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ARTICLE

# Taxonomic status of *Tamarinus imperator subgrisescens* (Lönnberg, 1940) (Cebidae, Callitrichinae)

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**Abstract.** The emperor tamarin, *Tamarinus imperator,* is composed of two subspecies, the nominal type, *T. i. imperator,* distributed between the Acre and Purus Rivers, whose range is limited between the Brazilian state of Acre and Peru are unbounded, and *T. i. subgrisescens,* occurring in Peru, Bolivia, and Brazil, in the Brazilian states of Acre and Amazonas. Morphologically, both taxa are easily identifiable by the pelage pattern (chromogenetic fields), and even being easily distinguishable, both lineages are considered subspecies according to the criterion based on the Biological Concept of Species from the 1970s, even without presenting some necessary criteria, such as the intergradation zone. Here we analyzed pelage traits, cranial morphometry, Cytochrome-*b* divergence, and distributional pattern data applying the premises of integrative taxonomy to elucidate the taxonomic status of both lineages. We hypothesize that both lineages are considered full species through a series of criteria for species recognition, such as distinguishability, level of phenotypical divergences of several morphological complexes with congruence among them, and some genetic divergence. The hybridization is unknown and the low or the lack of sampling in target areas does not allow us to determine whether a hybridization or even contact zone between the two lineages exists indeed. All character sets analyzed were congruent with each other and reinforced the high level of divergences between the two subspecies including several pelage differences, morphometry (descriptive statistics, PCA, and MANOVA), and mitochondrial DNA Cytochrome-*b* divergence. Most of the distribution in both lineages are allopatric, and the levels of intra-lineage phenotypical variation are much lower than between the lineages.

**Keywords.** Emperor tamarin; Morphology; Cytochrome-*b;* Systematics; Species delimitation.

## INTRODUCTION

Marmosets, Tamarins, and Goeldi's monkeys' treatise of Hershkovitz (1977) defined the taxonomic arrangement of the family Callitrichidae for decades, but within the last thirty years, the systematics of this small-bodied Neotropical primate group is still found in intense debate (*e.g.,* Rylands *et al.,* 2016) to elucidate phylogenetic relationships inside the genera and mainly to delimit the taxa at the species level. Many of the lineages are still considered subspecies, a conservative scenario from the 1970s based on the Biological Concept of Species (de Queiroz, 2007) and does not take into account criteria, such as the verification of intergradation zones. Non-hybridization zones can be due to several reasons, such as the absence of samples or studies in target areas or the real absence of hybridization.

In respect to *Saguinus* (*lato sensu*), Hershkovitz (1977) recognized 10 species, clustering them into three species groups based on the facial morphology: haired, mottled, and bare-faced tamarins. Based on the high level of molecular divergence, Cropp *et al.* (1999) suggested that *Saguinus* should be divided into two genera: *Leontocebus,* grouping the small-bodied species (= *S. nigricollis* group); and *Saguinus,* for the remaining species. This arrangement was formalized later by Rylands *et al.* (2016). Later, Garbino & Martins-Junior (2017) divided *Saguinus* into three species groups and ranked them at the subgeneric

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level based on morphological and molecular markers: *Saguinus, Leontocebus,* and *Tamarinus.* Some authors refused *Leontocebus* and *Tamarinus* as distinct genera; the subgenus *Tamarinus* as recognized included *S. imperator, S. labiatus,* and *S. mystax.* Lastly, Brcko *et al.* (2022) again revised the tamarins' supraspecific taxonomic arrangements and based on a phylogenetic analysis of 44 nuclear and mitochondrial markers, restructured the group elevating once again *Leontocebus* to the full genus rank and proposed three species groups for *Saguinus,* each one representing a different lineage that would represent a distinct genus: *Tamarinus* (including *inustus* and *mystax* groups), *Saguinus* (*bicolor* and *midas* groups), and *Oedipomidas* (*oedipus* group).

Although there are many phylogenetic hypotheses for tamarins (Jacobs *et al.,* 1998; Cropp *et al.,* 1999; Tagliaro *et al.,* 2005; Matauschek *et al.,* 2011; Cunha *et al.,* 2011; Buckner *et al.,* 2015; Athaydes *et al.,* 2021; Brcko *et al.,* 2022), the most of last taxonomic decisions, that is, the delimitation of species was not based on those phylogenies and has used the distinguishability criteria to define the taxa. Currently, tamarins comprise 22 species and 20 subspecies (Rylands *et al.,* 2016). After Hershkovitz (1977), several studies were addressed to reassess the taxonomy of particular species groups resulting in the recognition of some subspecies as full species, such as *Saguinus tripartius* (Milne-Edwards, 1878), *S. geoffroyi* (Pucheran, 1845), *S. niger* (Geoffroy, 1803), *S. ursulus* (Hoffmansegg, 1807), *S. lagonotus* (Jiménez de la Espada, 1870), *S. nigrifrons* (Geoffroy, 1851), *S. weddelli* (Deville, 1849), and *S. cruzlimai* Hershkovitz, 1966 (Thorington, 1988; Natori & Hanihara, 1988; Moore & Cheverud, 1992; Matauschek *et al.,* 2011; Gregorin & Vivo, 2013; Sampaio *et al.,* 2015).

*Tamarinus imperator* (Goeldi), following the last generic arrangement proposed by Brcko *et al.* (2022), is one of the seven polytypic species for tamarins (Rylands *et al.,* 2016). Its distribution ranges from southeastern Peru and northwestern Bolivia to western Brazil in the states of Amazonas and Acre (Rylands *et al.,* 1993). *Tamarinus imperator* is defined by a conspicuous, long, white mustache, and two subspecies are recognized. *Tamarinus i. imperator,* occurs between the right bank of the Purus River and the left bank of the Acre River. This subspecies is represented by scarce records, and its original description was based on a low number of voucher specimens available both in collections and field observations. *Tamarinus i. subgrisescens* (Lönnberg, 1940) occurs from the right bank of the upper Juruá River, in the Brazilian states of Amazonas and Acre, to the upper Ucayali Basin, Peru (Hershkovitz, 1979), and Pando, Bolivia (Buchanan-Smith *et al.,* 2000); it is better represented by vouchers in collections, and there are more available data of field records and ecology.

*Tamarinus i. imperator* and *T. i. subgrisescens* were delimited by Hershkovitz (1977, 1979) using discrete pelage traits and parapatric distribution. Both taxa are easily distinguishable by the color of dorsal and tail pelage, and the presence or not of a white beard. No hybridization zone was evident, one of the premises to support the subspecific level. Indeed, no clear barrier is observed separating both taxa by their distribution, and the limit of these subspecies along the upper Acre, Purus, and Iaco Rivers, in Acre, is unclear.

Here, we used a comparative analysis of pelage, skull morphometrics, DNA evidence, and the distributional pattern to clarify the taxonomic status of these two lineages. Even with the low sampling of *T. i. imperator,* our study brings novelties regarding new diagnostic pelage characteristics to define both taxa besides consistent Cyt-*b* divergence among three individuals representing both taxa and new evidence on the morphology of type series material of *T. i. imperator.* We also updated the distribution of both taxa based on additional studied specimens and other sources of information as personal and literature reports.

## MATERIAL AND METHODS

### Material examined

The analyzed material included skins (denoted by sn) and skulls (denoted by sl) of 66 specimens housed in the following institutions: American Museum of Natural History, New York, USA (AMNH), Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA), Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ), Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG), Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP), Museum of Vertebrate Zoology, Berkeley, USA (MVZ), Swedish Museum of Natural History, Stockholm, Sweden (NRM), and The Field Museum, Chicago, USA (FMNH). We directly analyzed 60 specimens, including the lectotype of *T. i. imperator* housed at the MPEG, and five topotypes of *T. i. subgrisescens* housed in MZUSP and MNRJ. Six specimens, including all type series of *T. i. subgrisescens* (lectotype and paralectotypes), were studied using photographs sent by researchers and curators.

### Pelage

We carried out the study of pelage coloration considering the chromogenetic fields as defined by Hershkovitz (1977). The chromogenetic fields were the head (crown), face, chin, scapular region, mid-dorsal portion (back), rump, chest, belly, inner and outer sides of the fore and hindlimbs, and the dorsal and ventral portions of the tail. Differential distribution of hairs on the chin and upper border of the ears was also analyzed.

### Morphometrical characters

We took 13 cranio-dentary measurements of 35 adult specimens only for descriptive statistics. We considered adult specimens when all permanent teeth erupted and the basisphenoid-basioccipital suture fused (Gregorin & Vivo, 2013). Only three skulls of *T. i. imperator* were available for study and 32 of *T. i. subgrisescens.* The

morphometrical variables, their acronyms, and definitions followed Gregorin & Vivo (2013): 1) greatest length of the skull (GLS), 2) breadth of the braincase (BRB), 3) condyle-basal length (CBL), 4) palatal length (PAL), 5) post-orbital constriction (POC), 6) breadth between outer orbital limits (ORB), 7) total length from upper canine to last upper molar (C-M), 8) palatal breadth (PAB), 9) upper canine breadth (C-C), 10) upper molar breadth (M-M), 11) mandible height (MAH), 12) greatest length of the mandible (MAL), and 13) total length from lower canine (anterior face) to the last molar (posterior face) (c-m) (Fig. 1).

