mantle tamarin lies within the boundaries of the Parque Nacional Yasuní and the Reserva Indígena Huaorani (p.102).

The western limits to the range of *S. tripartitus* in Ecuador are not clearly defined but Albuja (1994) extended it as far as the middle reaches of the ríos Tiputini and Curaray (*S. f. lagonotus* occurs at the headwaters of the Río Curaray), the upper Río Cononaco, and the entire basins of the Ríos Yasuní and Nashiño (Fig. 4.3).

Concerning the information on *S. tripartitus* north of the Napo in Peru, we also contemplate the possibility that this is a new taxon that is phenotypically similar to, but distinct from, *S. tripartitus*.

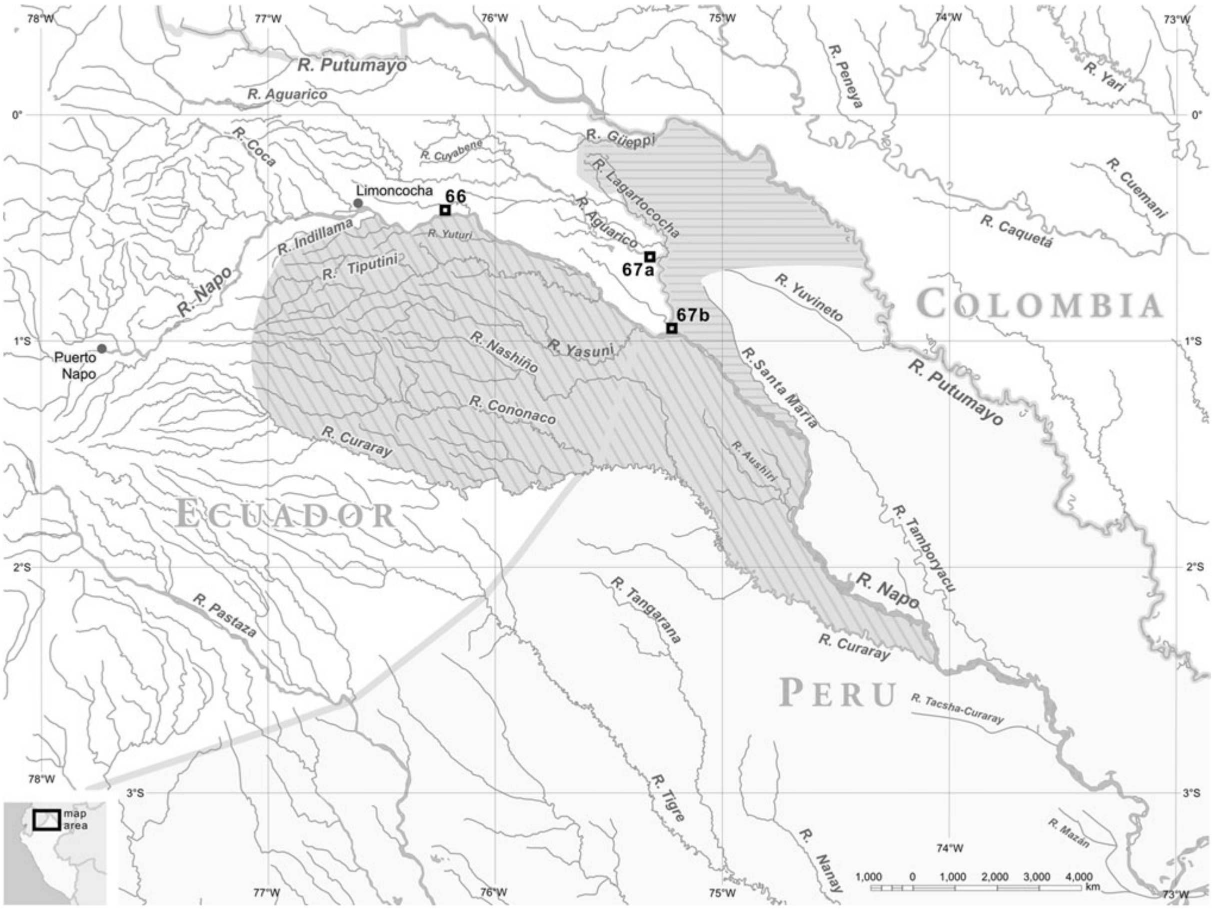

Fig. 4.3 Hypothetical distribution of *Saguinus tripartitus* (shaded). Its occurrence in the shaded area north of the Río Napo is doubtful. Map © Kellee Koenig / Conservation International.

4.3 Sympatry

The question of sympatry is central to understanding the taxonomic status of *S. tripartitus*. There are three tamarins in the region which, in the literature, have been considered to be partially sympatric.

4.3.1 Saguinus fuscicollis lagonotus (Jiménez de la Espada, 1870)

In Peru, this saddleback tamarin occurs south of the ríos Napo and Curaray, east to the Río Amazonas, and north of the Río Marañón (Aquino and Encarnación, 1994). Hershkovitz (1977) and Tirira (2007) indicated that the western limit to its range was the Río Santiago, a north (left) bank tributary of the Marañón. In their text, Aquino and Encarnación (1994, p.14) indicated its occurrence further west and south to the Río Chinchipe, although their map (p.108) takes it only as far west as the basin of the Río Cenepa (both also north (left) bank tributaries of the Marañón) (Fig. 4.4). The Peruvian range is quite well documented in terms of collecting localit ies, with Hershkovitz (1977) listing specimens from the Ríos Nanay, Tigre, Pastaza and upper Santiago. Significantly, Hershkovitz (1977) gives no localities north of the Río Curaray (one at the mouth of the Curaray was collected by the Olalla Bros.1926 and presumably from the south [right bank] of the river [Heymann, 2000]). Its type locality is based on syntypes from the three localities; two in Ecuador: La Coca, Río Napo and Humuyacu, Río Napo, nearby (both plotted as locality 63, Fig. XIII.4, p.916, in Hershkovitz [1977]) and Tarapoto in Peru, a tributary of the left bank of the Napo near and on the opposite bank of the mouth of the Curaray (locality 79, Fig. XIII.4, p.916, in Hershkovitz [1977]). Taropoto is also a locality for *S. n. graellsi* as is the Río Curaray, and the reason why Hershkovitz (1982) indicated sympatry between *S. n. graellsi* and *S. f. lagonotus*. If we follow the assertion of de la Torre (1996), it does not occur in eastern Ecuador between the Ríos Curaray and Tiputini, where *S. tripartitus* is present. Albuja (1994), on the other hand, said that *S. f. lagonotus* and *S. tripartitus* were both present in the Tiputini and Tambococha localities he reported (see Table 4.2). The range map of *S. f. lagonotus* provided by Tirira (2007) covers the entire range of *S. tripartitus* in Ecuador, and Tirira stated (p.119), without explanation or reference, that the two tamarins are sympatric in the north of the range of *tripartitus* near the Río Napo (Fig. 4.5).

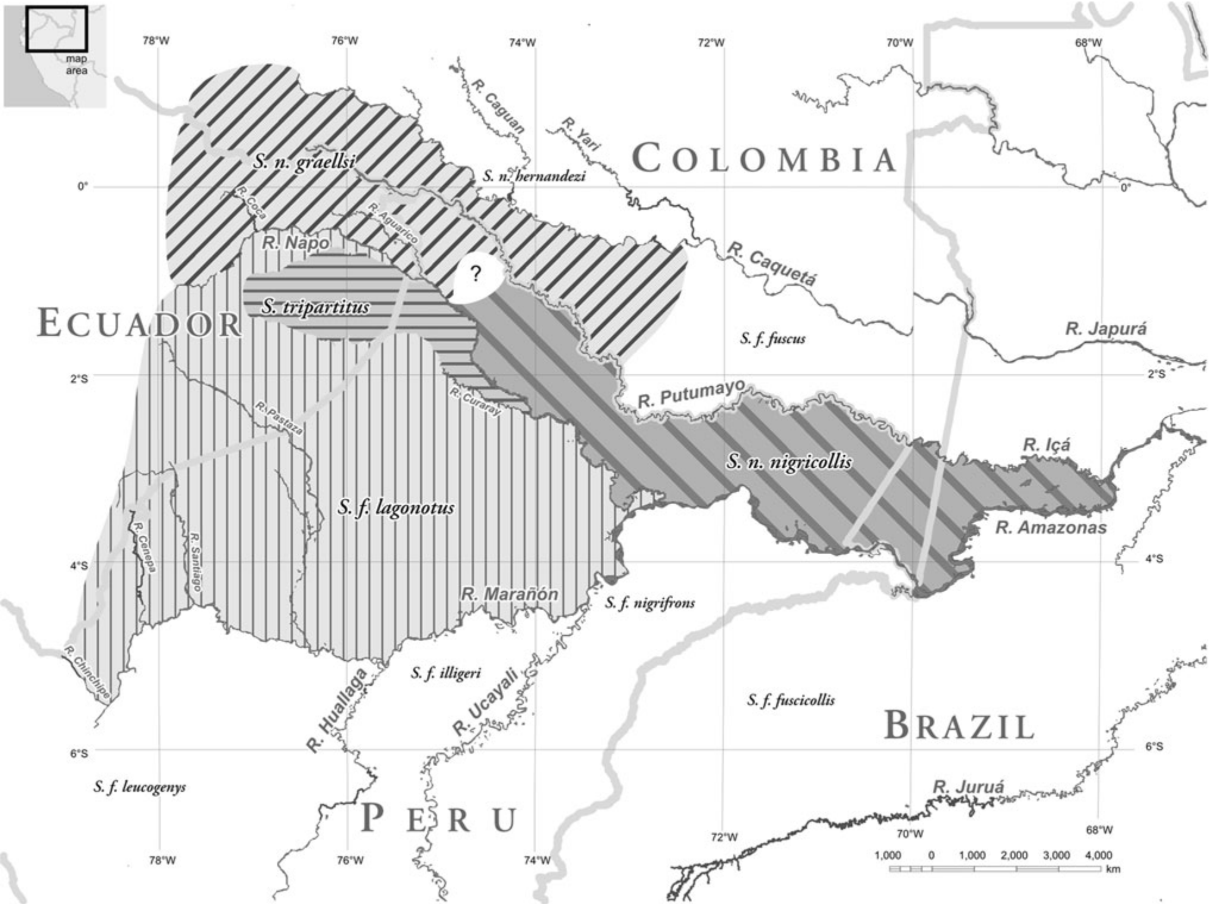

Fig. 4.4 Hypothetical distributions of four tamarins in the northeastern Amazon: *Saguinus nigricollis nigricollis*, *S. n. graellsi*, *S. tripartitus*, and *S. fuscicollis lagonotus*. Map © Kellee Koenig / Conservation International.

Table 4.2 Localities for *Saguinus tripartitus* in Ecuador and Peru

	Coordinates	Notes	Reference
Ecuador Tambococha, Río Tambococha, tributary of the Río Jatuncocha, right bank of the Río Napo, 5 km south of the mouth of the Río Tiputini	75º35'58"W, 00º54'12"S, alt. 187 m	Nov. 1991, obs. (6 groups). *S. fuscicollis lagonotus* also reported	Albuja (1994)
Río Tivacuno, mouth of, south bank tributary of the middle Río Tiputini	-	Mar. 1994, obs. (3 groups). *S. fuscicollis lagonotus* also reported	R. Muñoz, in Albuja (1994)
Yasuní Research Station, Yasuní National Park, north of Río Yasuni	-	Observation of 4 groups	de la Torre (1996)
Pompeya Sur – Río Iro highway, between Río Indillama (Río Tiputini) and Río Yasuní	-	Observation	de la Torre (1996), Kostrub (1997)
Yasuní National Park, Proyecto Primates Study Site	Near 76°33’W, 0°48’S	Observation	Kostrub (1997)
Tiputini Biodiversity Station (Universidad San Francisco), north bank of Río Tiputini, north of Yasuni National Park	76°20'W, 0°40'S	Observation	Kostrub (1997)

Peru Bellavista, Río Yuvineto, south of Río Putumayo	74°33'W, 02°02'S	Aug. 1978, obs. (2 groups)	Aquino & Encarnación (1996)
Puerto Elvira, south bank of Río Napo	74°32'W, 02°02'S	Jan. 1983, obs. (1 group), Nov. 1989 (2 groups); Aug. 1992 (1 group) + 1 specimen collected	Aquino & Encarnación (1996)
San Rafael, north bank of Río Curaray, near mouth	74°08'W, 02°22'S	Feb. 1983, obs. (3 groups), 1 specimen collected	Aquino & Encarnación (1996)
Correviento, north bank of Río Curaray	73°32'W, 02°15'S	Feb. 1983. obs. (1 group)	Aquino & Encarnación (1996)
Soledad, north bank of Río Curaray	74°25'W, 02°17'S	Feb. 1983, obs. (2 groups)	Aquino & Encarnación (1996)
Aushiri, Río Aushiri, south bank of Río Napo	74°44'W, 02°14'S	Dec. 1983, obs (1 group), 1 specimen collected	Aquino & Encarnación (1996)
Tempestad, south bank of Río Napo	74°52'W, 01°15'S	Dec. 1989, obs. (1 group)	Aquino & Encarnación (1996)
Ingano, south bank of Río Napo	74°11'W, 02°08'S	Dec.1991, obs. (2 groups), 1 specimen collected (pet infant male)	Aquino & Encarnación (1996)
Puerto Arica, north bank of Río Curaray	75°12'W, 01°28'S	Aug. 1992, 1 specimen collected	Aquino & Encarnación (1996)
Playa, Río Curaray, north (left) bank	74°53'W, 02°02'S	Sep. 1999, obs. (1 group)	Heymann (2000), Heymann et al. (2002)
Soledad, Río Curaray, north (left) bank	74°26'W, 02°16'S	Sep. 1999, obs. (1 group)	Heymann (2000), Heymann et al. (2002)
Vencedores, Río Napo, right bank	75°02’W 01°12’S	Jun. 2007, obs. (5 groups)	Matauschek (in prep.)
Campo Serio, Río Napo, right bank	74°70’W 01°80’S	Jun. 2007, obs. (1 group, 1 pet)	Matauschek (in prep.)

