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Morphological and molecular identification of *Callithrix* sp. hybrids

Abstract

The common marmoset (*Callithrix jacchus*) and black-tufted marmoset (*Callithrix penicillata*) are endemic species of Brazil. Currently, both species are distributed in different regions of the country, being the main cause the illegal trafficking. The introduction of exotic animals is the second biggest cause of biodiversity loss globally and can lead to hybridization. In Bauru, São Paulo (SP), there is a record of both species and animals showing intermediate patterns between them. Thus, this work aimed to identify individuals of the genus *Callithrix* present in the Municipal Botanical Garden of Bauru (MBGB) as either pure or hybrids, through morphological and molecular markers. Ten individuals were sampled, 4 of which were pure species for control and six free-living, which were photographed, morphometrically measured, and from which the fragments of the mitochondrial genes COI and D-loop region were amplified. As a result, all animals showed intermediate patterns between both species. After analyzing the sequences obtained for D-loop and obtaining a high haplotypic diversity (h), low nucleotide diversity (π), Tajima D and Fu's Fs statistic, it is possible to infer that the animals in the area continue receiving gene flow and the population began from a founder effect. Morphological and molecular data indicate that the individuals sampled are hybrids. From the data generated by this research, it is possible to plan the insertion of management of these animals in the Conservation Unit (UC), to avoid population growth and its outcomes.

Keywords: primate, hybrid, anthropogenic hybridization, molecular biology

Volume 6 Issue 1 - 2023

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Received: November 25, 2023 | **Published:** December 08, 2023

Introduction

Callithrix[1] is a genus of primates (marmosets) belonging to the genus and are restricted to the Brazilian territory.[2] The common marmoset[3] and the black-tufted-ear marmoset *Callithrix penicillata* Étienne Geoffroy, 1812 are small animals that base their feeding on plant exudates (gums, saps, latex), little arthropods, fruits and flowers.[4,5] Individuals belonging to *C. penicillata* are endemic to the East Central and part of Northeastern Brazil, occurring in the states of Bahia, Minas Gerais, Goiás, Mato Grosso do Sul, the Federal District, the southwestern tip of Piauí, Maranhão and the north of São Paulo (north of the Tietê and Piracicaba Rivers). This species commonly occurs in the Cerrado, and Atlantic Forest in areas such as gallery forest and dry forest, beyond Caatinga (forest patches).[6,7] On the other hand, *C. jacchus* individuals are endemic to the Northeast region of Brazil and can be found in the semideciduous and deciduous scrub forest in Caatinga, besides gallery forest and humid Atlantic forest of Northeastern Brazil, in the states of Ceará, Rio Grande do Norte, Paraíba, Alagoas, Pernambuco and Piauí.[6] Both species were naturally separated from each other according to geographic barriers such as rivers, climate, relief and vegetation type,[8–10] have non-overlapping natural ranges, and therefore have experienced allopatric specialization. Nowadays these species are also distributed throughout the North and Southeast of Brazil,[8] due to escape from captivity, release and introduction into inappropriate environments and wild animal trafficking.[11,12]

The introduction of exotic animals can interfere with the environment in different ways, altering important ecological processes, being considered the second biggest cause of biodiversity loss in the world.[13–15] Some exotic species settles in the new environment at the detriment of native species, altering their habitat, preying on them or competing for resources, thus making their existence unfeasible, potentially leading to extinction.[3] Researches to understand the outcomes of *Callithrix* sp. as exotic animals had shown individuals of *C. penicillata* and *C. jacchus* as predators of birds, like *Turdus leucomelas* (pale-breasted thrush), *Polioptila plumbea* (tropical gnatcatcher), *Zenaida auriculata* (eared dove), and *Mimus saturninus* (chalk-browed mockingbird), besides a high predation rate on artificial eggs in nests, revealing that the presence of these exotic animals may be negatively impacting the ornithological community.[14,16,17] Moreover these works focused in avifauna, in 2006, a research done in Rio de Janeiro state showed that in two areas where golden lion tamarins *Leontopithecus rosalia*[3] were reintroduced, there were more marmosets (*Callithrix* spp.) than tamarins, and both animals have similar diets and ecology, competing for resources.[3]