Before performing the multivariate analysis, we evaluated the sexual dimorphism of each variable by applying a Shapiro-Wilk test (Table 2) to check the normality of each variable, and then we performed a *t*-test (parametric) or a Mann-Whitney U (non-parametric) according to the distribution of the variables. We consider $p \leq 0.05$ as significant for all statistical tests. Because the three specimens of *T. i. imperator* are male, statistical tests verifying sexual differences were applied only to *T. i. subgrisescens* (11 females and nine males). Subsequently, we performed a Principal Component Analysis (PCA) to verify the distribution of the set of variables for each individual at the vectorial space using a variance-covariance matrix (Cadima & Jolliffe, 1996). Lastly, we applied MANOVA using Hotteling's value with Bonferroni corrected. Among the three skulls of *T. i. imperator,* one was partially damaged, thus for multivariate analyses, we worked with a reduced dataset

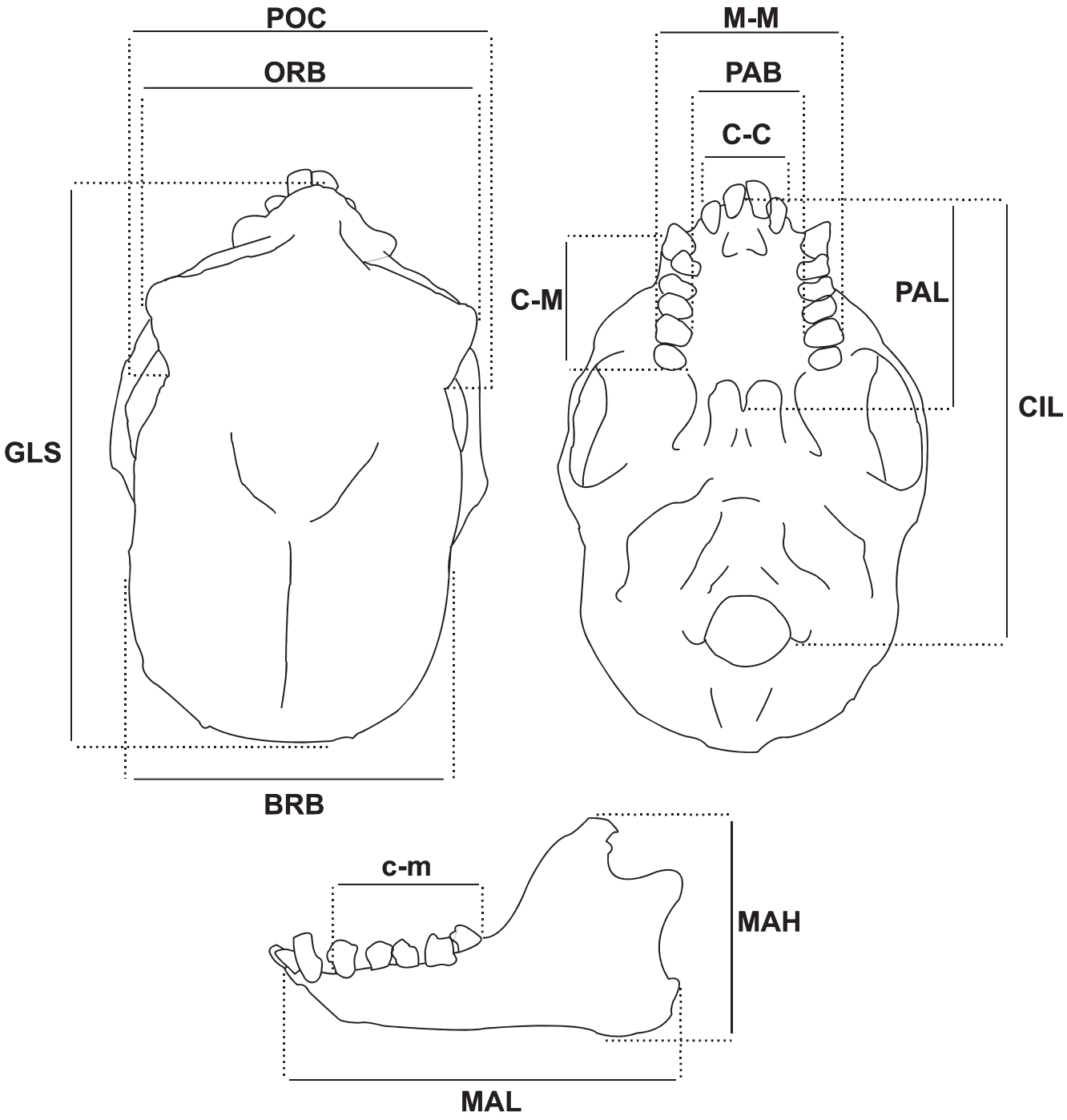


**Figure 1.** Schematic skull of *Tamarinus* showing the delimitation of the measures used in the morphometric analyses.

composed of nine measurements (variables 4, 5, 6, 7, 8, 9, 11, 12, and 13) to include all three skulls of *T. i. imperator* for the multivariate analyses. Both analyses as described above were performed in PAST© version 4.0.

## Mitochondrial DNA

We used two mitochondrial markers widely available in Genbank, Cytochrome-*b* and 16S, for estimative, molecular, phylogenetic relationships. We extracted DNA from the muscle of two *T. i. imperator* specimens (Table 1) through the phenol-chloroform method (Sambrook & Russel, 2001). We re-suspended the extracted DNA in 50 µL of TE buffer. We amplified the 5'region of the Cytochrome-*b* gene using the primers CytB1 AATGATATGAAAAACCATCGTTGTA and Cytochrome-*b* TTTCAGCTTTGGGTGTTGATG (Matauschek *et al.,* 2011). That of the 16S was amplified using the primers L1987-5' GCCTCGCCTGTTTACCAAAAAC 3' and H2609-5' CCGGTCTGAACTCA GATCACGT 3' (Araripe *et al.,* 2008). We amplified both genes in a 25 µL polymerase chain reaction (PCR) mix, including 0.3 units of Taq DNA polymerase (Invitrogen Platinum™ Taq DNA Polymerase), 2 mM $MgCl_2$ in 1× PCR buffer, 0.5 µMol per primer, 2.5 mM dNTPs, and about 20 ng of genomic DNA. We carried out the amplification in a thermocycler using a program consisting of 5 min of denaturation at 94°C, followed by 37 cycles – 30 seconds at 94°C, 30 s at 50°C, 1 min at 72°C, and a final extension for 10 min at 72°C. We visualized the PCR products in a 2% agarose gel. Subsequently, we purified the products of positive reactions using polyethyleneglycol 20% (PEG 20%) (Santos-Júnior *et al.,* 2015), we sequenced the purified PCR products in both directions with the same primers used in the PCR using the BigDye terminator sequencing kit (Applied Biosystems, Waltham, Massachusetts), and then we analyzed them with an ABI 3130xl (Applied Biosystems). We obtained the consensus sequences with SeqScape v.2.6. All laboratory procedures were conducted at the Laboratório de Biodiversidade e Evolução Molecular, Universidade Federal de Minas Gerais, Brazil (LBEM). In addition to the three sequences generated here (one CytB, and two 16S sequences), 40 Cytochrome-*b* and 16S sequences available in the GenBank were employed for 20 species belonging to Callitrichinae (Table 1).

For molecular data analyses, we aligned each gene individually using the algorithm MAFFT in the online server (https://mafft.cbrc.jp/alignment/server; Katoh *et al.,* 2017) assuming the default parameters. Subsequently, we submitted the 16S sequences to Gblocks 0.91b (Castresana, 2000) to search for and remove regions with ambiguous alignment. We used the default settings, except for the "allowed gap positions" option, which was set to 'with half'. After that, we concatenated the "two genes" alignments using the software SequenceMatrix v.1.8 (Vaidya *et al.,* 2011). We explored the best partitioning schemes and substitution models simultaneously, using PartitionFinder v.2.1.1 (Lanfear *et al.,* 2017) under a Bayesian information criterion for the entire matrix. The branch lengths were unlinked, the criterion for model selection was the corrected Akaike information criterion, and the search was done for all possible partitioning schemes.

We performed the phylogenetic analyses using a concatenated matrix with Cytochrome-*b* and 16S gene data, including a total of 20 species. We generated the phylogenies through Bayesian Inference (BI) in MrBayes 3.2.7 (Ronquist *et al.,* 2012) using two sets of Markov chains, each containing three hot chains and one cold, temperature set to 0.05 with 20 million generations and a 25% burn-in, to seek for convergence to the same subset of best trees. Convergence of the runs was assessed using the following statistics: standard deviation of split

**Table 1.** Vouchers for each molecular dataset employed, with the respective DNA sequence access numbers in the GenBank.

| Taxon | Voucher number | |
|---|---|---|
| | 16S | Cytochrome-*b* |
| *Tamarinus i. imperator* | MZUSP 1238 | — |
| *Tamarinus i. imperator* | MZUSP 1241 | This study |
| *Tamarinus i. subgrisescens* | EU497288.1 | HM368019.1 |
| *Tamarinus inustus* (Schwars) | — | KM370853.1 |
| *Tamarinus labiatus* | EU497289.1 | HM367998.1 |
| *Tamarinus mystax* | EU497295.1 | HM368073.1 |
| *Saguinus bicolor* (Spix) | EU497280.1 | KR528403.1 |
| *Saguinus midas* | EU497273.1 | EU232712.1 |
| *Saguinus niger* (Geoffroy) | EU497268.1 | — |
| *Oedipomidas oedipus* | NC_021960.1 | HM368007.1 |
| *Oedipomidas geoffroyi* | U39008.1 | AF001931.1 |
| *Oedipomidas leucopus* (Günther) | EU497286.1 | — |
| *Saguinus martinsi* (Thomas) | EU497277.1 | — |
| *Leontocebus fuscicollis* (Spix) | EU497285.1 | HM368072.1 |
| *Callithrix geoffroyi* (Humboldt) | NC_021941.1 | HM368005.1 |
| *Callithrix penicillata* (Geoffroy) | NC_030788.1 | NC_030788.1 |
| *Cebuella pygmaea* (Spix) | NC_021942.1 | NC_021942.1 |
| *Callimico goeldii* (Thomas) | KC592391.1 | KC592391.1 |
| *Leontopithecus chrysomelas* (Kuhl) | — | KR528398.1 |
| *Leontopithecus rosalia* (Linnaeus) | NC_021952.1 | NC_021952.1 |
| *Callibella humilis* Roosmalen, Roosmalen, Mittermeir & Fonseca | FJ769145.1 | — |
| *Mico mauesi* Mittermeier, Schwarz & Ayres | FJ769147.1 | AF245051.1 |