It was on the basis of supposed sympatry with *S. f. lagonotus* (through apparently coincident collecting localities) that Thorington (1988) argued that *S. tripartitus* should be considered a species. The key locality for Thorington’s argument of sympatry, however, was Puerto Indiana at the mouth of the Napo, but, as discussed above, Aquino and Encarnación (1996) have failed to find any evidence that *S. tripartitus* ever occurred there. Albuja (1994), however, noting that *S. f. lagonotus* was present at the two localities he recorded for *S. tripartitus* (see Table 4.2), supported Thorington’s (1988) thesis that *S. tripartitus* should be considered a distinct species. De La Torre (1996), Kostrub (1997) and Heymann et al. (2002), on the other hand, found no evidence of sympatry. In Ecuador, they would apparently be sympatric south of the Río Napo, but de la Torre (1996) stated that to date, there are no reports of sites where any two of the (Ecuadorian) tamarin species live in sympatry (p.88). She observed *S. f. lagonotus* south of the Río Napo in areas close to the Jatun Sacha Research Station, the Añangococha Lake and along the Pompeya Sur Río Iro highway as far south as, but not south of, the Río Indillama, a south bank tributary of the Napo. *S. tripartitus* occurs south of the Río Indillama to the Río Curaray. Heymann et al. (2002) recorded a clear separation of *S. tripartitus* (left bank of the Río Curaray) and *S. f. lagonotus* (right bank), and concluded that the ecological similarity [of *S. tripartitus*] with *Saguinus fuscicollis* and the lack of hard evidence for sympatry argue against it being a separate species (p.198). They agreed with Thorington (1988), however, in indicating that if *S. tripartitus* should be maintained as a distinct species, other *S. fuscicollis* subspecies should also be considered full species.

Although Aquino and Encarnación (1994) indicated that *S. n. graellsi* occurred south of the lower Napo to the Río Nanay, we believe that it is improbable (see below). We believe that the geographic ranges of *S. f. lagonotus* and *S. n. graellsi* do not overlap. During a survey in 2007, Matauschek (in prep.) encountered only *S. f. lagonotus* at all locations he visited on the right bank of the Napo, south of the Curaray in Peru. He saw both wild animals and pets (in three different villages). Also, local people clearly identified the resident tamarins on the pictures shown to them. There was no evidence for *S. f. lagonotus* occurring on the right bank of the Napo above the mouth of the Curaray.

4.3.2 Saguinus nigricollis nigricollis (Spix, 1823)

Information on the distribution of Spix's black-mantled tamarin, *S. n. nigricollis*, is confused and conflicting. Its type locality is the north bank of the Río Solimões, near São Paulo de Olivença, Amazonas, Brazil, and Hershkovitz (1977, 1982) placed it between the Ríos Solimões- Amazonas and Içá-Putumayo, at least as far west as the mouth of the Río Napo. Encarnación et al. (1990) and Aquino and Encarnación (1996) suggested its occurrence west from there along the left (north) bank of the Río Napo in Peru, upstream to the ríos Lagartococha and Güeppi on the border with Ecuador, where according to Hershkovitz (1982) it is replaced by *S. nigricollis graellsi*. In a recent exploration along the Río Napo, C. Matauschek (in prep.) encountered *S. n. nigricollis* on the north bank of the Napo opposite San Felipe, a village 270 km down the Rio Napo from the Ecuadorian border, and from there further east on different locations north of the Napo and the Amazon (for example, the ríos Ampiyacu and Apayacu, see Fig. 4.2). Montenegro and Escobedo (2004) saw black-mantled tamarins, which they presumed were *S. n. nigricollis* not *graellsi*, between the ríos Amazonas and Putumayo in Peru, in numerous localities they surveyed in the basins of the Río Yaguas, a south (right bank) tributary of the Putumayo, and the ríos Apayacu and Ampiyacu, both (left bank) tributaries of the Amazonas (see Fig. 4.1).

In Colombia, Hernández-Camacho and Cooper (1976) and Defler (1994) reported that it occurs north of the Río Putumayo to the Río Caquetá, and east to the Brazilian border, indicating its, as yet undocumented, presence between the ríos Japurá and Iça in Brazil (Hershkovitz, 1977, 1982). Evidence for its occurrence north of the Río Putumayo in Colombia is sparse, however. As discussed in detail by Defler (2003, 2004), the occurrence of *S. n. nigricollis* in Puerto Leguízamo (Fig. 4.2) was based on a specimen in the collection of the Instituto de Ciencias Naturales (ICN), registered as from the Quebrada El Hacha, a left (north) bank affluent of the Putumayo (collectors H. Granados and H. Arévalo). A number of specimens in the same collection are labeled as from between the ríos Caqueta and Putumayo. Hernández-Camacho and Cooper (1976) noted, however, that the population of the upper Putumayo River has a dull and brownish cast to the lower back and hind limbs, as well as some grizzled yellow and black in the saddle and concluded that This population is thus more reminiscent of *S. fuscicollis* than is the lower Putumayo and Leticia population, which has a rich ferruginous cast to the lower back and hind limbs and no yellowish tones in the saddle area (pp.37–39). Evaluating this, Defler (2003, 2004) concluded that the tamarins thought to be *S. n. nigricollis* to the north of the Río Putumayo are in fact dull specimens of *S. fucicollis fuscus*, and recorded that Philip Hershkovitz, in a personal communication to Defler, believed the same.

South of the Río Putumayo the range of *S. n. nigricollis* perhaps overlaps with *S. tripartitus* between the ríos Yuvineto and Güeppi, as argued by Encarnación et al. (1990) and Aquino and Encarnación (1996). Bravo and Borman (2008) carried out mammal surveys at five sites between the ríos Napo and Putumayo in Ecuador and Peru: west of the middle Río Lagartococha (Garzacocha), on the south (right) bank of the upper Río Güeppi (Güeppicillo) (both in the Cuyabeno Faunal Production Reserve in Ecuador), on the right (east) bank of the Río Lagartacocha (Redondococha) in the proposed Airo Pai Communal Reserve (Peru), the south (right) bank of the Río Güeppi in the proposed Güepi National Park (Peru), and on the upper reaches of the Río Peneya (Aguas Negras), a right bank tributary of the Putumayo (Peru) (see Fig. 4.2). Bravo and Borman (2008) reported *S. nigricollis* in all these sites. They made no mention of subspecies, but published a photograph of an infant/juvenile (their Fig. 8A) that appears to be *S. n. nigricollis* not *graellsi*. They made no mention of *S. tripartitus*.

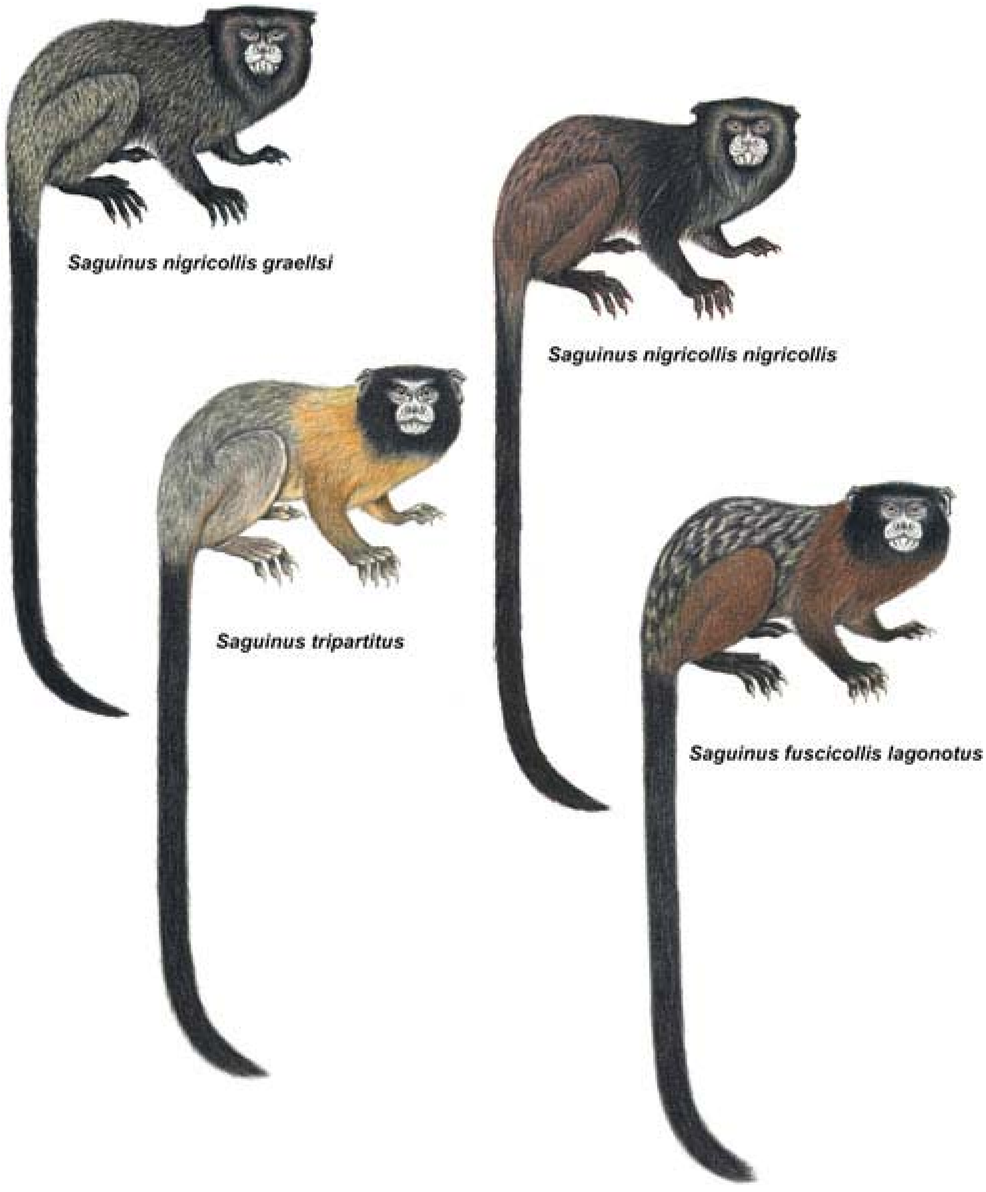

Fig. 4.5 Graells' black-mantle tamarin (*Saguinus nigricollis graellsi*), Spix's black-mantle tamarin (*Saguinus nigricollis nigricollis*), red-mantle saddleback tamarin (*Saguinus fuscicollis lagonotus*), and the golden-mantle saddleback tamarin (*Saguinus tripartitus*). Stephen D. Nash © Conservation International.

4.3.3 *Saguinus nigricollis graellsi (Jiménez de la Espada, 1870)*

Graells' black-mantle tamarin occurs in the upper Amazon, in southern Colombia, eastern Ecuador and northeastern Peru. Its range is not well known, however, and our interpretation of the current evidence indicates that it is probably more restricted than is indicated by

Hershkovitz (1977, 1982). The type locality is banks of Río Napo near Tarapoto, and Destacamento, near confluence with the Marañón, Loreto, Peru (Hershkovitz, 1977). Cabrera (1958) restricted it to Tarapoto, and Hershkovitz (1977) restricted it further to right bank Río Napo, opposite Tarapoto and above the mouth of the Río Curaray (locality 79, p.629). Destacamento is plotted by Hershkovitz as on the right bank of the Río Napo, just above its mouth (locality 91, map, p.626). The Río Tarapoto is a left bank (north) tributary of the Río Napo.