Furthermore, such introduction of species outside their natural range can even drive the occurrence of hybridization with an autochthonous species.[18] According to Zinner, Arnold and Roos,[19] natural hybrids occur for several reasons and can be considered an evolutionary factor, however, when hybridization is induced by human activity, such as animal trafficking, releasing and introduction into inappropriate environment and due to escape from captivity, it may have implications for the conservation. For mainly of non-human primates (NHP), it may lead to a reversal speciation (the fusion of parental species through interbreeding of them or interbreeding of them with hybrids - backcrossing) and introgression (exchanging of alleles between two species or one species receiving alleles of the other). Despite this, studies on hybridization in *Callithrix* sp. and hybridization zones are still scarce.[18]

As reported by De Paula,[20] there is a record of *C. penicillata* and *C. jacchus*, in an Environmental Protection Area (EPA), in Bauru, SP, Southeast of Brazil. These animals were identified by interviewing people who affirmed had seen them, thereafter searching for scarification marks on trees and *in loco* search in the areas informed, where it was possible to document around 150 marmosets. In 2009, *C.*

jacchus were reported by Silva[14] in The Municipal Botanical Garden of Bauru (MBGB), which is part of the EPA and is an institution dedicated to plant conservation.[21] Its area of natural vegetation covers two global biodiversity hotspots for conservation priorities: Cerrado and Atlantic Forest,[22] additionally, it is part of the Wildlife Refuge (RVS) Aimorés, a classification of the National System of Conservation Units (SNUG) of Integral protection. This study aimed to characterize individuals of the genus *Callithrix* present in the MBGB, in order to provide biological data and subsidies for the conservation programs of the biomes and native species involved, associating molecular biology and morphology tools. In order to characterize properly, the biomolecular markers Cytochrome C Oxidase I (COI) and displacement-loop (D-loop) region of mitochondrial DNA (mtDNA) were used. COI is a 'barcode' region (short DNA sequence for species recognition and discrimination) located at the 5' of the gene while D-loop is the most variable region of mtDNA, used to identify genetically discrete populations.

Methods

The standard samples were obtained from blood and hair of 6 animals (3 *C. jacchus* and 3 *C. penicillata*) from a legalized animal breeding (Sagui Legal, based in Cotia, SP) and from hair of 5 animals (2 *C. jacchus*, 2 possível hybrids and 1 *C. penicillata*) from the Center for Medicine and Research in Wild Animals (CEMPAS), FMVZ, UNESP, SP, in order to standardize the following protocols used in this study. Approximately 0.2 – 0.5 mL of whole blood were collected from each animal in Vacutainer® tube, preserved in EDTA, stored at -20ºC and sent to the Animal Genetics Laboratory (Sao Paulo State University). The hair needed to retain their follicles for successful DNA extraction; therefore, they were removed with single pulls of around 200 hair strand to minimize additional stress on the animal. Field work was carried out within a radius of 100m at the Municipal Botanical Garden of Bauru (MBGB) (22º20'30"S, 49º00'30"W) in the central-west region of the state of São Paulo, Brazil (Figure 1) in November, 2020. Eight individuals were sampled, 6 adults and 2 juveniles. The animals were captured with auto-close traps, Tomahawk-style (34cm x 13cm x 12cm), covered with black cloth to decrease animal stress besides traps baited with a mix of bananas, paçoca (Brazilian sweet made with peanuts), and corn flour. All the samples were collected from free-range marmosets and all steps were done by biologists and veterinarians under the approval of the competent authorities in the country (Ethics Committee on Animal Experiments of São Paulo State University) (CEUA - 0157/2019), (COTEC - 292/2020 D31/2020 WLS) and (SisBio 71758-2). The animals were transported to a laboratory inside MBGB where they were monitored by a veterinarian. They were weighed and immobilized with an injection of ketamine (12 mg/kg) and midazolam (0,6 mg/kg) in the intramuscular region of the outer thigh. Then, they were measured, photographed and the biological samples (hair and blood) were collected as mentioned before.