**Table 2.** Results of Shapiro-Wilk (SW) and *t*-test (for parametric distribution), and Mann-Whitney (for non-parametric distribution) (P) tests between males and females of *T. i. subgrisescens.*

| Variable | Mean and (Standard deviation) ♂ (n = 9) | Mean and (Standard deviation) ♀ (n = 11) | Shapiro-Wilk | P |
|---|---|---|---|---|
| GLS | 49.919 (1.848) | 50.321 (1.648) | 0.557 | 0.613 |
| BRB | 28.184 (0.913) | 28.331 (1.089) | 0.699 | 0.752 |
| CBL | 38.996 (1.559) | 39.441 (1.707) | 0.135 | 0.554 |
| PAL | 17.710 (1.735) | 17.503 (0.959) | 0.897 | 0.738 |
| POC | 23.767 (0.635) | 23.713 (0.729) | 0.658 | 0.864 |
| ORB | 27.174 (1.110) | 27.526 (0.994) | 0.082 | 0.464 |
| C-M | 9.906 (0.780) | 9.969 (0.720) | 0.078 | 0.852 |
| PAB | 11.141 (0.510) | 11.183 (0.552) | 0.351 | 0.864 |
| M-M | 16.889 (0.738) | 16.993 (0.701) | 0.161 | 0.751 |
| C-C | 15.483 (0.774) | 15.329 (0.850) | 0.253 | 0.680 |
| MAH | 18.648 (1.277) | 19.444 (1.293) | 0.843 | 0.185 |
| MAL | 32.066 (1.678) | 32.445 (1.795) | 0.596 | 0.635 |
| c-m | 11.427 (0.853) | 11.257 (0.695) | 0.025 | 0.630 |

frequencies, potential scale reduction factor (PSRF), and estimated sample size (ESS) for each parameter in Tracer 1.7.1 (Rambaut *et al.,* 2018).

We obtained the interspecific genetic distances with Mega X, using the parameters of the Kimura 2 model-K2P and variance estimation Bootstrap method with 500 replications (Kumar *et al.,* 2018). We performed two analyses with different data sets, one using the Cytochrome-*b* gene (1010 pb) and the other using the 16S gene (459 pb). We chose to use a data set for each gene because when both were used in the same data set, the amount of missing data prevented the achievement of a satisfactory result.

### Geographic distribution

To update the distribution, we considered data from museum labels, personal communication with photographic records, and literature with records in which we considered suitable (Izawa & Bejarano, 1981; Terborgh *et al.,* 1984; Bicca-Marques *et al.,* 1997; Lopes & Regh, 2003; Buchanan-Smith *et al.,* 2000; Matauschek *et al.,* 2011). We plotted the occurrence records on a map built using Quantum GIS v.3.22.2 software (https://www.qgis.org).

## RESULTS AND DISCUSSION

### Pelage coloration

We confirmed the five differences in the chromogenetic fields as already described by Hershkovitz (1979), in which the author delimited both subspecies. They are 1) a chin with just two patches of short, white hairs in *T. i. imperator* (Fig. 2) and a tufted chin with long, white hairs in *T. i. subgrisescens* (Fig. 2); 2) a chin markedly triangular and blackish in *T. i. imperator* (Fig. 2) and incipient in *T. i. subgrisescens* (Fig. 2); 3) brownish mingled with white hairs on both the chest and belly in *T. i. subgrisescens* (Fig. 3) and reddish, orange, and white hairs mixed in *T. i. imperator* (Fig. 4); 4) the back and lateral fringe of hairs are dark agouti and brownish or somewhat yellowish in *T. i. subgrisescens,* and light grayish or buffy fringe of hairs in *T. i. imperator;* and 5) silvery, light brown-grayish hairs along the inner side of forelimbs in *T. i. subgrisescens* (Fig. 2) and silvery orange in *T. i. imperator* (Fig. 2).

In addition, we observed three other consistent pelage traits that distinguish individuals of *T. i. subgrisescens* and *T. i. imperator:* 6) grayish or whitish agouti on the rump and base of the tail pelage, dorsally, similar to the thighs in *T. i. imperator* (Figs. 3 and 5) and orange in *T. i. subgrisescens* (Figs. 4 and 5); 7) tail, in general, predominantly grayish-brown or blackish with some yellowish or whitish hairs mixed (Figs. 3 and 4), in *T. i. imperator,* but orange or rufous on the ventral side close to the genitalia; rufous tail, orange on the tail with pelage from the middle and distal portions that eventually mingled with scattered blackish hairs, mainly at the terminal hairbrush, in *T. i. subgrisescens* (Figs. 4 and 5); and 8) the presence of a long (11-12 mm), dense tuff of black hairs on the upper and posterior border of the ears in *T. i. imperator* (Fig. 6) and naked or sparsely haired in *T. i. subgrisescens* (Fig. 6).

### Skull morphometry

Skulls of *S. i. imperator* proved to be smaller than *T. i. subgrisescens* in all measured variables (Fig. 7, Table 3). Even considering the discrepancy in the sampling between the two subspecies, it is possible to notice that the level of divergence of the measures between *T. i. imperator* and *T. i. subgrisescens* is larger than other sister species pairs, such as *S. niger* and *S. umbratus* (Gregorin

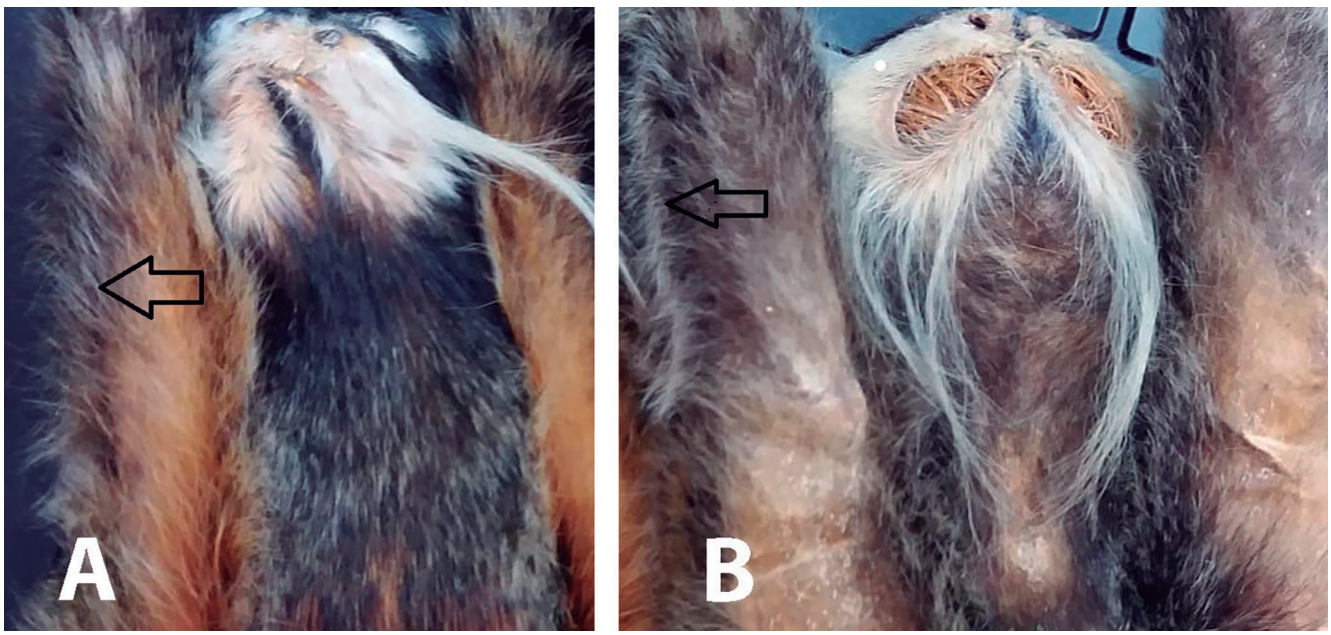


**Figure 2.** Comparison of some characters between *T. i. imperator* (A) and *T. i. subgrisescens* (B) (ventral view). Note the short white hairs tuft, blackish chin, and greyish-brown hairs on the lateral of the arm in A (arrow), and a long tuft of hairs, brown chin, and the lateral fringe of hairs silvery greyish in B (arrow).