Hernández-Camacho and Cooper (1976) wrote that in Colombia it occurs south from the right (south) bank of the upper Río Caquetá to the Ríos Sucumbíos and Putumayo on the border with Ecuador. They recorded that it could also be found in the neighborhood of Puerto Asís on the upper Putumayo, east to the vicinity of Puerto Leguízamo, north bank of the Río Putumayo. As discussed above for *S. n. nigricollis,* these authors indicated a probability that the form *graellsi* is sympatric with *S. n. nigricollis* in the region of Puerto Leguízamo, and Hernández- Camacho and Cooper (1976) and Defler (1989, 1994) listed *graellsi* as a full species as result. The conclusion of Defler (2003, 2004) and Hershkovitz (pers. comm. to Defler 2003, 2004), however, was that the specimens considered to be *S. n. nigricollis* were in fact just dull-coloured *S. fuscicollis fuscus*. In his most recent assessment Defler (2003, 2004) listed *graellsi* as a subspecies of *S. nigricollis*. Genetic data (Matauschek et al., submitted) support this assessment. Groves (2001, 2005) maintained *graellsi* as a full species based on Hernández-Camacho and Cooper's (1976) supposition of its sympatry with *nigricollis*.

Hershkovitz (1982) argued that there is no definite evidence for *S. n. graellsi* occurring north of Ecuador, and suggested that black-mantled tamarins reported by Hernández-Camacho and Cooper (1976) may be either *S. n. nigricollis* or *S. n. hernandezi*. He restricted its northern limit to the Ríos Putumayo and Sucumbíos (Río San Miguel), indicating as such that it does not occur in Colombia at all.

According to Hershkovitz (1982), in Ecuador *S. n. graellsi* extends throughout a large part of the Amazon region, south from the Ríos Putumayo and San Miguel, west to the foothills of the Andes. The altitudinal range is between 100 m and 1000 m (Hershkovitz, 1982). *Saguinus n. graellsi* is supposed to occur north of the Río Napo in Ecuador, and to extend east only as far the ríos Güepi and Lagartococha on the frontier with Peru (Hershkovitz, 1982), where it should be replaced by *S. n. nigricollis* (see Hershkovitz, 1982; Aquino and Encarnación, 1996). However, in Peru, Matauschek (in prep.) encountered tamarins north of the Napo between

Pantoja and Torres Causana that phenotypically matched *S. n. graellsi* exclusively. Consequently the border between *S. n. graellsi* and *S. n. nigricollis* must lie further south-east in Peru. The only larger river in this area is the Río Tamboryacu. The river itself seems unlikely to form a barrier for the tamarins because of its course, which is more or less parallel to the Río Napo, leaving much space for dispersal between the Putumayo and the Tamboryacu. It seems more likely that the large area of low, seasonal-flooded *várzea*-forest surrounding the vast stream-network of the Tamboryacu could form a barrier for the tamarins, which are scarce and rarely seen in this type of forest. The approximate area is marked in white with a question mark in Figure 4.4.

It has been recorded recently in a number of localities in northern Ecuador between the Ríos Napo and Putumayo, including the basins of the ríos Aguarico, Cuyabeno and Pacuyacu (S. de la Torre, in litt. 1996). Borman (2002) recorded *S. nigricollis* (presumably *graellsi*) at Sinangoe on the Río Cofanes (a left bank tributary of the upper Aguarico), and from the upper Río Bermejo (a right bank tributary of the Putumayo) in Ecuador. However it has not been found in the Yasuní National Park, covering the basin of the Río Yasuní, where it would seem that only *S. tripartitus* and, according to Albuja (1994), but not de la Torre (pers. obs. 1996), *S. fuscicollis lagonotus* occur.

Hershkovitz (1977) suggested that the range in Ecuador may extend as far south as the right bank of the upper Río Santiago, although in his later publication (1982) he was more conservative, giving the north (right) bank of the Río Pastaza as the limit. The specimens from the ríos Pastaza and Tigre are from their uppermost reaches in Ecuador. Hershkovitz (1982) showed the four localities, numbers 19–22, on his Figure 3 (p.653), and listed them as follows:

(19) Yana Rumi (Río), mouth of Río Pindo Yacu, 1º38'S, 76º59'W. R. Olalla, December, 1934, February, 1935;

(20) Pindo Yacu (Río), joins Río Cunambo, upper Río Tigre at 2º 379 08'S, 76º04'W. R. Olalla, October, 1934, above junction with Río Cunambo at 250 m;

(21) Pastaza (Río), 2º05'S, 500 m. C. S. Webb; and

(22) Capahuara or Capihuara (Río), mouth at Río Pastaza, 2º03'S, 76º51'W. R. Olalla, November 1934, above mouth at 300 m.

These are the only records south of the Río Napo in Ecuador, and the reason why Hershkovitz (1982) tentatively extended the range to the entire lowland Amazonian region of Peru between the ríos Napo and Pastaza to the ríos Amazonas and Marañón. Both de la Torre (1996) and Tirira (2007) restrict *S. n. graellsi* to the north of the Napo in Ecuador. Tirira (2007) discounted the Pastaza and Tigre records for this species listed by Hershkovitz (1982) saying only that: "The validity of certain populations to the south of the Río Napo, in the provinces of Orellana and Pastaza, is motive for controversy" (p.118).

Aquino and Encarnación (1994) reported, however, that *S. n. graellsi* has never been found along the ríos Tigre and Pastaza despite a number of primatological surveys along these rivers between 1981 and 1986. They indicated that *S. n. graellsi* extends eastwards from Ecuador into Peru along the right (south) bank of the Río Napo and that the range in Peru is restricted to the region between the ríos Nanay and Napo-Curaray. Matauschek (in prep.) could not find any evidence for *S. n. graellsi* along the Río Nanay (Santa María, Diamante Azul) in 2007 and 2008. Aquino et al. (2005) surveyed the Río Aushiri (right bank affluent of the Napo) in 2005 and in the area between the ríos Curaray and Nashiño (left bank affluent of the Curaray) in 2007 and 2008. They did not find any evidence for the presence of *S. n. graellsi*, encountering only *S. tripartitus*.

Hershkovitz (1982) gave just three localities for *S. n. graellsi* in Peru. Two of them are type localities quite distant from each other. The third is Curaray (Río), boca (= mouth) at Río Napo collected by Olalla and Sons in 1925. Heymann's (2000; Heymann et al., 2002) finding that *S. n. graellsi* does not occur along either bank of the Río Curaray puts the Tarapoto and mouth of the Curaray localities (both number 4, p.655) in doubt. Likewise, Hershkovitz (1977, p.629) gives the (type) locality of Destacamento as "near confluence with Marañón" but mapped it at the confluence of the Río Napo. The third Peruvian locality of Hershkovitz (1982) is listed as Destacamento (= Francisco Orellana), Río Napo, at the junction with Río Amazonas (Hershkovitz, 1977, locality 91, p.626; 1982, locality 5, p.652). There is another Francisco de Orellana (Puerto), which is at the mouth of the Río Coca, an affluent of the Napo, in Ecuador (Fig. 4.2), a region where it would seem that *S. n. graellsi* certainly does occur, and which, suggestively, is also near or at (the same locality as) the type locality of *S. f. lagonotus*, also described by Jiménez de la Espada. Francisco de Orellana at the mouth of the Río Coca is also known as Coca.

If we accept that *S. n. graellsi* is limited to the north of the Río Napo in Ecuador, and does not occur between the ríos Curaray and Napo in Ecuador or Peru, and does not occur along the right bank of the Curaray in Peru, it is very difficult to accept that it occurs between the lower Río Napo and Río Nanay in Peru. This supposition is either based on erroneous localities (either incorrect labels or incorrect interpretation of them), or, if tamarins considered to be *graellsi* have been seen there, that they are in fact a similar but distinct taxon.

Saguinus n. graellsi is largely restricted to Ecuador north of the Río Napo including the basin of the Río Cuyabeno, and, probably occurring in Peru between the ríos Napo and Putumayo west possibly as far the upper reaches of the Río Tamboryacu. Evidence for its presence in Colombia is based on a preserved specimen from the Comisaría of Putumayo, and a number of reliable sightings (for example, on the right bank of the Río Guamués, reported in Moynihan [1976], who said that their appearance conformed to the description *S. n. graellsi* provided by Hershkovitz (1966); but no specimens were obtained), and captive specimens from Puerto Asís, east to the vicinity of Puerto Leguízamo (Hernández-Camacho and Cooper 1976).

The distribution of *S. n. graellsi* has yet to be clearly defined. Hernández-Camacho and Cooper (1976) informed that, in Colombia between the upper reaches of the ríos Putumayo and Caquetá, *S. graellsi* is undoubtedly sympatric with *S. fuscicollis fuscus* throughout its range (p.39) and this is confirmed by Defler (2003, 2004). It does not occur between the Ríos Curaray and Napo in Peru and Ecuador, and is not, therefore, sympatric with *S. tripartitus*.

4.4 Sympatry, Body Size and Ecological Niches

In order to share the same habitat, organisms must differ in some dimension of their realized ecological niche (Putman, 1994). This is true independent of whether niche differences are brought about through interspecific competition, predation or other biotic interactions, or whether differences result from inherently diverging fundamental niches (Keddy, 1989; Putman, 1994). In the context of examining the geographic distribution of different taxa from the genus *Saguinus*, it is appropriate to address the question of their niche differentiation and the implications for possible sympatric occurrence or exclusion.

Body size is an important life-history parameter with direct implications for the ecological niche occupied by an organism. Ecological theory considers body size as an important factor for structuring communities (Schoener, 1984, 1988). It is a structuring factor for Neotropical primate communities through its implications for diet, foraging strategies, 447 predator avoidance, locomotion, and metabolic needs (Terborgh, 1983). *Saguinus* species which undoubtedly live in sympatry (*Saguinus fuscicollis* with *Saguinus imperator*, or *Saguinus labiatus*, or *Saguinus mystax*) differ in body mass by 21%–49%, and in head-body length by 8%–17% (Heymann, 1997). This difference is linked to differences in locomotor style, substrate use, vertical space use and perhaps more important to different strategies of foraging for animal prey (Bicca- Marques, 1999; Buchanan-Smith, 1990, 1999; Garber, 1991, 1993; Nickle and Heymann, 1996; Nyakatura and Heymann, 2010; Peres, 1992; Pook and Pook, 1982; Terborgh, 1983; Yoneda, 1981). Different prey foraging strategies in turn result in a larger divergence in the animal component of the diet between sympatric tamarin species compared to the plant component (Heymann et al., 2000; Nickle and Heymann, 1996). This has been suggested as a mechanism which allows not only for sympatric coexistence but also for the formation of stable mixed-species groups in tamarin monkeys (Heymann, 1997; Heymann and Buchanan-Smith, 2000). In contrast to the body size differences in sympatric *Saguinus* species, differences between doubtful sympatric taxa (*Saguinus fuscicollis lagonotus* – *Saguinus tripartitus*; *Saguinus fuscicollis fuscus* – *Saguinus nigricollis nigricollis*) amount for only 1–4% in head-body length (Heymann, 1997). Head-body length is similar or identical for other taxa of the *Saguinus fuscicollis/nigricollis* clade with debated sympatry (Table 4.3). Unfortunately, no body mass data from wild animals are available for these taxa for more detailed analyses.