While the animals were sedated, sex was checked (male or female) and age (juvenile or adult) was predicted based on body measurements (weight, tail length and body length), behavior and number of teeth. Information about body characteristics as color patterns (on head, back, legs and tail), besides dispositions of tufts as pre and/or post-auricular were collected. Body length, tail length, head length, chest and neck circumferences (the widest parte) were measured with a measuring tape; hands, feet and ears were always measured with a 50 cm ruler, on the right side. A plastic organizer box (36 cm x 28 cm x 23 cm) was adapted with led lights, holes and lined with covered cardboard, to be used as a photography studio. Every individual was photographed in portrait, lateral, ventral and dorsal decubitus, using a Nikon Coolpix L810 camera and an Asus Zenfone Max Shot.[23] Whole blood was collect as previous informed. At the end of data collection, trichotomy of the medial part of the tail was performed and the hair at the end of the tail and right tuft were dyed with pararosalin chloride in order to avoid recapture of the individuals. Afterward, animals were returned to cages and released at the same place they were captured after they got recovered from anesthesia (about 2h). During the procedures, vital parameters such as body temperature, respiratory and heart rates were continuously monitored and recorded in an individual record.

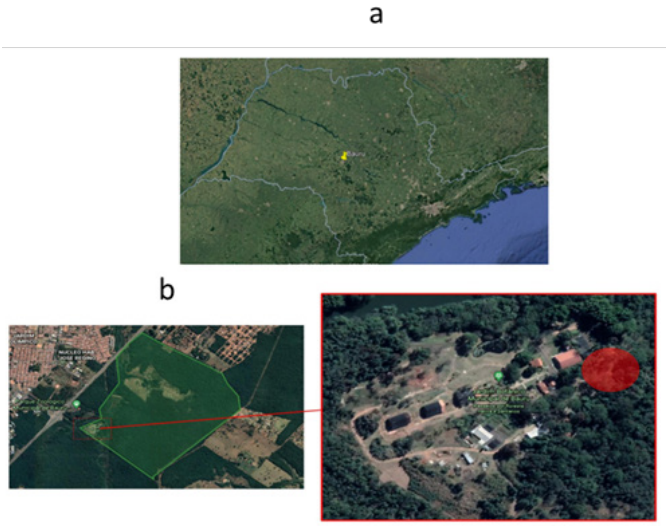

Figure 1 Study site. (a) Map of Brazil, in yellow dot highlighting the municipality of Bauru;

(b) Delimitation of the Bauru Municipal Botanical Garden and sample capture area highlighted in a red circle (Google Earth).

DNA from the sample was extracted using a standard proteinase K/phenol/chloroform protocol[24] with adaptations and later quantity and quality were verified on a spectrophotometer (NanoDrop ND-1000 Spectrophotometer – Thermo Fisher Scientific) by 260/280 nm absorbance. Nested Polymerase Chain Reactions (PCR) were performed using primer pairs for the fragments Cytochrome C Oxidase I (COI) and displacement-loop (D-loop) region of mitochondrial DNA (mtDNA), in Thermocycler (Eppendorf® Mastercycler® Nexus X2). The COI 700 bp fragment region was amplified using the primer pairs F1 forward 5'TTTTCAACCAACCACAAAGACATCGG3') e F5reverse (5'ACTTCTGGGTGGCCGAAAAATCAGAA 3'),[25] while the 1200bp fragment of the D-loop gene was amplified with the pair of primers cal_dloopF1 (5'CCCTAGTAGCTGACCTATTAAC3') and cal_dloopR2 (5'TGAGGTATGCGAGGAGTAAC3').[8] Later, the Nested PCR reactions were performed for both genes for improving the quality of the PCR for later sequencing, obtaining around 180bp COI fragment using the same primer F1forward and F1reverse (5'AATAAATGCGTGAGATGTGACGAT3');[25] and 1036bp D-loop fragment using the same primer cal_dloopF1 and HVIR (5'ATTCAATATCAGGCGCGATGATAG3'), as described by Malukiewicz.[8]