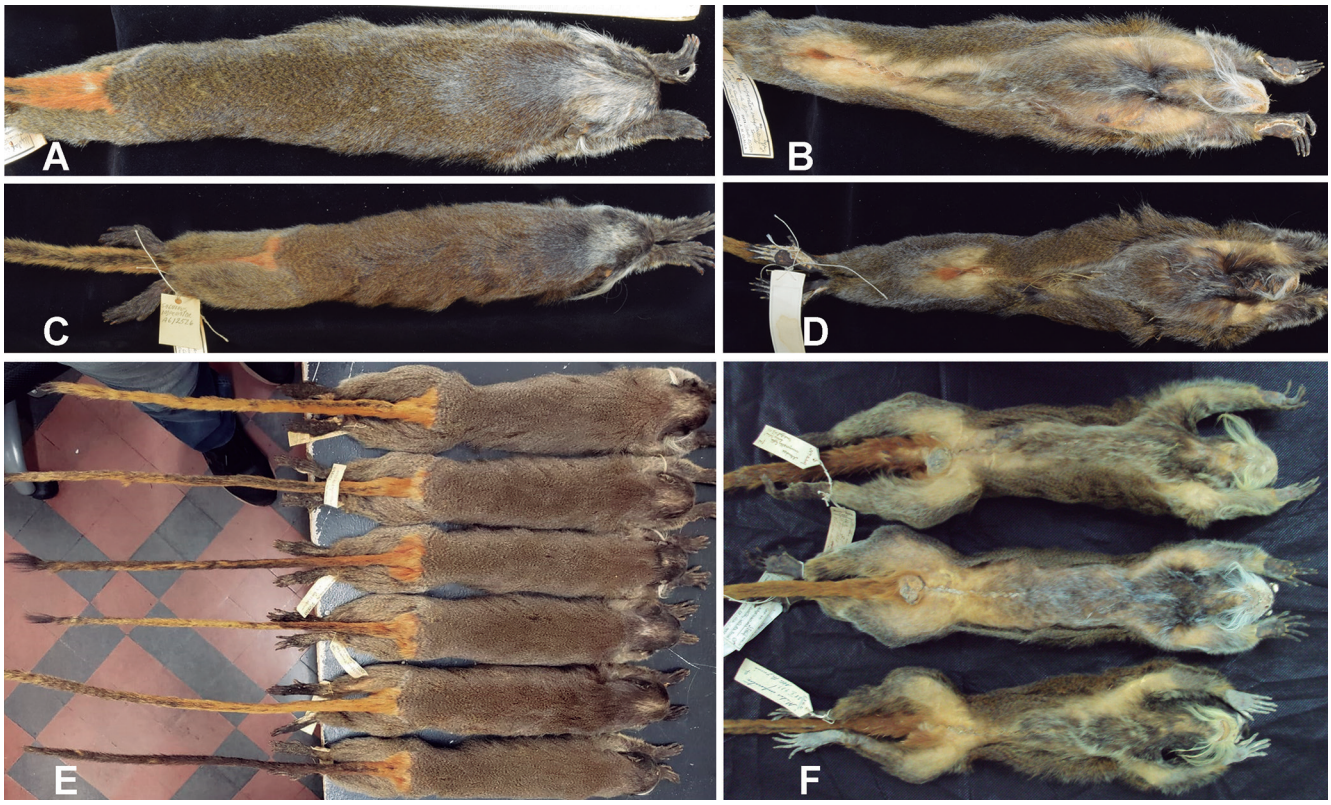


**Figure 3.** Pelage patterns in *T. i. subgrisescens,* left row, dorsal view, right row, ventral view. (A and B) RNM 632525, holotype; (C and D) RNM 612542, paratype; (E) series from Urubamba River, Peru (top to bottom: AMNH 75918, 75919, 75920, 75921, 76009, 76010); (F) series from Acre, Brazil (top to bottom: MPEG 1342, 22964, and 229). Note the rump and tail rufous (A, E, F), yellowish-brown belly (B, F), beard and mustache, and brown chin and throat (B, F).

**Table 3.** Descriptive statistics comparing *T. i. imperator, T. i. subgrisescens* from Brazil, between Juruá and Purus Rivers, and *T. i. subgrisescens* from Peru (Urubamba River). Upper line: mean and sampling (n); lower line: minimum-maximum.

| Variable/Taxon | *T. i. imperator* | *T. i. subgrisescens* Peru | *T. i. subgrisescens* Brazil |
|---|---|---|---|
| GLS | 46.7 (2)<br>46.6-46.8 | 50.6 (13)<br>49.2-52.5 | 50.2 (21)<br>47.1-53.1 |
| BRB | 26.9 (2)<br>26.7-27.0 | 27.5 (13)<br>26.6-28.4 | 28.4 (21)<br>25.3-31.6 |
| CBL | 35.2 (2)<br>35.0-35.6 | 41.2 (13)<br>39.6-43.4 | 39.5 (20)<br>35.3-41.6 |
| PAL | 16.5 (3)<br>(15.0-18.5) | 16.2 (13)<br>15.6-16.8 | 17.8 (21)<br>15.1-19.8 |
| POC | 23.3 (3)<br>22.5-24.5 | 23.9 (13)<br>23.1-24.7 | 23.8 (21)<br>22.8-25.3 |
| ORB | 25.6 (3)<br>25.0-26.6 | 26.9 (13)<br>25.6-28.2 | 27.4 (21)<br>25.5-28.6 |
| C-M | 8.9 (3)<br>8.5-9.2 | 9.8 (13)<br>9.2-10.7 | 10.0 (21)<br>8.2-11.00 |
| PAB | 9.5 (3)<br>8.9-10.0 | 11.3 (13)<br>10.2-11.8 | 11.2 (21)<br>10.0-12.4 |
| M-M | 15.6 (3)<br>15.0-16.0 | 16.0 (13)<br>15.8-17.3 | 17.0 (21)<br>15.9-18.1 |
| C-C | 13.2 (2)<br>13.1-13.3 | 15.4 (13)<br>14.0-17.8 | 15.5 (20)<br>14.5-17.0 |
| MAH | 16.7 (3)<br>15.3-18.0 | 19.0 (13)<br>17.0-20.0 | 19.0 (21)<br>16.8-22.2 |
| MAL | 29.3 (3)<br>28.7-30.0 | 32.1 (13)<br>29.7-34.0 | 32.4 (21)<br>29.8-35.2 |
| c-m | 10.2 (3)<br>9.9-10.5 | 13.4 (13)<br>11.6-15.0 | 11.4 (21)<br>9.7-12.6 |

**Table 4.** Scores and contributions of each variable in two first principal components (PC) and MANOVA (DF).

| Variable | PC 1 | PC 2 | DF1 | DF2 |
|---|---|---|---|---|
| CP | 0.145 | -0.668 | 0.099 | 0.544 |
| CPO | 0.164 | 0.071 | 0.525 | -0.849 |
| LO | 0.333 | -0.047 | 0.087 | 0.698 |
| CMS | 0.128 | 0.028 | 0.698 | 0.877 |
| LEM | 0.177 | 0.061 | 1.509 | 0.812 |
| LAM | 0.219 | -0.067 | 0.449 | 0.484 |
| AM | 0.513 | -0.004 | -0.318 | -0.098 |
| CM | 0.656 | -0.088 | 0.096 | -0.073 |
| CMI | 0.222 | 0.727 | -0.946 | -0.199 |
| Variation | 47.074% | 23.999% | | |
| Eingenvalue | 5.166 | 2.633 | 2.712 | 1.587 |

& Vivo, 2013) or *L. cruzlimai* and the subspecies of *L. fuscicollis* (*L. f. avilapiresi, L. f. primitivus, L. f. mura, L. w. weddelli,* and *L. w. melanoleucus* – Sampaio *et al.,* 2015). The scores of the PCA and MANOVA are in Table 4. Figure 8 shows that in both analyses (PCA and MANOVA), there was the formation of two clusters on axis 1, representing *S. i. imperator* and *S. i. subgrisescens.* Axis 1 is influenced by size and shows the differences in dimensions between the two groups of individuals representing the two already recognized lineages, with *S. i. imperator* being the smaller form. The variables that most contributed to this grouping in PCA were the height and length of the mandible. Axis 2 indicates some level of variation in *S. i. subgrisescens* when comparing samples from the Brazilian Amazon and Peru.

### Molecular analyses

We removed a total of 74 pb of ambiguous alignment from the 16S gene (533 pb with the ambiguous alignment regions). The concatenation of the genic regions resulted in a matrix with 1599 pb (Cytochrome-*b* – 1140 pb and 16S – 459 pb, Table 5). The best partitioning schemes and substitution models are presented in Table 6.

The phylogenetic analysis (BI) (Fig. 9) recovered *Saguinus* with high support of posterior probabilities (PP = 1), as previously found from morphological (Hershkovitz, 1977) and molecular analyses (Canavez *et al.,* 1999; Perelman *et al.,* 2011; Buckner *et al.,* 2015). The basal division of *Saguinus* into two groups as determined by Hershkovitz (1977) and subsequently confirmed by other studies (Canavez *et al.,* 1999; Cropp *et al.,*


**Figure 4.** Pelage patterns in *T. i. imperator.* (A, B, and C) MPEG 914, lectotype. (D, E, and F) MZUSP 4931 and 5012 from Manuel Urbano, Acre, Brazil. Note the dark greyish-brown tail (E), rufous belly (C, F), blackish throat and chin, no beard (F), and haired ear (B).