Table 4.3 Head-body length of taxa from the *Saguinus fuscicollis/nigricollis*-clade with debated sympatry (source: Hershkovitz 1977; Appendix. Table 1)

Species	Measurements
Saguinus n. graellsi	223 mm (n = 5)
Saguinus n. nigricollis	223 mm (n = 2)
Saguinus f. fuscus	226 mm (n = 16)
Saguinus tripartitus	229 mm (n = 2)
Saguinus f. lagonotus	232 mm (n =11)

When organisms occur in sympatry they may differ in morphological, physiological or ecological parameters more strongly than if they occur allopatrically, a phenomenon called character displacement (Schoener, 1988). It can therefore be asked whether the observed differences between sympatric *Saguinus* species result from such character displacement or whether they are part of the fundamental ecological niche. Only one kind of data is available for such a comparison, namely the patterns of vertical space use. The use of vertical space has been compared between *Saguinus fuscicollis* subspecies living sympatrically and allopatrically with another *Saguinus* species by Buchanan-Smith (1999) and Heymann (2000). The strongly similar to almost identical patterns of vertical space use of *S. fuscicollis weddelli* at sites where it lives sympatrically with either *S. labiatus* or *S. imperator* compared to sites where it occurs alone, and of *S. fuscicollis nigrifrons* (sympatric with *S. mystax*) and *S. fuscicollis illigeri* (no sympatric congener) suggests that this is part of the fundamental niche and not influenced by the presence of a congeneric species. Examination of the few data available on vertical space use by other members of the small-bodied *Saguinus* clade (*sensu* Cropp et al. [1999]; that is *S. n. nigricollis*, *S. n. graellsi*, *S. n. hernandezi*, *S. tripartitus*) also reveals similar or identical patterns of vertical space use. These taxa perform most of their activities at lower strata of the forest, like *Saguinus fuscicollis* (de la Torre et al., 1995; Heymann, 2000; Ulloa Vaca, 1988; Vargas Tovar, 1994). Qualitative and quantitative descriptions of the patterns of foraging also suggest that the different subspecies of *S. fuscicollis* and *S. nigricollis*, and *S. tripartitus* are also very similar or identical in this aspect (Heymann, 2000; Izawa, 1978; Peres, 1993; Soini, 1982). In summary, we can say that (1) all taxa of the *Saguinus fuscicollis/nigricollis*-clade for which information is available, be they sympatric or allopatric with another *Saguinus* species, show similar or identical patterns of vertical space use; (2) *Saguinus* species which certainly are sympatric, even forming mixed species groups, clearly differ in body size, while allopatric or doubtfully sympatric taxa have a very similar to identical body size (Table 4.3).

Given the similarity in vertical space use and body size of the taxa of the *Saguinus fuscicollis/nigricollis*-clade, it is unlikely, and for theoretical reasons implausible, that they can co-exist in the same forests, even though their geographic distributions overlap.

4.5 Geographic Ranges, Sympatry, and the Taxonomy of *Saguinus nigricollis* and *S. fuscicollis*

The definitions of these tamarin species and their range limits are, of course, hypotheses. The confusion concerning the geographic distributions of the northwestern Amazonian tamarins arises not only from the relatively few locality records upon which they are based (Hershkovitz was able to use five for *S. tripartitus*) but also, even when precise, the uncertainty that the labels attached to museum specimens accurately state the location where the specimens were actually obtained. As Hershkovitz (1977) fully recognized, knowing from which bank of a river a specimen was obtained is fundamental, and so often can only be inferred from other locations where the species has been recorded. The collector, receiving the specimen from a hunter, may not have known or even cared to ask the exact provenance of the specimen, believing that it occurred everywhere in the region and that the base camp was a sufficient reference, even though many miles away. Many place names are confused or now difficult to identify or locate. These difficulties concerning interpretation are well known, but we also have the additional confounding factor of the definition of the species in question. For example, in a closely related group of callitrichids, the marmosets, Hershkovitz (1977) recognized six taxa in his *argentata* group (two species, *humeralifer* and *argentata*, each with three subspecies); today we list 14 within the geographic range he circumscribed (Rylands et al., 2008, 2009). With our understanding of the importance of rivers in demarcating the geographic distributions of so many of the Amazonian primates, it is possible to suppose that with further investigation, we would find that tamarins identified as *tripartitus* north of the Napo are in fact a distinct and different taxon. Lastly, we have the simple problem of assertions of the presence of a species being unknown to the readerbased merely on the authority of a distribution map; considered a fact rather than a hypothesis. The numerous sources of inexactitudes have come together to confound our true understanding of the ranges of these tamarins, and our suppositions are based to a large extent on surveys which failed to find evidence of the occurrence of a species where it should be, on our understanding of the ecology and patterns of sympatry of these small insectivore frugivores, and on distribution patterns found elsewhere in the Amazon, with geographic ranges being delimited even by minor tributaries.

Here we conclude that *S. tripartitus* occurs between the Ríos Napo and Curaray and that there there is no strong evidence that it occurs in sympatry with *S. f. lagonotus*, *S. n. nigricollis* or *S. n. graellsi*. The population north of the Napo around the headwaters of the Río Santa María and along the left (west) bank of the Río Yuvineto (Encarnación et al., 1990) means that it is potentially sympatric with *S. nigricollis*, but only one group was seen and further investigation is needed. It may be a distinct taxon, and we believe that sympatry is unlikely.

Concurring with other authors such as Tirira (2007), we argue that the range of *S. n. graellsi* is much smaller than was indicated by Hershkovitz (1977, 1982) and limited to the north of the Napo in Ecuador. This implies considerable confusion in the museum specimen localities for *S. n. graellsi* and the interpretation of them, as is also the case for *tripartitus* and *lagonotus*. Specimens cited by Hershkovitz from the upper ríos Tigre and Pastaza in Ecuador require investigation (Tirira, 2007). *Saguinus n. graellsi* extends into Peru perhaps as far as the low, seasonal-flooded *várzea*-forest surrounding the Río Tamboryacu. Beyond that would be the domain of *S. n. nigricollis*, which otherwise occurs in the middle and lower interfluvium of the Amazonas-Napo and Putumayo-Içá.

According to Defler (2004), *S. n. graellsi* also occurs between the upper ríos Caquetá and Putumayo in Colombia. The middle and lower interfluvium of the ríos Caquetá and Putumayo is otherwise occupied by *S. f. fuscus*. *Saguinus n. hernandezi* extends through areas to the north of the Caquetá, north to the upper Río Guaviare, and east to the Río Yari (Hernández-Camacho and Cooper, 1976; Hershkovitz, 1977; Defler, 2003, 2004). The removal of *S. tripartitus* from the Napo-Putumayo interfluvium (except for the Río Yuvineto locality of Encarnación et al. [1990]) means that *S. f. fuscus* as such is geographically isolated from other saddleback tamarins a reason to raise eyebrows and questions concerning its taxonomic status and even perhaps the distinction of the black-mantled and saddleback tamarin groups. The geographical proximity of *S. f. fuscus* and *S. nigricollis* is concordant with the phylogenetic affinity found by Cropp et al. (1999), who suggested that *fuscus* should be considered a separate species as a result; an argument also supported by Moore and Cheverud (1992), who studied facial morphology. Notable, however, is the sighting of *S. fuscicollis* on the Río Yaguas (between the Putumayo and Amazonas in Peru) by Montenegro and Escobedo (2004).

Cropp et al. (1999) were also concerned with their finding that *S. tripartitus,* considered a distinct species on the basis of sympatry with *S. f. lagonotus,* was, based on mtDNA sequencing, phylogenetically so very close to it (sister taxa). It would seem that the sympatry in this case cannot be the determining factor for considering *S. tripartitus* to be a species. Whether it should be considered again a subspecies, however, is a question which requires a review of the taxonomy of the *Saguinus nigricollis* group of Hershkovitz (1977), and further research on the geographic distributions of the 17 component taxa (Rylands et al., 2008; Röhe et al., 2009).

Acknowledgements

Our sincere thanks to two people who reviewed this article, one anonymous, the other Colin P. Groves, for their comments and wisdom. We are most grateful to Kellee Koenig, Conservation International, for drawing the maps, figures 4.1-4.4, and to Stephen D. Nash, Stony Brook University and Conservation International, for the illustrations of the four tamarins (figure 4.5).

5 General Discussion

5.1 Summary of results

Tamarins of the genus *Saguinus*, subfamily Callitrichinae, represent one of the most diverse primate radiations. So far, about 35 taxa have been described, but detailed information about their taxonomy and phylogeny is still lacking. To further elucidate the phylogenetic relationships and the biogeographic history within the genus, and to contribute to a more reliable classification of its taxa, we sequenced complete mitochondrial genomes, the cytochrome *b* gene and the hypervariable region I of the D-loop. Therefore, we mainly used fecal samples from wild tamarins collected during two expeditions to the Peruvian Amazon, an area of high tamarin diversity.

Phylogenetic relationships within the genus *Saguinus*:

- What is the position of *Saguinus* within the Callitrichinae?

Saguinus branched off first within the Callitrichinae. They separated around 13 mya. *Leontopithecus* branched off second and forms a sister lineage to *Callimico* and the marmosets.

- How are the phylogenetic relationships among the different tamarin species groups?

Within *Saguinus* we find a well supported deep initial split between the small-bodied *S. fuscicollis*[1] and *S. nigricollis* on the one hand and all other larger-bodied representatives of the *S. mystax-, S. bicolor-, S. midas-* and *S. oedipus* species groups on the other hand. Within the large-bodied clade we find a separation of South Amazonian (*S. mystax* group) and North Amazonian (*S. bicolor-, S. midas-* and *S. oedipus* group) lineages. *S. labiatus* (representing the *S. mystax* group) branches off first, while *S. midas* and *S. bicolor* form a clade to the exclusion of *S. oedipus*.

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[1] Although we propose the genus name *Leontocebus* for the *S. nigricollis* species group (see Chapter 2), the old genus name *Saguinus* is provisionally kept until our results are published and discussed by the scientific community.

- Was the ancestral tamarin more similar to the *S. nigricollis*- or to the *S. midas*-species group?

Within *Saguinus* the *S. nigricollis* group branched off first. Within the large-bodied lineage the *S. midas* and *S. bicolor* group are the most recent offshoots. Thus, the hypothesis of phyletic dwarfism and a basal position of *S. midas* as proposed by Ferrari (1993) cannot be confirmed.

- Which taxonomic implications can be made on genus and subgenus level?

We found much older and deeper phylogenetic splits between different species groups of *Saguinus* than between the different genera of marmosets. The *S. nigricollis* group split off from other tamarins in the late Miocene around 9.2 mya. The other species groups diverged between 5.3 and 7.3 mya. The taxonomic nomenclature of the different species groups should be revised. On a mainly time-based genus concept we recommend an elevation to generic level for at least the *S. nigricollis* species group and subgeneric status for the *S. mystax-, S. oedipus*-, and the *S. bicolor/S. midas* groups.

Phylogeny of the *S. nigricollis*-species group:

- Are *S. fuscicollis* and *S. nigricollis* monophyletic species?

We could show, that in the currently recognized taxonomy, *S. fuscicollis* and *S. nigricollis* are polyphyletic taxa. Taxa, which are currently recognized as species are closer related with some subspecies of *S. fuscicollis* than these with each other.

- Can we delimitate species within the diverse *S. nigricollis* group

We could confirm most of the existing taxa as distinct entities and could identify various clusters, which are mainly consistent with described differences in coat coloration. On the basis of the phylogenetic species concept (PSC) we suggest species status for the following taxa: *fuscicollis, illigeri, lagonotus, leucogenys, nigricollis, nigrifrons*, *tripartitus* and *weddelli.*

- What is the taxonomic status of the proposed species *S. graellsi, S. tripartitus* and *S. melanoleucus,* and their relationship to the remaining Peruvian tamarin taxa?

Our genetic data do not support a separate status for the taxa *melanoleucus* and *graellsi*, as they are too closely related with *S. weddelli* and *S. nigricollis*, respectively. But due to clearly defined differences in fur coloration, we give them subspecies status. *S. tripartitus* is clearly distinct in coloration and in the genetic data, and therefore deserves species status.

- Can we detect any sympatry between taxa of the *S. nigricollis* group?

We conclude that despite the existence of a number of specimens with collecting localities which indicate overlap in their geographic ranges, the fact that these tamarins are of similar size and almost identical in their feeding habits and other aspects of their ecology militates strongly against the occurrence of sympatry among them. We also could not find any evidence for sympatry between different taxa of the *S. nigricollis* group during field surveys in Peru.

Biogeography of *Saguinus*:

- What is the geographic origin and direction of the *Saguinus* radiation?

Our data suggest that the numerous taxa of the *S. nigricollis* species group are derived from a common ancestor that separated from the other representatives of the genus ~9.2 mya. Most taxa of the *S. nigricollis* group form monophyletic clusters, which mainly originated in a single rapid radiation ~2.9 mya. The species group most likely originated in western Amazonia and diversified during the decline of the Acre wetland and the formation of the Amazonian river system.

- Can we find eventual coincidence with palaeogeographic history of the Amazon basin?

Our data clearly match geological events like the establishment of the Amazon channel around seven to 10 mya (split of the south-Amazonian *S. mystax* group from the north-Amazonian species groups), the establishment of the great Acre mega-wetland, which covered large parts of western Amazonia could have isolated the ancestral *S. nigricollis* stock in the western part from. The decline of the mega-wetland and the formation of the Amazonian river system enhanced the radiation of the *S. nigricollis* group and brought them back into contact with the *S. mystax* lineage.

- Did *Saguinus* cross the main Amazon channel or was it more or less a parallel dispersal north and south of the Amazon?