The PCR reactions were carried out in a final volume of 50µl, containing: 25µl of 2.0x Taq DNA Polymerase Master Mix (Ampliqon©) (2.0 mM $MgCl_2$), 25pmol of each primer, 50ng of genomic DNA and additional ultrapure water to complete the final volume. Amplification reactions of COI gene were carried out with initial denaturation at 94ºC for 5 minutes, followed by 35 cycles at

94ºC for 30 seconds, 55ºC for 45 seconds, 72ºC for 2 minutes and final extension at 72ºC for 7 minutes, while amplification reactions for D-loop gene were carried out with 5 minutes initial denaturation at 95ºC; 35 cycles of 95ºC for 45 seconds, 57ºC for 30 seconds, 72ºC for 90 seconds; and a final extension at 72ºC for 4 minutes and 30 seconds.

Both fragments obtained were identified by electrophoresis in 1% agarose gel, stained with Gel Red® (Uniscience) (0,1 µl/10 ml), run in 1x Tris- acetate-EDTA (TAE) buffer and then visualized in transilluminator (Benchtop UV Transilluminator, Cambridge, UK) with the UVP® VisionWorksLS™ (LifeScience Software) software. The amplified products were purified after the first PCR of each gene using the Illustra Kit GFX PCR DNA and Gel Band Purification (GE Healthcare), and after used in the Nested PCRs, following the same instructions given. Thereafter, all the amplified products in Nested PCR were observed in an electrophoresis gel and purified with the same kit as used before. Fragments were sequenced in both directions using the 2 µl Big Dye Terminator Cycle Sequencing Ready Reaction Kit versão 3.1 (Applied Biosystems, Foster City, CA), 2 µl of 2,5 Save Money (400 mM Tris-HCl ph 9, 0,10 mM MgCl2) and 5pmol of each primer in ABI3100 Genetic Analyzer (Applied Biosystems) following manufacturer's instructions.

The analyzed fragments are of conserved domain and consist of the hypervariable region 1 (HVR1) and part of the hypervariable region 2 (HVR2). The obtained sequences and the electropherograms were analyzed (Geneious v.10.0.9; Biomatters, Auckland, New Zealand), aligned with the Muscle algorithm,[26] and manually checked. The sequences were compared with the gene sequences that belong to the genus and *C. jacchus* and *C. penicillata* species using the blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Haplotype diversity, nucleotide diversity, number of polymorphic sites, number of haplotypes, and their frequency were estimated using DnaSP version 5.0 program[27] and Arlequin 3.0 software.[28] The same software was used to do the Fu's Fs statistic and the Tajima D tests.[29,30] The haplotype network design was made utilizing Phylogenetic Network 4.2.0.1.[31]

Results

The body measurements of all individuals samples are showed in Table 1. The mean and standard deviation (mean SD) for weight, body length and tail length of the captured *Callithrix* sp. (6 individuals) adults are 328.33±24.35g, 17.83±1.37cm and 31.33±4.12cm, respectively. The color patterns of the marmosets were compared with the ones already known for *C. jacchus* and *C. penicillata*[1] and all of them showed characteristics of both species, or even intermediate characteristics between them (Figure 2).

The tail sometimes showed a ringed pattern and others just a mix of colors consisting of wide dark gray and narrow white rings, sometimes with orange spots (Figure 3). The body showed two different patterns, being the first dark gray, with orange and white or light gray spots and the second light gray with orange and dark gray spots. Some had the upper back darker than the rest of the body. Most strands of hair in their bodies carried more than one color (dark gray and light gray/white or orange, dark gray and light gray/white). The tufts were represented by dark gray with few light gray/white hair strands, average pattern or light gray/white with few dark gray hair strands. It is also possible to notice that some strands are mixed themselves, having 2 colors in the same strand (possible to notice clearly on Figure 2 on individuals C1 and C3.