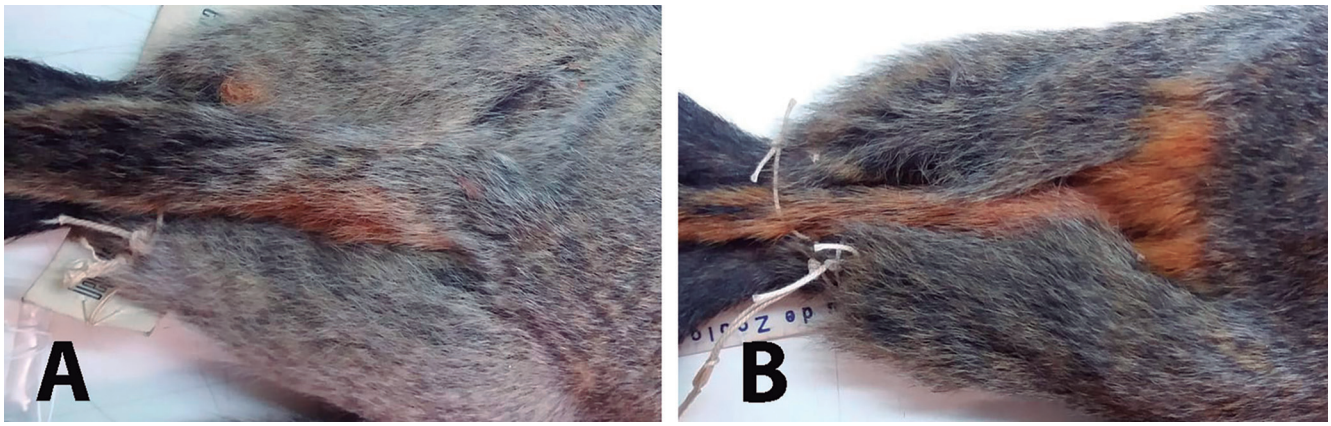


**Figure 5.** Rump and dorsal base of the tail grayish in *T. i. imperator* (A) and rufous in *T. i. subgisescens* (B).

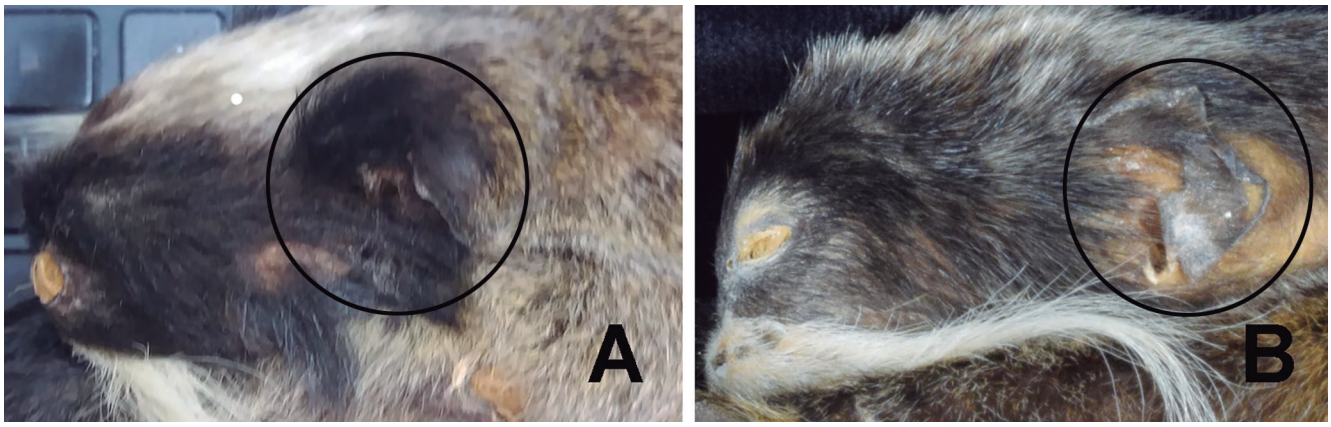


**Figure 6.** Lateral of the face. Note the hairy ear in *T. i. imperator* (A) and naked in *T. i. subgrisescens* (B).

**Table 5.** Full-length concatenated alignment data was used in the phylogenetic analysis. Numbers between parenthesis followed by 'N' refer to nucleotide base is not known.

| Taxon | Total (pb) | 16S | Cytochrome-*b* |
|---|---|---|---|
| *Tamarinus i. imperator* | 1471 | 459 | 1012 |
| *Tamarinus i. subgrisescens* | 1599 | 459 | 1140 |
| *Saguinus bicolor* | 1599 | 459 | 1140 |
| *Leontocebus fuscicollis* | 1599 | 459 | 1140 |
| *Oedipomidas geoffroyi* | 781 | 459 | 322 |
| *Tamarinus inustus* | 773 | (No data) | 773 (1 'N') |
| *Tamarinus labiatus* | 1599 | 459 | 1140 |
| *Oedipomidas leucopus* | 459 | 459 | (No data) |
| *Saguinus martinsi* | 459 | 459 | (No data) |
| *Saguinus midas* | 1599 | 459 | 1140 |
| *Tamarinus mystax* | 1599 | 459 | 1140 |
| *Saguinus niger* | 459 | 459 | (No data) |
| *Oedipomidas oedipus* | 1599 | 459 | 1140 |
| *Cebuella pygmaea* | 1599 | 459 | 1140 |
| *Callimico goeldii* | 1599 | 459 | 1140 |
| *Callithrix penicillata* | 1599 | 459 | 1140 |
| *Callithrix geoffroyi* | 1599 | 459 | 1140 |
| *Callibella humilis* | 459 | 459 | (No data) |
| *Mico mauesi* | 839 | 459 | 380 |
| *Leontopithecus chrysomelas* | 1140 | (No data) | 1140 |
| *Leontopithecus rosalia* | 1599 | 459 | 1140 |

**Table 6.** Best partitioning schemes and substitution models.

| Subset | Best Model | Subset Partitions | Subset Sites |
|---|---|---|---|
| 1 | GTR+I+G | Cytochrome-*b* 1 and 16S | 460-1599\3 1-459 |
| 2 | HKY+I | Cytochrome-*b* 2 | 461-1599\3 |
| 3 | GTR+I+G | Cytochrome-*b* 3 | 462-1599\3 |

1999; Ackermann & Cheverud, 2002; Tagliaro *et al.,* 2005; Matauschek *et al.,* 2011; Buckner *et al.,* 2015; Brcko *et al.,* 2022) was found: one clade composed by the small-bodied black mantle marmosets *(nigricollis* group, represented here by *S. fuscicollis)* and another clade including large-bodied species, with the *mystax, midas, bicolor,* and *oedipus* groups also supported for 1 PP.

Even considering that the low sampling of *T. imperator* does not permit testing for monophyly, the most important information for this study is the levels of genetic divergence, particularly Cyt-*b* among the three individuals of *T. imperator,* each representing the subspecies. The smaller genetic distances among pairs of evolutionary closed taxa of tamarins using Cyt-*b* were *T. labiatus* × *T. mystax* (4.31) and *T. i. imperator* × *T. i. subgrisescens* (4.42) (Table 7). Cytochrome-*b* has been a very useful mitochondrial marker for defining species in mammals, though defining them based uniquely on only one gene is not easy (Vallinoto *et al.,* 2006). Cropp *et al.* (1999), using a poll of three mitochondrial regions (D-loop, Cytochrome-*b,* and 16S) found some similar genetic divergence levels for some sister species as compared to our data, such as 3.4 for *S. midas* × *S. niger,* 5.1 for *L. tripartitus* × *L. nigricollis,* and 4.9 for *O. oedipus* × *O. geoffroyi*. Thus, strictly regarding Cyt-*b,* our data is following the literature, and they are compatible in recognizing both taxa of *T. imperator* as full species based on genetic

**Table 7.** Genetic distances for Cytochrome-*b* sequences (%) between species of *Saguinus.* GD = genetic distance; SE = Standard error.

| Taxa-pair | GD | SE |
|---|---|---|
| *T. labiatus* × *T. mystax* | 4.31 | 0.71 |
| *T. i. imperator* × *T. i. subgrisescens* | 4.42 | 0.68 |
| *S. bicolor* × *S. midas* | 7.81 | 0.94 |
| *T. labiatus* × *T. i. subgrisescens* | 8.95 | 1.02 |
| *T. mystax* × *T. i. subgrisescens* | 9.29 | 1.01 |
| *T. i. subgrisescens* × *O. labiatus* | 9.53 | 1.05 |
| *T. i. subgrisescens* × *T. mystax* | 9.75 | 1.05 |
| *S. bicolor* × *S. oedipus* | 12.32 | 1.24 |
| *S. bicolor* × *T. labiatus* | 13.76 | 1.30 |
| *S. midas* × *O. oedipus* | 13.99 | 1.36 |
| *L. fuscicollis* × *T. labiatus* | 14.19 | 1.27 |
| *S. bicolor* × *T. mystax* | 14.25 | 1.37 |
| *S. bicolor* × *T. i. imperator* | 14.46 | 1.32 |
| *S. midas* × *T. mystax* | 14.46 | 1.38 |
| *L. fuscicollis* × *T. i. imperator* | 14.53 | 1.34 |
| *L. fuscicollis* × *T. i. imperator* | 14.54 | 1.31 |
| *L. fuscicollis* × *T. mystax* | 14.55 | 1.33 |
| *T. labiatus* × *S. midas* | 14.72 | 1.38 |
| *S. bicolor* × *L. fuscicollis* | 14.88 | 1.32 |
| *T. labiatus* × *O. oedipus* | 15.42 | 1.38 |
| *S. bicolor* × *T. i. subgrisescens* | 15.48 | 1.38 |
| *L. fuscicollis* × *O. oedipus* | 15.51 | 1.37 |
| *L. fuscicollis* × *S. midas* | 15.59 | 1.39 |
| *T. mystax* × *O. oedipus* | 15.68 | 1.45 |
| *T. i. subgrisescens* × *O. oedipus* | 15.72 | 1.38 |
| *T. i. subgrisescens* × *S. midas* | 15.80 | 1.38 |
| *S. midas* × *T. i. imperator* | 15.81 | 1.46 |
| *O. oedipus* × *T. i. imperator* | 15.86 | 1.44 |

divergence. However, we reiterate that the use of a few individuals of *T. imperator* for molecular analysis is insufficient to express the intragroup variation and the possibility of these divergences being altered. On the other hand, this study is the first to provide comparative sequences between the two subspecies of *T. imperator* and in congruence with the other characters, the genetic data becomes informative.

## Taxonomy

Several studies were addressed to delimit species in tamarins, and they resulted in the recognition of some subspecies as full species (Gregorin & Vivo, 2013; Matauschek *et al.,* 2011). Many species that were recently recognized based on pelage (Hershkovitz, 1977; Vivo, 1991; Ferrari & Lopes, 1992; Mittermeier *et al.,* 1998) were validated with other sources of evidence such as osteological and dental complexes (Natori, 1986; Natori & Hanihara, 1992), morphometrical analyses (Gregorin & Vivo, 2013), and molecular markers (Vallinoto *et al.,* 2006; Ferrari *et al.,* 2010; Sampaio *et al.,* 2015; Costa-Araújo *et al.,* 2019). Our taxonomic decision was based on an integrative approach (Dayrat, 2005; Padial *et al.,* 2010) that considered the congruence of several traits including chromogenetic fields in pelage, morphometry, and Cyt-*b* divergence to recognize the lineages.