The development of the Amazon river channel seems to have separated the *S. mystax* group in the South. North of the Amazon river our data indicate one dispersal route into the north, leading to the *S. oedipus* group and another heading to the east, leading to the closely related *S. midas* and *S. bicolor* groups.

5.2 The importance of extant field sampling for phylogenetic studies

For appropriate phylogenetic studies, especially of such a wide-spread and diverse group as the tamarins, an area-wide dense sampling is essential. Samples of museum specimens are extremely difficult to analyze. Most of the museum material is treated with chemicals for preservation. These chemicals, like the often-used borax and formalin, normally destroy the DNA and inhibit any successful extraction (Coombs et al., 1999). In older material, which is not treated with chemicals, only dried or salted, the DNA is often degraded and highly fragmented (Wandeler et al., 2007). Another problem with museum specimens, that they are often mislabeled or the old names of the localities cannot be recovered today. However, for accurate phylogenetic and biogeographic interpretations it is important to know the exact provenance of a sample, for example which side of the river, as rivers, especially in the Amazon basin, often form barriers between different taxa or populations (Ayres and Clutton-Brock, 1992). Sometimes the old collectors bought the skins on local markets, where hunters often sell animals from various locations. Often used captive or zoo animals today are normally bred over generations in captivity. The origin of the founder animals is in most cases not comprehensible (Willis, 1993).

Therefore, samples from wild animals with certain localities across their distribution range are necessary to resolve the phylogenetic relationships of such a diverse genus like *Saguinus* and in particular the *S. nigricollis* species group. As mentioned above the numerous taxa of this species group are distributed throughout the western Amazon region of South America, a vast area covered with lowland rain forest containing a network of rivers and swamps. For a comprehensive sampling, Peru offers the unique possibility to encounter 13 different taxa of tamarins within one country. The area of the Peruvian Amazon is mainly not developed by roads or other infrastructure. The only public transport in this area is local cargo boats which travel the main rivers. To reach also smaller rivers and side-arms the only possibility is to travel by small boats or canoes, which makes expeditions in these areas extremely difficult and logistically extensive. Drug farming, gold mining and guerrilla activities bear considerable risks for strangers, which makes safe travelling and working for foreign researchers extremely risky and in some areas impossible. These are probably reasons, why comprehensive phylogenetic studies of most New World primates are very scarce and often incomplete. This study is so far the most complete assessment of the highly diverse *S. nigricollis* species complex using almost exclusively samples from wild tamarin populations with confirmed sampling localities.

5.3 Methodical approach: Can mitochondrial DNA serve as a successful tool in tamarin taxonomy?

The present study is based on mitochondrial DNA (mtDNA) sequence data. Mitochondrial DNA is only maternally inherited. Analyses on mitochondrial sequence data thus only reflect maternal lineages. MtDNA functions as a single locus. Therefore it is difficult to rule out sorting failures of ancestral polymorphisms (incomplete lineage sorting) (Funk and Omland, 2003). In general mtDNA is likely to sort faster than nuclear DNA (Avise, 2004). Our phylogeny of callitrichine genera and *Saguinus* species groups seems to be confirmed extensively by ongoing nuclear data analyses on the generic level (Roos, pers. comm.). The results of the present study clearly demonstrated that mtDNA provides sufficient information to identify distinct clusters and lineages within the genus *Saguinus* and in particular the various taxa of the *S. nigricollis* species group. The mitochondrial lineages reflect in most cases the taxonomic arrangements of Hershkovitz (1977) based on fur coloration. Exceptions are as mentioned above, the white saddle-back tamarin *S. melanoleucus,* which is closely related to *S. weddelli* and the northern Andean saddle-back tamarins, which were originally described as *leucogenys* but match genetically and phenotypically more *illigeri*.

In recent years hybridization has been increasingly considered as an important factor in species evolution (Arnold and Meyer, 2006). It was for example described for African cichlid fish (Egger et al., 2007). Meanwhile molecular genetic studies uncovered various examples for hybridization as speciation factor also in primates. The recently described highland mangabey (*Rungwecebus kipunji*) appears to be the result of an ancient hybridization between baboons (*Papio*) and mangabeys (*Lophocebus*) (Burrell et al., 2009). Ancient hybridization was also detected in Asian Colobine monkeys (Ting et al., 2008). Currently, stable hybridization zones are known in baboons (Zinner et al., 2009; Keller et al., 2010). To detect such hybridization events, mitochondrial DNA is not sufficient. Therefore analyses of nuclear and Y-chromosomal markers are necessary. In this perspective, tamarin fecal samples proved to be difficult to analyze. High quality DNA from blood or tissue would be more suitable for laboratory analyses, but difficult to obtain from the wild without harming the animals. In the tamarins studied here, this would be of special interest, to examine the situation of the northern *leucogenys* clade, which are mitochondrial closer related to *illigeri* than to the nominate form of (southern) *leucogenys* and show an intermediate or even more *illigeri*-like phenotype.

5.4 Species concepts and species delimitations and their effect on conservation issues

For all taxonomic studies the choice of the species concept is always a crucial point and one of the most disputed questions in biology in general, as the traditional biological species concept proposed by Ernst Mayr (1963) is proven to be insufficient (De Queiroz, 2007). Meanwhile there are various other species concepts on the taxonomic market (Groves, 2004). Most of them are theoretical approaches to meet the challenge of putting a dynamic system into static categories. A new approach, a concept of species as population level lineages eliminates the discussion about species concepts. The only necessary property of a species is the property as separately evolving metapopulation lineages (De Queiroz, 2007). But this will not solve the problems related to species delimitation in practice. We should not confuse our understanding of the nature of a species with our criteria for detecting them (Hey 2006).

Recently, the application of the so-called phylogenetic species concept (PSC) prevailed in primate taxonomy. It defines a species as ”*the smallest cluster of individual organisms within there is a parental pattern of ancestry and descent and that is diagnosably distinct from other such clusters by a unique combination of fixed character states*“ (Cracraft, 1983). It was adapted for example by Groves (2001, 2004) and addresses the more practical part of the “species problem”. The main advantage of the PSC is that the amount of difference is not important, so that it can be applied on the basis of any available data set, but does not exclude the comprehensive and doubtless accurate approach of DeQueiroz (2007). The consequence of the application of the PSC was a dramatic increase in primate species numbers. Nevertheless, at present it is widely used and applied in most recent studies on primate taxonomy. In platyrrhine primates, almost all former subspecies of titi monkeys (*Callicebus*) (van Roosmalen, 2002) and marmosets (*Callithrix* and *Mico*) (see Groves, 2001) have been elevated to full species. Especially in lemurs, a drastic increase in species numbers, for example in mouse lemurs (*Microcebus*) or sportive lemurs (*Lepilemur*), can be observed in recent years (Tattersall, 2007; Mittermeier et al., 2010).

So far, it has been applied to the tamarins, one of the most diverse genera, only on a limited scale. In our studies, we showed that the PSC was proven to be adaptable to the tamarins as well, as we could define distinct genetic lineages which are in concordance with taxa, previously described on the basis of fur coloration.

The taxonomic term "species" is, despite all theoretical problems, the currency in biology, which is understood by scientists and non-scientists as well and the basis for all political decisions concerning conservation, animal trade and welfare, landscape protection, etc. Facing the rapid decline of biodiversity and growing threats, it is necessary to find practical solutions to the "species problem" to provide a solid basis to deal with decision makers and stake holders, but always being aware of advancing to a "political species concept".

For the evaluation the conservation status especially of such widely distributed genera, like these tamarins, it is highly important to know the taxonomic status and the phylogenetic relationships between the different populations. The conservation status of a species can change dramatically, if it turns out, that there are a lot of small populations which are distinct evolutionary units and deserve a higher taxonomic status. In the current IUCN red list only one of 15 listed *Saguinus* species (*S. oedipus*) is listed as critically endangered, one as endangered (*S. bicolor*), and one as vulnerable (*S. niger*). All others are listed under the category "least concern", including all taxa which are featured as subspecies of *S. fuscicollis* and *S. nigricollis* (IUCN, 2010). This is surely true if one considers all the different taxa as one species. But if the single taxa are considered as distinct species, their conservation status has to be revised as well. For most of the taxa the status "least concern" remains appropriate for the moment, as throughout western Amazonia large areas are still covered with relative intact rainforest. The capability of the tamarins to cope with degraded habitats enables them to survive in areas with strong human disturbances (Johns and Skorupa, 1987). But the pressure on the Amazonian rainforests is growing rapidly. The human demands for oil, tropical timber, and land for agriculture and cattle farming is causes rapid deforestation and lead to more and more fragmented habitats for forest animals (Strier, 2007). Already 72% of the Peruvian Amazon is covered by blocks for oil and gas exploration (Finer et al., 2009). Some areas, like the district San Martín in the lower Andes on the edge of the Amazonian lowland, are already nearly completely deforested. Only in some steep areas small forest patches are left. These are the areas, from where the samples of the northern *leucogenys* originated. The tamarins share these last refugias with the endemic Andean titi monkey (*Callicebus oenanthe*), which is one of the most endangered Peruvian primates. The high fragmentation of the habitat and the isolation of the remaining populations are a major threat for their survival (Bóveda-Penalba et al., 2009). As the tamarins in this area are faced with the same problems, further research is needed to determine, if the tamarins there are in fact *leucogenys*, *illigeri* or even form an own evolutionary unit.

5.5 The relevance of molecular studies for the understanding of Amazonia's diversity and biogeography

The understanding of the extremely rich biodiversity of Amazonia requires the interdisciplinary collaboration of a broad range of scientific fields (Hoorn et al., 2010). With the examination of fossil records and molecular phylogenetic studies the field of biology can provide useful evidence for the geological and evolutionary history of a bioregion. The combination of various phylogenetic studies on a broad range of organisms, for instance, clearly showed that the most often highlighted Pleistocene refugia (e.g. Haffer, 1969, 1997) are rather sampling artifacts than the main driving mechanism for the evolution of the Amazonian biodiversity (Nelson et al., 1990). For most groups of organisms the time of enhanced diversification clearly predates the Pleistocene. They rather indicate an increased proliferation of biodiversity during middle and late Miocene, where an accelerated Andean uplift and the draining of the Amazon basin provided the basis for accelerated evolution and speciation (Hoorn et al., 2010). This is strongly reflected by our data on tamarin evolution. Our data on tamarin phylogeny clearly match current findings of the geological history of the Amazon basin (Acre mega-wetland, Amazon river channel). This shows that molecular phylogenetic studies with divergence time estimates, especially on diverse and widely distributed groups of animals can contribute to the understanding of the biogeography and biodiversity.

5.6 Outlook

An establishment of nuclear and Y-chromosomal markers to detect ancient and recent hybridizations would be desirable. On the generic level, these studies are already on the way (Roos, pers. comm.) but they could also provide interesting insights on species group and species level, if markers with sufficient variability can be identified. In particular this would be important to clarify the situation and relationship between *S. illigeri/S. leucogenys* from the southern and western Peruvian Amazon. To further examine the status of *S. illigeri* and *S. leucogenys* in the north-western part of their distribution area additional sampling would be desirable to detect distribution limits, possible hybrid zones or zones of phenotypic intergradations. This would also be highly necessary to examine the conservation status of the tamarins in this threatened and strongly fragmented habitat.

To complete the picture of tamarin diversity the inclusion of samples from Ecuadorian (*S. f. fuscus, S. n. hernandezi*) and Brazilian taxa, especially from the *S. melanoleucus* complex ("*crandalli", "acrensis*") and possible hybrids of them with *S. fuscicollis* from Brazil and the Peruvian border (Alto Purus) would be of great interest.

Similar studies on other diverse and wide-spread New World primate taxa, could provide invaluable information to find accordance and to detect patterns for primate evolution in Amazonia. A comparable study on titi monkeys (*Callicebus*), which live sympatrically with the tamarins and show a similar diversity, could offer further opportunities to confirm our findings and to uncover Amazonia's biogeographic history.

Summary

The Callitrichinae are small bodied New World primates, including two of the most diverse groups of primates, the marmosets (genera *Callithrix* and *Mico*) and tamarins (genus *Saguinus*). So far, about 35 tamarin taxa have been described. The phylogenetic relationships within this subfamily and in particular the phylogeny of the six species groups of tamarins (*Saguinus*) and their position within the Callitrichinae is disputed.

The first part of this thesis illuminates the phylogenetic relationships within the Callitrichinae on a broader scale. We sequenced 13 complete mitochondrial genomes of nearly all callitrichine genera and *Saguinus* species groups. Based on our phylogenetic reconstructions, *Saguinus* branched off first among Callitrichinae, followed by *Leontopithecus.* Among the remaining genera, *Callimico* is basal, and *Mico* and *Cebuella* form sister genera to the exclusion of *Callithrix*. The genus *Saguinus* is further divided into various, unexpectedly old lineages. We found much older and deeper phylogenetic splits between different species groups of *Saguinus* than between the different genera of marmosets. The *S. nigricollis* group split off from other tamarins in the late Miocene around 9.2 mya. The other species groups diverged between 5.3 and 7.3 mya. The taxonomic nomenclature of the different species groups should be revised and we recommend an elevation to generic level for at least the *S. nigricollis* species group. We bring our data together with the current standard of knowledge of Amazonian geology. Our data support a West-Amazonian origin and an eastward dispersal of the genus *Saguinus*.

The second part goes more into detail by focusing on the *S. nigricollis* species group, which is the most diverse group and with its numerous taxa, subspecies and/or color variants and cryptic distribution areas surely one of the most complicate genera of New World primates. To further elucidate the phylogenetic relationships and the biogeographic history within this species group, and to contribute to a more reliable classification of its taxa, we sequenced the complete mitochondrial cytochrome *b* gene and the hypervariable region I of the D-loop. Therefore, we mainly used fecal samples from about 100 wild tamarins collected during two expeditions to the Peruvian Amazon, an area of high tamarin diversity. Most taxa of the *S. nigricollis* group form monophyletic clusters, which mainly originated in a single rapid radiation ~2.9 mya. *S. fuscicollis* and *S. nigricollis* appear as polyphyletic taxa, but we could

identify various clusters, which are mainly consistent with differences in coat coloration. We could confirm most of the existing taxa as distinct entities and suggest species status for *fuscicollis, illigeri, lagonotus, leucogenys, nigricollis, nigrifrons*, *tripartitus* and *weddelli*. Our genetic data do not support a separate status for *melanoleucus* and *graellsi*, but due to differences in fur coloration, we give them subspecies status. The species group most likely originated in western Amazonia and diversified during the decline of the Acre wetland and the formation of the Amazonian river system.

The third part is bringing together the information on distribution areas, distribution limits and sympatry, obtained during our extant field surveys in the Peruvian Amazon, with the evidence of other field researchers and the current knowledge provided by museum specimens and historic reports. A detailed understanding of the range of the golden-mantle tamarin, *Saguinus tripartitus,* in Amazonian Peru and Ecuador is of particular relevance, not only because it is poorly known but also because it was on the basis of its supposed sympatry with the saddleback tamarin (*Saguinus fuscicollis lagonotus*). A number of surveys have been carried out since 1988 in the supposed range of *Saguinus tripartitus*, in both Ecuador and Peru. Here we summarize and discuss these, and provide a new suggestion for the geographic range of this species; that is, between the ríos Napo and Curaray in Peru, extending east into Ecuador. We also review current evidence for the distributions of Spix's black-mantle tamarin (*S. nigricollis nigricollis*), Graells' black-mantle tamarin (*S. n. graellsi*) and the saddleback tamarin (*S. fuscicollis lagonotus*), which are also poorly known, and examine the evidence regarding sympatry between them. We conclude that despite the existence of a number of specimens with collecting localities which indicate overlap in their geographic ranges, the fact that the four tamarins are of similar size and undoubtedly very similar in their feeding habits militates strongly against the occurrence of sympatry among them.

Zusammenfassung

Die Krallenaffen (Callitrichinae) sind kleine Neuwelt-Affen. Zu ihnen zählen zwei der formenreichsten Primatengruppen, die Marmosetten (*Callithrix* und *Mico*) und die Tamarine (*Saguinus*). Bisher wurden etwa 35 verschiedene Tamarintaxa beschrieben. Die phylogenetischen Beziehungen innerhalb der Unterfamilie und insbesondere zwischen den sechs Artengruppen der Tamarine und deren Position innerhalb der Krallenaffen ist weitgehend ungeklärt und teilweise umstritten.

Der erste Teil dieser Dissertation befasst sich mit der Phylogenie innerhalb der Krallenaffen. Dazu sequenzierten wir insgesamt 13 komplette mitochondrielle Genome von Vertretern aller Krallenaffengattungen und Tamarin- Artengruppen. Nach unseren phylogenetischen Rekonstruktionen spalteten sich die Tamarine als erstes innerhalb der Callitrichinae ab, gefolgt von den Löwenaffen (*Leontopithecus*). Unter den verbleibenden Gattungen bildet *Callimico* eine Schwesterlinie basal zu den Marmosetten. Innerhalb der Marmosetten sind die Amazonischen Marmosetten (*Mico*) näher mit den Zwergmarmosetten (*Cebuella*) verwandt, als mit den Atlantischen Formen (*Callithrix*). Die Gattung *Saguinus* teilt sich in mehrere unerwartet alte Linien. Wir fanden innerhalb der Tamarine tiefere und ältere phylogenetische Aufspaltungen als zwischen den verschiedenen Gattungen von Marmosetten. Die *S. nigricollis* Artengruppe spaltete sich bereits im späten Miozän vor etwa 9,2 Millionen Jahren von den anderen Tamarinen ab. Die anderen Artengruppen trennten sich zwischen 5.3 und 7.3 Millionen Jahren voneinander. Daher sollte die Nomenklatur der verschiedenen Artengruppen überarbeitet werden und wir empfehlen Gattungsrang zumindest für die *S. nigricollis* Artengruppe. Die phylogenetischen Daten bringen wir weiterhin zusammen mit dem neuesten Kenntnisstand über die geologische Geschichte Amazoniens. Unsere Daten deuten auf einen westamazonischen Ursprung der Gattung *Saguinus* mit einer östlichen Ausbreitung hin.

Der zweite Teil der Arbeit beschäftigt sich detaillierter mit der formenreichsten Gruppe, der *S. nigricollis* Artengruppe, die mit ihren zahlreichen Taxa, Unterarten oder Farbvarianten, sowie oftmals unbekannten Verbreitungsgebieten sicherlich die komplizierteste Gruppe darstellen. Um die Phylogenie und Biogeographie dieser Artengruppe aufzuklären, sequenzierten wir das komplette Cytochrom *b*-Gen und die hypervariable Region des mitochondriellen D-

Loops. Dafür verwendeten wir fast ausschließlich georeferenzierte Kotproben von etwa 100 freilebenden Tamarinen. Die Proben wurden während zwei Expeditionen im peruanischen Amazonasgebiet gesammelt, einem Gebiet mit besonders hoher Tamarindiversität. Unsere Daten ergaben, dass sich die zahlreichen Taxa dieser Artengruppe innerhalb einer schnellen Radiation vor etwa 2,9 Millionen Jahren entstanden. Wir konnten klar abgegrenzte Kladen definieren, die zumeist mit bereits anhand der Fellfärbungen beschriebenen Einteilungen übereinstimmen. Deshalb schlagen wir Artstatus für die Taxa *fuscicollis, illigeri, lagonotus, leucogenys, nigricollis, nigrifrons*, *tripartitus* und *weddelli* vor. Der Artstatus von *melanoleucus* und *graellsi* konnte von unseren genetischen Daten nicht bestätigt werden. Aufgrund der deutlichen Unterschiede in der Fellfärbung schlagen wir Unterartstatus für diese Taxa vor. Die Artgruppe entstand wahrscheinlich im westlichen Amazonasgebiet und verbreitete sich mit dem Verschwinden eines ausgedehnten Sumpfgebietes (Acre mega-wetland) und der Ausbildung des heutigen amazonischen Flusssystems.

Der dritte Teil der Arbeit geht noch weiter ins Detail und bringt die Erkenntnisse aus den beiden Expeditionen ins peruanische Amazonasgebiet über Verbreitungsgebiete und –grenzen und Sympatrie mit den Ergebnissen anderer Feldforscher und dem aktuellen Kenntnisstand aus Museumssammlungen und historischen Berichten zusammen. Ein detailliertes Verständnis des Verbreitungsgebiets des Goldmantel-Tamarins (*S. tripartitus*) im nördlichen Peru und in Ecuador ist von besonderer Relevanz, weil es die Grundlage für mögliche Sympatrie zwischen zwei Taxa aus der *S. nigricollis* Gruppe, nämlich zwischen *S. tripartitus* und *S. fuscicollis lagonotus*, ist. Der Artstatus von *S. tripartitus* basiert auf dieser möglichen Sympatrie. Wir fassen auch den aktuellen Kenntnisstand zu den weitgehend unbekannten Verbreitungsgebieten von *S. n. nigricollis*, *S. n. graellsi* und *S. f. lagonotus* und potentieller Sympatrie zwischen diesen Taxa zusammen. Wir schlussfolgern, dass, trotz einiger Museumsexemplare mit überlappenden Herkunftsorten, Sympatrie zwischen diesen Taxa unwahrscheinlich ist, da sie sich aufgrund großer Ähnlichkeiten in Größe und Nahrungsökologie gegenseitig ausschließen würden.

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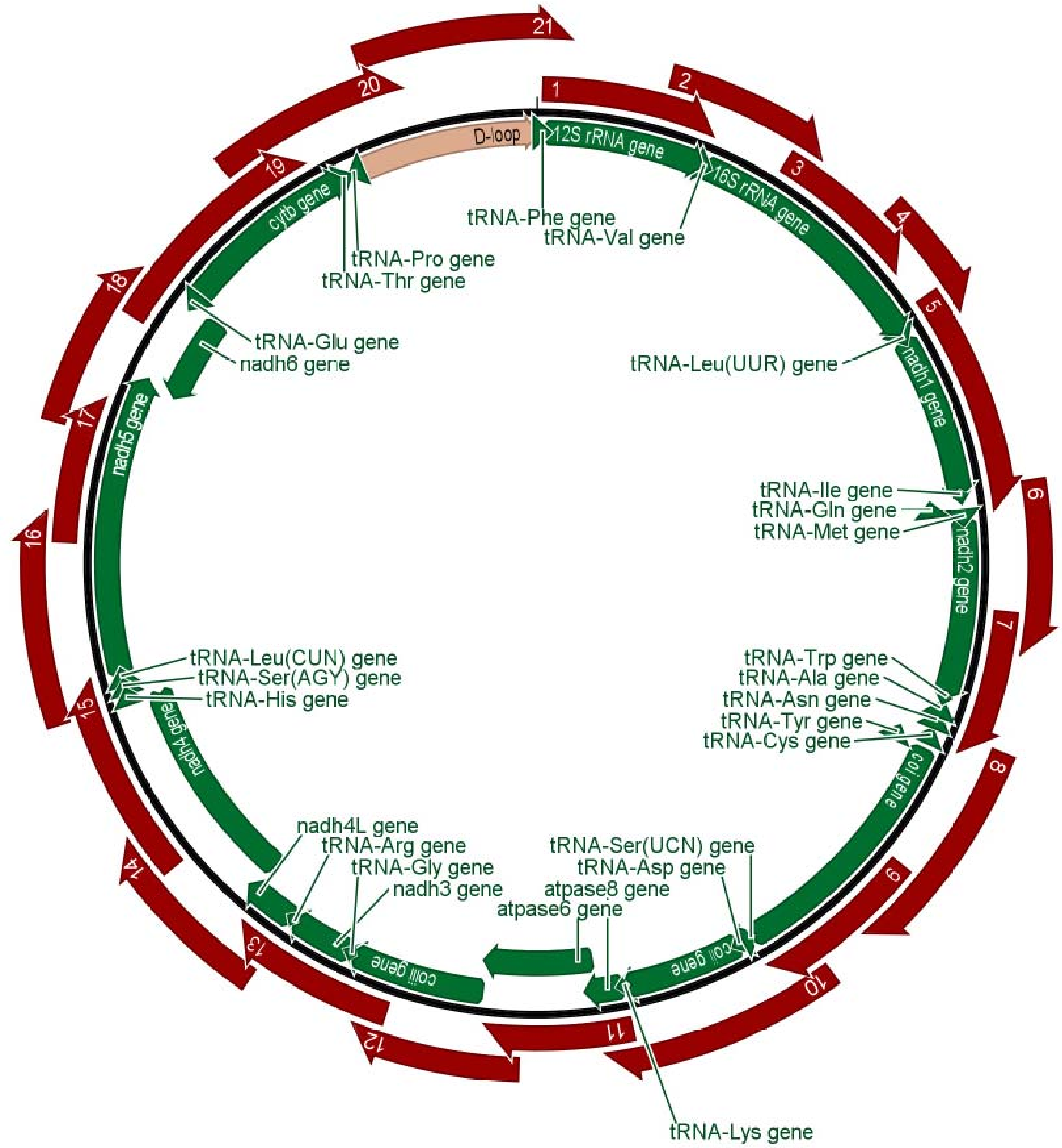
Appendix



Supplementary Figure 2.1: Scheme of a mammalian mitochondrial genome. Genes are indicated in green. Fragments, as amplified by primers used in this study, are indicated in red. For primer sequences see supplementary table 2.1.