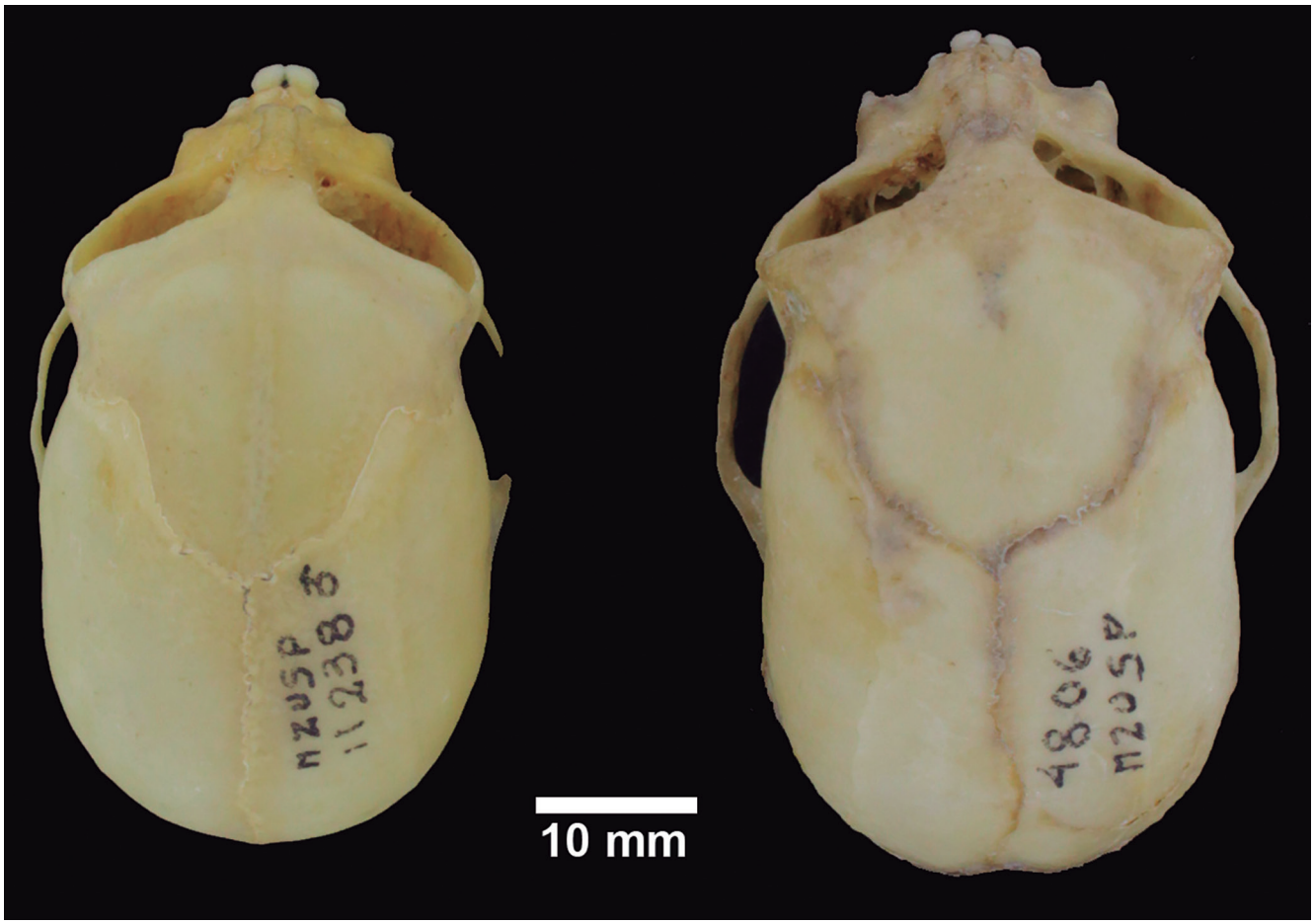


**Figure 7.** Top view of male specimens of the skulls of *T. i. imperator* (A = MZUSP 11238) and *T. i. subgrisescens* (B = MZUSP 4806), illustrating the difference in the general aspect. While in *T. i. imperator* the skull is more delicate and noticeably smaller, the skull of *T. i. subgrisescens* has both greater size and robustness.

Applying the unified concept of species (de Queiroz, 2007) with several sources of evidence not only provides robustness to the delimitation of lineages, and therefore species, but also allows inferences about more complex speciation processes to observe, such as parapatric speciation (Gao *et al.,* 2019). Considering that parapatric speciation occurs usually by the environmental gradient (dispersion – Florio *et al.,* 2012) and not by geographic/environmental ruptures as commonly postulated for allopatric speciation (Gao *et al.,* 2019), ecological studies of *T. imperator* and *T. subgrisescens* might corroborate our hypothesis of two lineages evolving independently (Blanckaert *et al.,* 2020). The second challenge is the delimitation of the contact zone between the two taxa on the Acre-Peru border and checking for the presence or absence of the hybridization zone, which could be due to secondary contact or evolutionary processes that could be recent and ongoing.

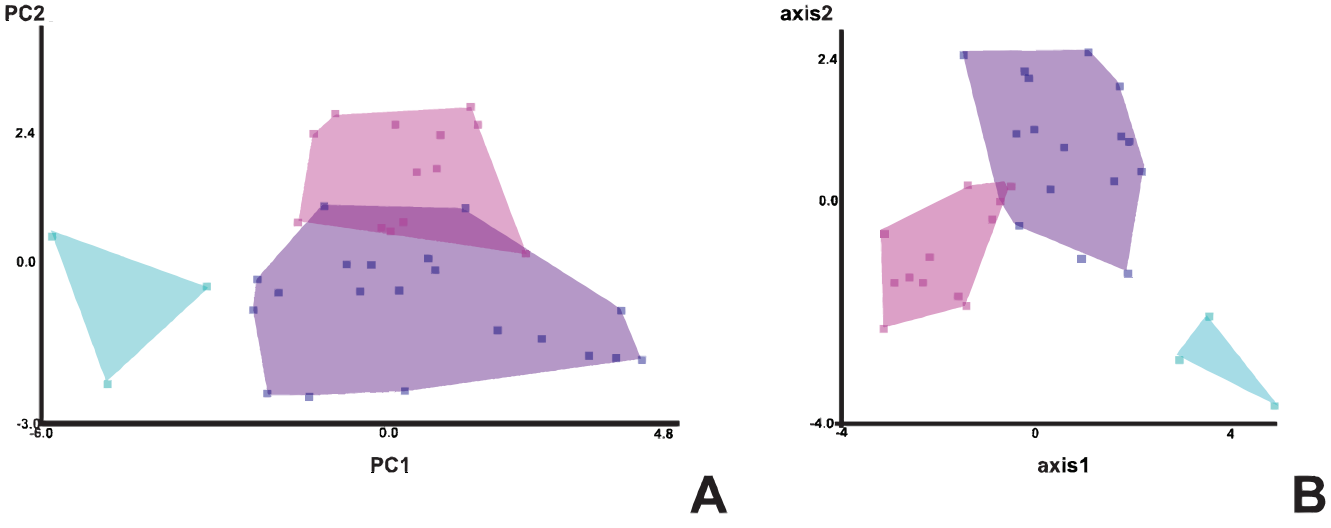


**Figure 8.** PCA and MANOVA scatterplot representing *S. i. imperator* (green) and *S. i. subgrisescens* from Peru (pink) and Brazilian Amazon (purple).

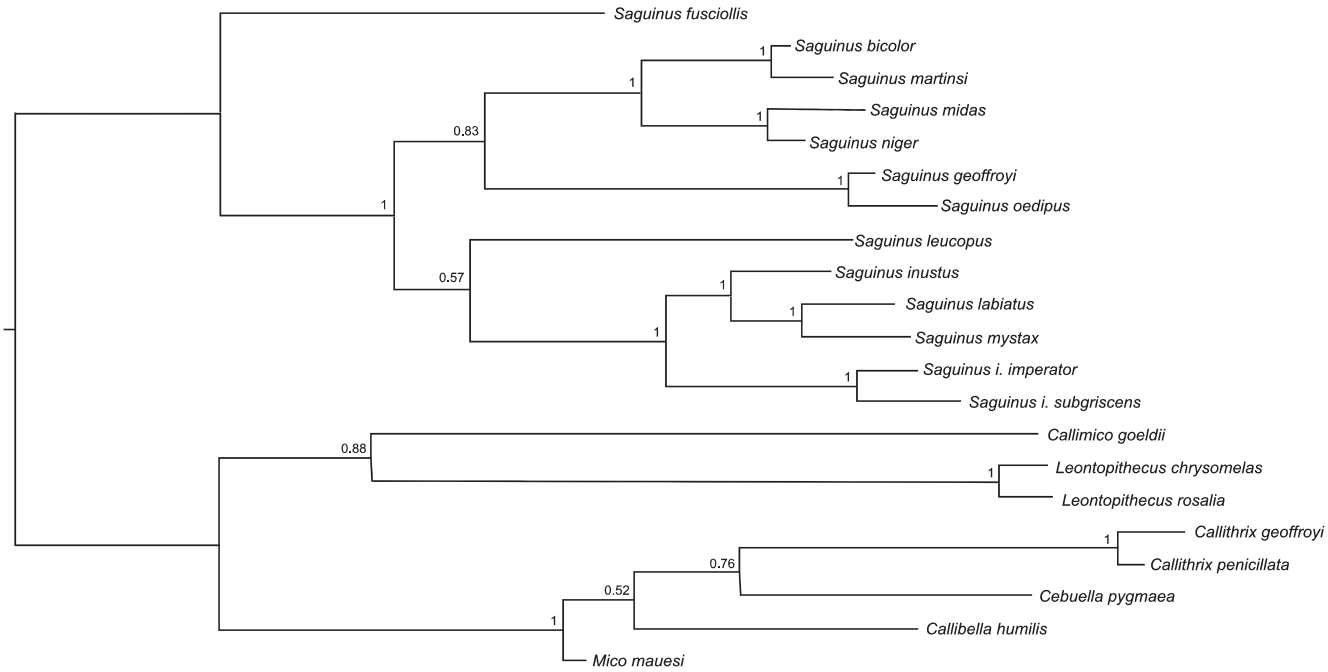


**Figure 9.** Bayesian phylogeny of the genus *Saguinus* inferred by Cytochrome-*b* abd 16S data. The posterior probability values are given above the branches. The outgroup was composed of *Callimico, Callibella, Callithrix, Cebuella, Mico,* and *Lentopithecus* species.