Suplementary Table 2.1: Primers used in this study for the amplification of the 21 fragments of the mitochondrial genome. Given are fragment number (f: forward; r: reverse), taxon, primer sequence, reference and annealing temperature (AT) in °C.

Fragment	Taxon	Primer sequence (5´-3´)	Reference	AT
1f	Callitrichinae	CAAGRCACTGAAAATGYCTAG	This study	58
1r	Callitrichinae	CTTACCATGTTACGACTTATC	This study	58
2f	*S. bicolor*	AAGGTGGATTTAGCAGTAAAC	This study	58
2f	Callitrichinae	ACGTTAGGTCAAGGTGCAG	This study	58
2r	*S. bicolor*	CAACTATGGTAGTTATATGGC	This study	58
2r	Callitrichinae	TGGCTGCTTTTARGCCAACT	This study	58
3f	Callitrichinae	AGCTGGTTGTCCAAGACAG	This study	58
3r	Callitrichinae	ATAGGATTGCGCTGTTATCC	This study	58
4f	Callitrichinae	GACGAGAAGACCCTATGGA	This study	58
4r	Callitrichinae	GRAGAGGAGTTGAACCTCTG	This study	58
5f	Callitrichinae	AATCCAGGTCGGTTTCTATC	This study	58
5r	Callitrichinae	AGCTTATTTAGCTGACCTTAC	This study	58
6f	*Callimico goeldii*	ATACCTGTTCTAACATCCGG	This study	58
6f	*S. fuscicollis*	TTAGACCGGTAATATTTGGGT	This study	58
6f	Callitrichinae	CCMCAAACRTAAGAAATATGT	This study	58
6r	*S. fuscicollis*	TTATAGGAATCGAACCTATACA	This study	58
6r	Callitrichinae	GTGGRATTATGGGTATTATTC	This study	58
7f	*S. fuscicollis*	TAGCAGTACTGCTCTACAAC	This study	58
7f	*S. fuscicollis*	TAGCAGTACTGCTCTACAAC	This study	58
7f	Callitrichinae	CAAACMCAACTWCGMAAAATC	This study	58
7r	*Callimico goeldii*	AGTAAGCATTAGACTGTAAATC	This study	58
7r	*S. fuscicollis*	CAGCTCTGAGGTGATTTATC	This study	58
7r	*S. fuscicollis*	CAGCTCTGAGGTGATTTATC	This study	58
7r	Callitrichinae	GCATTAGACTGTAAATCTAAA	This study	58
8f	*S. labiatus*	CTAATCAACTGGCTTCAATC	This study	58
8f	*S. bicolor*	AGCCCTTACTAGATCGATG	This study	58
8f	*Mico*	CTGGCTTCAATCTACTTCTC	This study	58
8f	*S. nigricollis*	CTGGCTTCAATCTACTTCTC	This study	58
8f	*Leonotopithecus*	ATGTGATAGTTCACCTCAGG	This study	58
8f	*S. labiatus*	CTAATCAACTGGCTTCAATC	This study	58

8f	Callitrichinae	GGCTTCAATCTAMTTCTCCC	This study	58
8r	*S. labiatus*	CCGGTAGGAATAGCAATGA	This study	58
8r	*S. bicolor*	TGTGAGATTATTCCGAATCCT	This study	58
8r	*Mico*	CGGTAGAATGAGAATATACAC	This study	58
8r	*S. nigricollis*	GAGATTATTCCGAATCCTGG	This study	58
8r	*Leonotopithecus*	GTGCAGTGTGGCTAATCAG	This study	58
8r	*S. labiatus*	CCGGTAGGAATAGCAATGA	This study	58
8r	Callitrichinae	GCRATRATTATRGTRGCTGAT	This study	58
9f	*S. fuscicollis*	GTACATGGGAATAGTCTGAG	This study	58
9f	*S. fuscicollis*	GTACATGGGAATAGTCTGAG	This study	58
9f	*Leonotopithecus*	ATATCTATTGGTTTCCTAGGC	This study	58
9f	Callitrichinae	AGCYGCYGGAATTACAATAC	This study	58
9r	*S. fuscicollis*	ACTCCAGGTTGGTCGATAG	This study	58
9r	*S. fuscicollis*	GGTTGGTCGATAGTTGCTC	This study	58
9r	Callitrichinae	TGGCTTGAAACCAATTTTWGG	This study	58
10f	*Callimico goeldii*	TCGAACAACTATCTACTAACC	This study	58
10f	*Saguinus*	AATRCCCCGACGATATTCAG	This study	58
10f	*Callithrix*	TGATTTGAGAAGCATTCTCCT	This study	58
10f	*Mico*	GATTTCAACCCACATCCTTC	This study	58
10f	*Saguinus*	AATRCCCCGACGATATTCAG	This study	58
10f	Callithrix	TGATTTGAGAAGCATTCTCCT	This study	58
10f	Callitrichinae	TRATYTGAGAAGCATTCTCYT	This study	58
10r	*Callimico goeldii*	TATGTGGAATGTGAAACCAAG	This study	58
10r	*Saguinus*	TTTGGTTCATTTTAGATTTCAAG	This study	58
10r	*Callithrix*	TTGGTTCATTTTAGTTCTCAAG	This study	58
10r	*Mico*	ACTGGATGATGTAGAATAAGG	This study	58
10r	*Saguinus*	TTTGGTTCATTTTAGATTTCAAG	This study	58
10r	Callithrix	TTGGTTCATTTTAGTTCTCAAG	This study	58
10r	Callitrichinae	TTGGTTCATTTTAGTTCTCAA	This study	58
11f	Callitrichinae	GCATTAACCTTTTAAGTTAAA	This study	58
11r	Callitrichinae	ARGCRTGTGTTTGGTGTGTC	This study	58
12f	*Callimico goeldii*	GCACTTATTCAAGCTTATGTG	This study	58
12f	*S. fuscicollis*	AATTATCATCGAAACAATCAGC	This study	58
12f	*S. bicolor*	CTAATACATCTACTTGGAGAC	This study	58

12f	*S. fuscicollis*	AATTATCATCGAAACAATCAGC	This study	58
12f	Callitrichinae	TAATYATTATYGAAACAATYA	This study	58
12r	*Callimico goeldii*	TACATGAAGACCGTGAAAGC	This study	58
12r	*S. fuscicollis*	CAGTCATACTACATCTACGAA	This study	58
12r	*S. bicolor*	TAATGACGTGAAGTCCGTG	This study	58
12r	*S. fuscicollis*	CAGTCATACTACATCTACGAA	This study	58
12r	Callitrichinae	TCTACRAARTGTCAGTATCAT	This study	58
13f	Callitrichinae	TTCTTTATRKCYACAGGYTTC	This study	58
13r	Callitrichinae	TGCRGCTTCRCATGCGGC	This study	58
14f	Callitrichinae	ACAAATGATTTCGACTCATTA	This study	58
14r	Callitrichinae	GTGATATTATCTGTTCATGATG	This study	59
15f	*S. bicolor*	TACCACCTAAGTAACTCAGG	This study	58
15f	*S. fuscicollis*	ACGCTTAAACGCTGGATCC	This study	58
15f	Callitrichinae	ATAGCCTTTATAGTHAAAATA	This study	58
15r	*S. fuscicollis*	TGGTAGCATGAGTTAGCAG	This study	58
15r	*S. bicolor*	TACTTGGAGTTGCACCAATG	This study	58
15r	Callitrichinae	TTRCTTTTATTTGGAGTTGCA	This study	58
16f	*Saguinus*	ATTAGATTGTGAATCTAATAATAG	This study	58
16f	*S. labiatus*	ACCATAGGATTAGTTCCCTG	This study	58
16f	*Saguinus*	ATTAGATTGTGAATCTAATAATAG	This study	58
16f	*S. labiatus*	ACCATAGGATTAGTTCCCTG	This study	58
16f	Callitrichinae	GATTGTGARTCTAATAAYAGA	This study	58
16r	*S. labiatus*	GATGCCAACTGTTACTATCAT	This study	58
16r	*S. labiatus*	GATGCCAACTGTTACTATCAT	This study	58
16r	Callitrichinae	CTARTTGGCTTGAGGTTGAG	This study	58
17f	*S. fuscicollis*	GACTCCCATCCGCCATAG	This study	58
17f	*S. fuscicollis*	GACTCCCATCCGCCATAG	This study	58
17f	Callitrichinae	CAGAHGCHAACACAGCAGC	This study	58
17r	*S. fuscicollis*	AATCAGAATAAGTCGAGTAGG	This study	58
17r	*S. fuscicollis*	AATCAGAATAAGTCGAGTAGG	This study	58
17r	Callitrichinae	RCCTAGYATRTTAGAGAAGTA	This study	58
18f	*Callimico goeldii*	TCAAGTAACCATACCATATTAC	This study	58
18f	*S. bicolor*	TAAGCGTAACCATTATAGGAC	This study	58
18f	Callitrichinae	TTYGCYGGGTTTCTTATTTC	This study	58

18r	*S. bicolor*	GTCATGGTTATAATCCATGTG	This study	58
18r	Callitrichinae	CTTATAGTTGAARTACAACRA	This study	58
19f	*S. labiatus*	ATCAATTGCTATAGCCGCAG	This study	58
19f	*S. bicolor*	ACGTAATCGCACAACCTACA	This study	58
19f	*Mico*	AGTAATAACACAACCTACAGC	This study	58
19f	*S. nigricollis*	AACGTAATAGCACAACCCAC	This study	58
19f	*S. labiatus*	ATCAATTGCTATAGCCGCAG	This study	58
19f	Callitrichinae	GCAGTRTARCCAAAAACMACC	This study	58
19r	*Callimico goeldii*	TACTTGAGCCTGTTTCATGC	This study	58
19r	*S. labiatus*	CGTTAGGAGATCGGCTACT	This study	58
19r	*S. bicolor*	ATAGGGTTAGGCTTATCAGG	This study	58
19r	*Mico*	GAATATTAGTCCGAGGATATC	This study	58
19r	*S. nigricollis*	TGTCTGACGTGTAGTGTATG	This study	58
19r	*S. labiatus*	CGTTAGGAGATCGGCTACT	This study	58
20f	*S. nigricollis*	CACGATTCTTCACTTTCCAC	This study	58
20f	*S. midas*	TGCACCTCACATTAAACCAG	This study	58
20f	*S. nigricollis*	CACGATTCTTCACTTTCCAC	This study	58
20f	Callitrichinae	CACGATTCTTTACCTTCCAC	This study	58
20r	Callitrichinae	GGTCTCTTAATCTACCWACC	This study	58
21f	*S. bicolor*	CACACGACTACCAAGCATG	This study	58
21f	Callitrichinae	CCATCAACACCCAAAGCTG	This study	58
21r	*S. bicolor*	GGCTAGGACCAAACCTATG	This study	58

Supplementary Table 3.1: Sampling localities, locality IDs, coordinates and GenBank accession numbers of cytochrome *b* and D-loop sequences from studied tamarins and other primates.