## Species account

### *Tamarinus imperator* (Goeldi, 1907)

*Midas imperator* Goeldi, 1907: 93. Description based on five specimens from upper Rio Purus and Rio Acre, Amazonas. Series mingled specimens from two subspecies (see discussion below). Lectotype: MPEG 914 (Fig. 4), adult female, specimen mounted at the MPEG, designated by Carvalho (1959: 460). A complete synonymy has already been provided by Hershkovitz (1979).

**Material examined (total 7):** Brazil: State of Acre: Manoel Urbano: MZUSP 11238 (sn, sl), 11239 (sn, sl); Rio Branco MZUSP 11340 (sn, sl), MPEG 7099 (sn), 7100 (sn). State of Amazonas: Upper Rio Purus, Bom Lugar or Monte Verde: MPEG 868 (sn); MPEG 914 (sn) (lectotype).

#### *Type locality and type series*

Rio Acre, opposite Bom Lugar, state of Amazonas, Brazil. In the description of this species, Goeldi (1907) mentioned two juvenile specimens from Rio Acre and three (two adults and one infant) from upper Purús River, and no type specimen was designated. The original label of the female lectotype indicates "upper Rio Purus, Brazilian State of Amazonas, in Bom Lugar or perhaps Monte Verde". Several subsequent attempts to restrict the type locality for *T. imperator* resulted in quite confusing scenarios and were summarized by Hershkovitz (1979) and Rylands *et al.* (1993). Elliot (1913) mentioned "Rio Purus, a tributary of the Amazon, western Brazil", and Lönnberg (1940) restricted it to "the upper Rio Purús". Cabrera (1958) considered the type locality of *T. imperator* as "Río Acre y Purús; aqui restringida ao río Acre", but criticized by Carvalho (1959), who stated that "upper Rio Purus" would be more realistic. Nonetheless, the confusion persists due to the uncertainty of the provenience of the lectotype that could be placed on either locality as indicated in the original label of the lectotype, the Purus (Monte Verde) and Acre (Bom Lugar) Rivers.

The restriction made by Carvalho (1959) was cautiously reviewed by Hershkovitz (1979) once Bom Lugar and Monte Verde would be out of the distribution range of *T. imperator:* Bom Lugar is placed at the right bank of the Acre River whereas Monte Verde at the western (left) margin of the Purus River. However, Hershkovitz (1979) stated that specimens could be collected on the opposite bank of the Acre and Purus Rivers, and those towns were used as the nearest reference. The same procedure was done regarding the records of *T. imperator* from Manoel Urbano, left margin of Purus River, State of Acre, collected by P.E. Vanzolini in September 1973 (MZUSP 11238 and 11239). We checked the travel diary of Vanzolini who collected material on both banks along the Purus River and there is no indication on which side of the Purus River the individuals of *T. imperator* were collected, except he indicated the town of Manoel Urbano as a sampling reference. Recently, Rylands *et al.* (2016) reinforced that neither Monte Verde nor Bom Lugar should be the type locality of the black-chinned emperor tamarin.

The confusion of type locality is because the material housed at MPEG, one adult female (MPEG 914), one adult male (MPEG 36604), and one infant (MPEG 868) of *T. imperator,* which Goeldi (1907) described, was collected by Snethlage (1908) in a long expedition through the upper Purus region, including collection from both Monte Verde (Purus River) and Bom Lugar (Acre River), places relatively close to Amazon dimensions but that may have relevant biogeographical significance depending on the side of the river to be considered. Two juveniles of the five specimens that Goeldi (1907) had in his hands

were sent to European museums and three were housed at the MPEG as stated by Goeldi (1907: 94) "the family with three individuals (♂♀ adults, and a young one) are already mounted in the Pará Museum". The label of one adult female (lectotype) stated the provenience as "Alto Rio Purús", in Monte Verde or Bom Lugar. After Goeldi, subsequent researchers that studied the type series from MPEG referred only to the female and the infant, but never mentioned the adult male (Carvalho, 1959; Hershkovitz, 1979). A detailed analysis of the adult male studied by us and housed in the type cabinet at MPEG revealed that the specimen presented a phenotype typical of *T. i. subgrisescens,* with a long, white mustache, brown chin, and a completely rufous tail. We observed that those traits have been previously noted by Goeldi (1907: 94) in his fig. 23, which illustrated a male individual with two long tufts of hairs on each side of the face (a "mustache" and a "beard") and stated: "white hairs of the circumbuccal zone extending over the whole area of the lower jaw, not including the chin and inferior side of the jaw. As a result, the old male appears bearded as well as mustached" [Goeldi (1907: 95)], and "Most aberrant is the coloring of the tail in the old male. From the very insertion, the bright rusty-red color predominates in its whole circumference throughout its entire length except for a dark terminal tuft" [Goeldi (1907: 96)]. The completely red tail of the adult male is shown in Goeldi's colored figure that was also reproduced by Sampaio *et al.* (2015).

Thus, we are hypothesizing that the three specimens that Goeldi (1907) had in his hands did not form a family as he thought, and there is a possibility that the adult male and female came from distinct places, perhaps the female from the Acre River (typical *T. imperator*) and the male with the phenotype of *T. subgrisescens* may be from the Purus River (there is no information on the label of specimen MPEG 36604 and the original one was probably lost). Therefore, the type locality of *T. imperator* could be the Acre River (left margin), opposite Bom Lugar in concordance with Cabrera (1958).

### *Geographic distribution*

Based on vouchers and literature, *T. imperator* has been recorded throughout the Brazilian states of Acre and Amazonas limited by the Purus and Acre Rivers (Fig. 10; Appendix 1). In Amazonas, *T. imperator* is limited by the confluence of the Acre (left bank) and Purus (right bank) Rivers as confirmed by the type series and discussed by Hershkovitz (1979). Records for the species in Acre are based on specimens from Manoel Urbano, Rio Branco, and São Pedro River Basin as observed by Izawa & Bejarano (1981). During analysis of material housed at the MPEG, it was noted that a specimen identified as *Midas imperator* (MPEG 264) has its provenience tagged as "Cobija Bolivia?". The specimen was identified as *S. i. imperator* by Hershkovitz (1979) but it undoubtedly has a phenotype