Taxon	Sampling locality	Locality ID	Coordinates	GenBank nr. cyt *b*	GenBank nr. HVI
S. graellsi	Pantoja, Rio Napo, left bank	P	S 0°94’ W 75°19’	P 1: HM368029 P 2: HM368030 P 3: HM368031 P 4: HM368032 P 5: HM368033 P 6: HM368034 P 7: HM368035	P 1: HM367929 P 2: HM367930 P 3: HM367931 P 4: HM367932 P 5: HM367933 P 6: HM367934 P 7: HM367935
S. nigricollis nigricollis	Tutapishcu, Rio Napo, left bank	TP	S 3°11’ W 73°14’	TP 1: HM368075	TP 1: HM367975
S. nigricollis nigricollis	Rio Apayacu, Amazonas, left bank	AP	S 3°27’ W 72°18’	AP 1: HM367984 AP 2: HM367985 AP 3: HM367986	AP 1: HM367884 AP 2: HM367885 AP 3: HM367886

				AP 4: HM367987 AP 5: HM367988 AP 6: HM367989 AP 7: HM367990 AP 8: HM367991 AP 9: HM367992	AP 4: HM367887 AP 5: HM367888 AP 6: HM367889 AP 7: HM367890 AP 8: HM367891 AP 9: HM367892
S. nigricollis nigricollis	Pevas, Amazonas, left bank	PV	S 3°20’ W 71°49’	PV 1: HM368046	PV 1: HM367946
S. tripartitus	Vencedores, Rio Napo, right bank	V	S 1°12’ W 75°02’	V 1: HM368076 V 2: HM368077	V 1: HM367976 V 2: HM367977
S. tripartitus	Campo Serio, Rio Napo, right bank	CS	S 1°80’ W 74°70’	CS 1: HM368006	CS 1: HM367906
S. fuscicollis lagonotus	Sergento Lores, Rio Napo, right bank	SL	S 2°48’ W 73°70’	SL 1: HM368059	SL 1: HM367959
S. fuscicollis lagonotus	Quebrada Huiririma, Rio Napo, right bank	QH	S 2°45’ W 73°79’	QH 1: HM368050 QH 2: HM368051 QH 3: HM368052	QH 1: HM367950 QH 2: HM367951 QH 3: HM367952
S. fuscicollis lagonotus	Nueva York, Rio Tigre, left bank	NY	S 4°32’ W 74°29’	NY 1: HM368026 NY 2: HM368027 NY 3: HM368028	NY 1: HM367926 NY 2: HM367927 NY 3: HM367928
S. fuscicollis lagonotus	Miraflores, Rio Marañon, left bank	MF	S 4°48’ W 74°08’	MF 1: HM368021	MF 1: HM367921
S. fuscicollis lagonotus	Pilpintuwasi, Rio Nanay, left bank	PW	S 3°70’ W 73°28’	PW 1: HM368047 PW 2: HM368048 PW 3: HM368049	PW 1: HM367947 PW 2: HM367948 PW 3: HM367949
S. fuscicollis lagonotus	Padre Cocha, Rio Nanay, left bank	PC	S 3°68’ W 73°28’	PC 1: HM368040	PC 1: HM367940
S. fuscicollis lagonotus	Rio Itaya, right bank	IT	S 4°00’ W 73°21’	IT 1: HM368013	IT 1: HM367913
S. fuscicollis lagonotus	Diamante Azul, Rio Nanay, left bank	NA	S 3°53’ W 73°47’	NA 1: HM368024 NA 2: HM368025	NA 1: HM367924 NA 2: HM367925
S. fuscicollis illigeri	Cahuana	CA	S 5°26’ W 74° 34’	CA 1: HM367999 CA 2: HM368000 CA 3: HM368001 CA 4: HM368002 CA 5: HM368003 CA 6: HM368004	CA 1: HM367899 CA 2: HM367900 CA 3: HM367901 CA 4: HM367902 CA 5: HM367903 CA 6: HM367904
S. fuscicollis illigeri	Aguas Calientes, Contamana, Rio Ucayali, right bank	AGC	S 7°13’ W 74°57’	AGC 1: HM367979 AGC 2: HM367980 AGC 3: HM367981	AGC 1: HM367879 AGC 2: HM367880 AGC 3: HM367881
S. fuscicollis illigeri	Rio Tapiche, left bank	TAPL	S 5°43’ W 74°02’	TAPL 1: HM368066 TAPL 2: HM368067 TAPL 3: HM368068 TAPL 4: HM368069	TAPL 1: HM367966 TAPL 2: HM367967 TAPL 3: HM367968 TAPL 4: HM367969
S. fuscicollis leucogenys	Pueblo Libre	PL	S 5°52’ W 77°08’	PL 1: HM368045	PL 1: HM367945
S. fuscicollis leucogenys	Rumi Pata	RP	S 6° 04’ W 76° 58’	RP 1: HM368058	RP 1: HM367958
S. fuscicollis leucogenys	Moyobamba, EPS	MOY	S 6°04’ W 76°58’	MOY 1: HM368022 MOY 2: HM368023	MOY 1: HM367922 MOY 2: HM367923
S. fuscicollis leucogenys	Tarapoto	TAR	S 6°29’ W 76°22’	TAR 1: HM368074	TAR 1: HM367974
S. fuscicollis leucogenys	Bellavista	BEL	S 7°01’ W 76°36’	BEL 1: HM367993 BEL 2: HM367994 BEL 3: HM367995	BEL 1: HM367893 BEL 2: HM367894 BEL 3: HM367895

S. fuscicollis leucogenys	Biological Station Panguana, Rio Pachitea	PA	S 9°37’ W 74°56’	PA 1: HM368036 PA 2: HM368037 PA 3: HM368038 PA 4: HM368039	PA 1: HM367936 PA 2: HM367937 PA 3: HM367938 PA 4: HM367939
S. fuscicollis weddelli	Rio Tambopata, left bank	TAM	S 12°57’ W 69°30’	TAM 1: HM368060 TAM 2: HM368061 TAM 3: HM368062 TAM 4: HM368063 TAM 5: HM368064 TAM 6: HM368065	TAM 1: HM367960 TAM 2: HM367961 TAM 3: HM367962 TAM 4: HM367963 TAM 5: HM367964 TAM 6: HM367965
S. fuscicollis weddelli	Biological Station Los Amigos, Rio Madre de Dios	LAM	S 12°34’ W 70°06’	LAM 1: HM368014 LAM 2: HM368015 LAM 3: HM368016 LAM 4: HM368017 LAM 5: HM368018	LAM 1: HM367914 LAM 2: HM367915 LAM 3: HM367916 LAM 4: HM367917 LAM 5: HM367918
S. fuscicollis nigrifrons	Rio Blanco, right bank	RBR	S 5°62’ W 73°87’	RBR 1: HM368053 RBR 2: HM368054 RBR 3: HM368055 RBR 4: HM368056	RBR 1: HM367953 RBR 2: HM367954 RBR 3: HM367955 RBR 4: HM367956
S. fuscicollis nigrifrons	Estaciŏn Biologica Quebrada Blanco	EBQB	S 4° 21’ W 73° 09’	EBQB 1: HM368008	EBQB 1: HM367908
S. fuscicollis fuscicollis	Rio Tapiche, right bank	TAPR	S 5°52’ W 74°00’	TAPR 1: HM368070 TAPR 2: HM368071 TAPR 3: HM368072	TAPR 1: HM367970 TAPR 2: HM367971 TAPR 3: HM367972
S. mystax mystax	Pijuyal, Rio Ucayali, right bank	PIJ	S 8°09’ W 74°19’	PIJ 1: HM368041 PIJ 2: HM368042 PIJ 3: HM368043 PIJ 4: HM368044	PIJ 1: HM367941 PIJ 2: HM367942 PIJ 3: HM367943 PIJ 4: HM367944
S. mystax mystax	Rio Blanco, right bank	RBR	S 5°62’ W 73°87’	RBR 5: HM368057	RBR 5: HM367957
S. mystax mystax	Estaciŏn Biologica Quebrada Blanco	EBQB	S 4° 21’ W 73° 09’	EBQB 2: HM368009 EBQB 3: HM368010 EBQB 4: HM368011 EBQB 5: HM368012	EBQB 2: HM367909 EBQB 3: HM367910 EBQB 4: HM367911 EBQB 5: HM367912
S. mystax mystax	Rio Tapiche, right bank	TAPR	S 5°52’ W 74°00’	TAPR 4: HM368073	TAPR 4: HM367973
S. mystax mystax	Aguas Calientes, Contamana, Rio Ucayali, right bank	AGC	S 7°13’ W 74°57’	AGC 4: HM367982 AGC 5: HM367983	AGC 4: HM367882 AGC 5: HM367883
S. imperator subgrisescens	Biological Station Los Amigos, Rio Madre de Dios	LAM	S 12°34’ W 70°06’	LAM 6: HM368019 LAM 7: HM368020	LAM 6: HM367919 LAM 7: HM367920
S. melanoleucus melanoleucus	Zoologische Staatssammlung, Munich	ZSM	-	ZSM 1: HM368078	ZSM 1: HM367978
S. labiatus labiatus	Bolivia, San Sebastian, Estación Biológica Tahuamanu	BOL	S 11°24’ W 69°01’	BOL 1: HM367996 BOL 2: HM367997 BOL 3: HM367998	BOL 1: HM367896 BOL 2: HM367897 BOL 3: HM367898
S. oedipus	German Primate Center (DPZ)	DPZ	-	DPZ: HM368007	DPZ: HM367907
Callithrix geoffroyi	Dresden Zoo	-	-	CAL: HM367905	CAL: HM368005
Cebus albifrons	GenBank	-	-	AJ309866.1	-
Saimiri boliviensis	GenBank	-	-	AJ315389.1	-
Aotus nancy-	GenBank	-	-	AJ489745.1	-

maae					
Cacajao calvus	GenBank	-	-	FJ531656.1	-
Pithecia monachus	GenBank	-	-	FJ531668.1	-
Callicebus torquatus	GenBank	-	-	AF524890.2	-
Alouatta seniculus	GenBank	-	-	AJ489759.1	-
Papio hamadryas	GenBank	-	-	Y18001.1	-
Macaca mulatta	GenBank	-	-	U38272.1	-
Pan troglodytes	GenBank	-	-	X93335.1	-
Homo sapiens	GenBank	-	-	DQ112959.2	-

Danksagung

Ich bedanke mich bei…

Eckhard W. Heymann, für die gute Betreuung, viele Anregungen und ein stets offenes Ohr für Fragen,

Christian Roos für die Geduld bei vielen, vielen Fragen im Labor, „bayrische Analysen“ und unzählige Grillfeiern,

Peter M. Kappeler für die Möglichkeit diese Arbeit in seiner Abteilung durchzuführen und allen Mitarbeitern der Abteilung Verhaltensökologie und Soziobiologie für die gute Zusammenarbeit,

Manuel Shahuano Tello, meinem Feldassistenten, ohne den die zahlreichen Proben mitsamt dem dazugehörigen Doktoranden nicht heil vom Amazonas nach Göttingen gekommen wären,

Stephan Behl und Peter Gottleuber für die unvergessliche Zeit als Vagabunden im Dschungel und so manche tapfer durchgestandene brenzlige Situation,

Allen Helfern, Assistenten, Bootsfahrern, Bürgermeistern, Gastgebern,… aus zahllosen Dörfern im peruanischen Regenwald,

Christina Oberdieck für die Hilfe und Unterstützung im Labor,

Marion Seiler für die Unterstützung und die vielen Sequenzen und Überstunden im Labor,

Stephen D. Nash für die schönen Illustrationen,

Dirk Meyer für die gemeinsam durchkämpfte Zeit im beschaulichen Gö, die Abenteuer zwischen Zürich und New York, all die Feierabendbiere und –diskussionen, und, und… (Rock`n Roll!), Sabine Hutschenreuther für jede Menge Ruhe und Geduld und Hilfe im Endphasen-Stress und bei last-minute Formatierungen, Yvan Lledo-Ferrer für die schöne und turbulente Zeit im Chaos-Büro („un aventura mas!“), Mojca Stojan-Dolar und Vanessa Mass für jede Menge Motivation und zahlreiche Ausflüge nach „Neverland“, Matthias Markolf, Susanne Schliehe-Dieks, Markus Port, Mr. Thinh van Ngoc und allen anderen Freunden und Kollegen, die die Zeit in Göttingen unvergesslich gemacht haben, Michael Pöhlmann, Benno Orthuber, Max Renner und Gertrud Spörl für den ständigen „Draht zur Außenwelt“ und die zwischendurch dringend notwendige „Erdung“,

Meinen Eltern und Großeltern, ohne deren Unterstützung diese Arbeit nicht möglich gewesen wäre.

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Erklärung über eigene Leistungen

Ich versichere, dass ich die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe. Die Stellen die anderen Werken wörtlich oder sinngemäß entnommen sind, sind als solche kenntlich gemacht. Eigene Beiträge im Verhältnis zu denen von Koautoren bei bereits publizierten oder zur Publikation eingereichten Teilen dieser Arbeit sind wie folgt:

Kapitel 2: Sieben mitochondrielle Genome wurden von Christian Matauschek zusammen mit Marion Seiler am DPZ sequenziert. Die in der Arbeit genannten spezifischen Primer wurden von Christian Matauschek für diese Studie angefertigt. Fünf mitochondrielle Genome wurden von Knut Finstermeier bearbeitet und für die Studie dankenswerterweise zur Verfügung gestellt. Die Datenauswertung und das Verfassen des Manuskripts wurden von Christian Matauschek durchgeführt.

Kapitel 3: Alle Tamarinproben wurden von Christian Matauschek im Peruanischen Amazonasgebiet gesammelt und im Labor bearbeitet. Die Datenauswertung und das Verfassen des Manuskripts wurden von Christian Matauschek durchgeführt.

Kapitel 4: Das Manuskript wurde im Wesentlichen von Anthony B. Rylands verfasst. Daten zu Verbreitungsgebieten und Sympatrie wurden von Christian Matauschek in Peru aufgenommen, ausgewertet und in das Manuskript eingearbeitet.

Ich versichere weiterhin, dass diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen hat.

Göttingen, Dezember, 2010