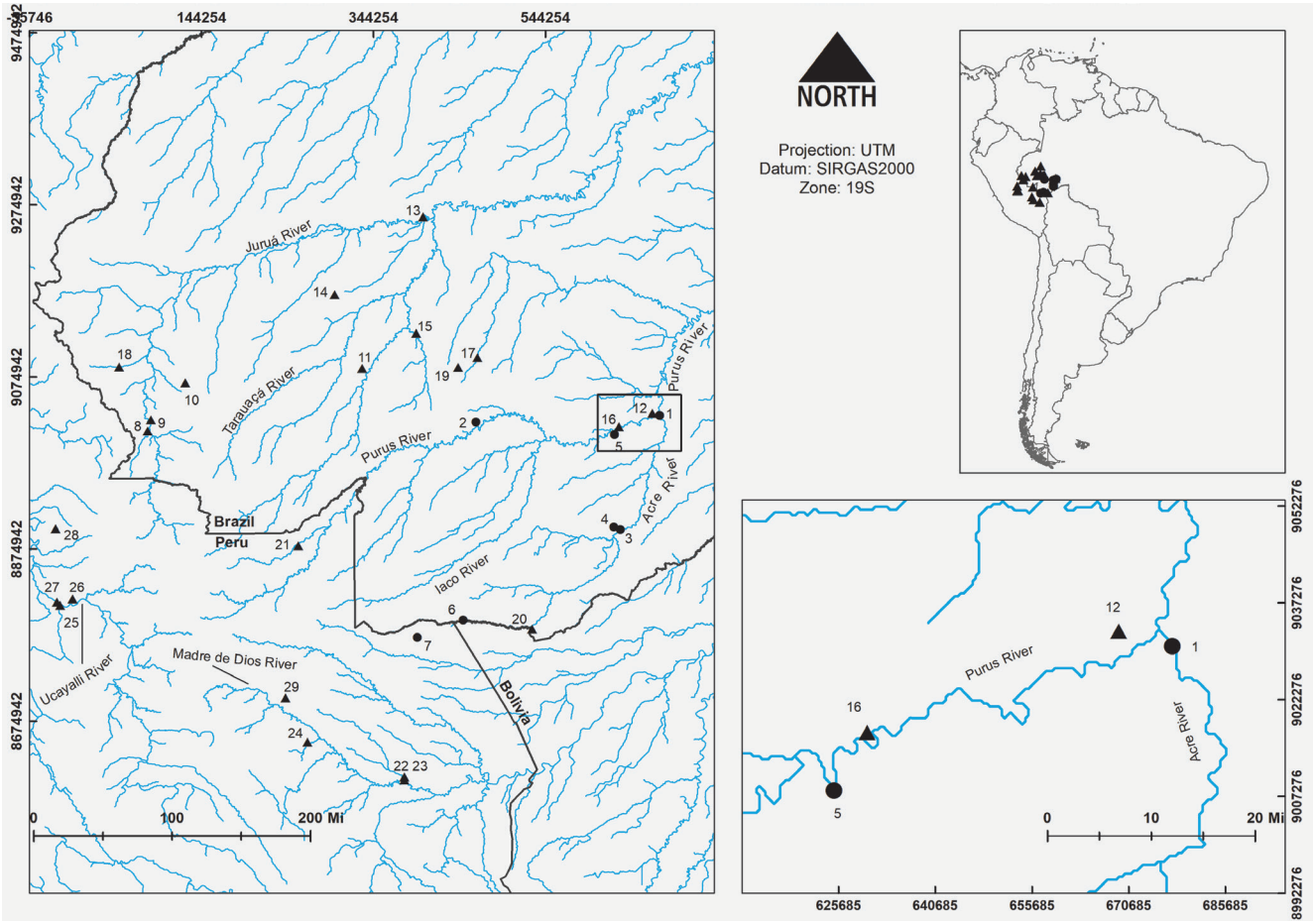


**Figure 10.** Map of the geographic distribution of *T. imperator* (circles) and *T. subgrisescens* (triangle).

of *T. subgrisescens*. Many primate surveys (Izawa & Bejarano, 1981; Freese *et al.,* 1982; Christen & Geissmann, 1994; Buchanan-Smith *et al.,* 2000) have not reliably recorded the species along the right bank of the Acre River. Indeed, Buchanan-Smith *et al.* (2000: 366, Table 1) indicated *T. imperator* in two places in the southern Acre River in Bolivia, but they just stated, "Although locals also reported *Saguinus imperator* (presumably *S. i. imperator*) to occur at two sites – Buena Vista and Los Campos – they are considered to be rare, and there has been no previous report of them in the area". Those records must be checked. All other records of emperor tamarins along the south margin of Rio Acre, including Cobija, clearly present the phenotype of *T. i. subgrisescens*. The southwestern limit of the species remains unknown.

### *Tamarinus subgrisescens* (Lönnberg, 1940)

*Mystax imperator subgrisescens* Lönnberg, 1940: 9. Description based on four specimens from Santo Antônio, left bank of Rio Eirú, near Rio Juruá, Amazonas. Holotype (Fig. 3 – designation based on information extracted from museum tag): RNM 632525, adult male, skin and skull, collected by A.M. Olalla on September 25, 1936. Paratypes: RNM 612543 and RNM 612542 (adult females, skin and skull, collected by A.M. Olalla on September 30, 1936), and RNM 612526 (adult male, skin and skull, collected by A.M. Olalla, September 26, 1936). A complete synonymy has already been provided by Hershkovitz (1979).

**Material examined (total 59):** Bolivia: Pando: Cobija (?): MPEG: 264 (sn, sl). Brazil: Acre: Pedra Preta: MPEG: 736; MZUSP: 9967 (sn, sl); Seringal Oriente: MPEG: 22965 (sn, sl), 735 (sn, sl); Poranga, Cruzeiro do Sul: MPEG: 22964 (sn, sl); Alto Rio Juruá: MPEG: 1342 (sn), 23202 (sl); Feijó: MPEG: 21848 (sn, sl). Amazonas: Santo Antônio, Rio Eiru: MZUSP: 4806 (sn, sl), 4812 (sn), 4931 (sn, sl) (topotypes); MNRJ: 5929 (sn, sl), 5930 (sn, sl) (topotypes); RNM, 612526, 612542, 632525, 632543 (sn, sl) (type series); Santa Cruz, Rio Eiru: MZUSP: 4864 (sn, sl), 4923 (sn, sl), 4925 (sn, sl), 4929 (sn), 4931 (sn, sl), 5012 (sn, sl), 5017 (sn, sl), 5023 (sn), 5024 (sn), 7115 (sn); Rio Jurupari: MPEG: 21846 (sn), 21847 (sn), 21849 (sn); Rio Juruá: MZUSP: 11386 (sn). Peru: Ucayali: Atalaya, Rio Urubamba: AMNH: 75918 (sn, sl), 75919 (sn, sl), 75920 (sn, sl), 75921 (sn, sl), 76009 (sn, sl), 76010 (sn), 76011 (sn, sl), 76012 (sn, sl), 76013 (sn), 76014 (sn, sl), 76015 (sn, sl), 76016 (sn, sl), 76017 (sn, sl), 76018 (sn, sl), 76019 (sn, sl), 147465 (sn); Rio Inuyo: AMNH: 99307 (sn), 98299 (sn), 98300 (sn, sl); Rio Tambo: AMNH: 99248 (sn), 147465 (sn); Loreto: Balta, Rio Curanja: MVZ: 136568 (sn), 136569 (sn). Madre de Dios: Zona Boca Amigo: FMNH: 84232 (sn), 84234 (sn); No locality: MPEG: 22963 (sl) and 36604 (sn).

#### *Type locality and geographic distribution*

Lönnberg (1940) indicated "Santo Antonio, western side of Rio Eiru near the confluence with Rio Juruá" as the provenience of the material used by him to describe his *Mystax imperator subgrisescens*.

*Tamarinus subgrisescens* occurs in the Brazilian states of Acre and Amazonas, the Peruvian departments of Madre de Dios and Ucayali, and the Bolivian department of Pando. In Bolivia (Fig. 10), this species is recorded in the Muyumanu River Basin, on the border of Peru, at the south Tahuamanu River, and at sites along the right (south) bank of the Acre River (Izawa & Bejarano, 1981; Buchanan-Smith *et al.,* 2000), including Cobija. In Peru, there are records of *T. subgrisescens* in localities along Madre de Dios and Manu Rivers, at the Curanja River (department of Ucayali), Atalaya, the mouth of Urubamba River, and Iñaperi (Encarnación & Castro, 1990); the western limit of the species seems to be the lowlands bordering the Fitzcarrald Arch. In Brazil, *T. subgrisescens* occurs along the right bank of the upper Juruá River (state of Acre and the Envira River, municipalities of Feijó and Cruzeiro do Sul) and the southwestern state of Amazonas, in Pauiní (west bank of Purus River), the northernmost reliable limits of the species to date (Fig. 10).

**AUTHORS' CONTRIBUTIONS:** RG: Conceptualization, Investigation; RG, DA, JESJ, TBA: Writing – original draft, DA, JESJ, TBA: Methodology. All authors actively participated in the discussion of the results; they reviewed and approved the final version of the paper.

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## APPENDIX 1

Records of *T. imperator* and *T. subgrisescens* based on studied specimens, except literature as indicated in parenthesis. Geographical coordinates and elevation are provided as possible. Map with localities in Fig. 10.

*Tamarinus imperator:* Brazil: 1) Monte Verde, mouth of Acre River (opposite side of Acre River), type locality (08°43′S, 67°20′W, 99 m) (see Hershkovitz, 1979); 2) Manoel Urbano (08°53′S, 69°19′W, 100 m); 3) Rio Branco (09°58′S, 67°48′W, 60 m); 4) Parque Zoobotânico da Universidade Federal do Acre (09°56′S, 67°52′W – Bicca-Marques *et al.,* 1997); 5) Reserva Extrativista Arapixi, righ bank of Purus River (08°58′14.2″S, 67°51′51″W); 6) Rio São Pedro (10°55′S, 69°28′W – Izawa & Bejarano, 1981). Peru: 7) Right margin Acre River (record needs confirmation) (11°06′S, 69°57′W – Izawa & Bejarano, 1981).

*Tamarinus subgrisescens:* Brazil: 8) Pedra Preta, near Taumaturgo (08°55′S, 72°48′W, 200 m); 9) Seringal Oriente (08°48′S, 72°46′W, 200 m); 10) Igarapé Porangaba (08°48′S, 72°46′W); 11) Feijó (08°16′S, 70°31′W, 153 m); 12) Monte Verde, upper Rio Purus (08°47′S, 67°25′W); 13) Santo Antônio, Rio Eiru, (type locality – 06°41′S, 69°53′W, 130 m); 14) Santa Cruz, Rio Eiru (07°30′S, 70°49′W, 130 m); 15) Rio Jurupari (07°54′S, 69°57′W, 150 m); 16) Reserva Extrativista Arapixi (05°83′S, 67°49′W) (Sampaio *et al.,* 2018); 17) Pauiní (07°42′S, 66°58′W). 18) Parque Nacional Serra do Divisor (08°16′S, 60°31′W); 19) Rio Paiuní (08°09′S, 69°18′W); Bolívia: 20) Rio Muyumanu (11°31′S, 69°03′W) (Buchanan-Smith *et al.,* 2000). Peru: 21) Balta, Rio Curanja (10°08′S, 71°13′W, 300 m); 22) Estação Biológica de Los Amigos (12°34′09″S, 70°06′00″W) (Matauschek *et al.,* 2011); 23) Zona Boca Amigo (12°36′S, 70°06′W); 24) "Altamira", Rio Manu (12°12′S, 71°08′W, 400 m); 25) Atalaya, mouth of Rio Urubamba (10°42′25″S, 73°45′W, 220 m); 26) Rio Inuya (10°40′S, 73°37′W, 228 m); 27) Rio Tambo (10°42′S, 73°47′W, 250 m); 28) Rio La Novia (12°34′09″S, 70°06′W, 259 m); 29) Estação Biológica Chocha Cashu (11°44′S, 71°22′W, 400 m – Terborgh *et al.,* 1984).

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