Table 1 Biometrical data from the MBGB individuals

Sample	C1	C2	C3	C4
Weight (g)	320	347	305	362
Age	Adult	Adult	Adult	Adult
Sex	Male	Male	Female	Male
Body lenght (cm)	17	20.5	17	18
Tail lenght (cm)	35	33.5	25.5	29
Chest circunference (cm)	15.8	15.5	14	17.5
Head circunference (cm)	3.5	4.5	3.5	6
Right foot lenght (cm)	6.2	5.9	6	5.5
Right hand lenght (cm)	3.5	4	3.9	4
Right ear lenght (cm)	2	1.5	1.5	1.7
Neck circunference (cm)	14.5	14.5	12	11
Sample	**C5**	**C6**	**C7**	**C8**
Weight (g)	212	343	254	293
Age	Juvenile	Adult	Juvenile	Adult
Sex	Female	Male	Male	Female
Body lenght (cm)	15.5	17	16.5	17.5
Tail lenght (cm)	29.5	29	30.9	36
Chest circunference (cm)	12	14.2	13	15
Head circunference (cm)	4	5	5.5	-
Right foot lenght (cm)	5.9	5.9	6	5.5
Right hand lenght (cm)	3.6	4	4.1	-
Right ear lenght (cm)	1.6	2	2	1.3
Neck circunference (cm)	11	13.3	12.5	-

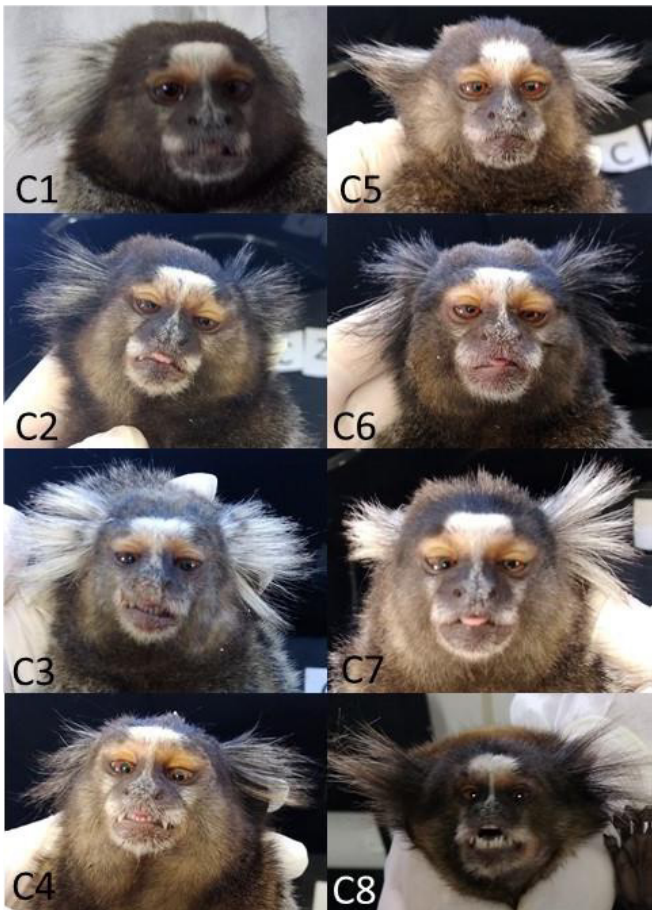

Figure 2 (C1 to C8) *Callithrix* sp. samples showing intermediate characteristics between *Callithrix jacchus* and *Callithrix penicillata.*

Citation: Santos BM, Almeida TRA, Rodrigues LP, et al. Morphological and molecular identification of *Callithrix* sp. hybrids. *Int J Mol Biol Open Access.* 2023;6(1):63–68. DOI: 10.15406/ijmboa.2023.06.00154

Figure 3 Individuals C2 and C6 (male-adults) showing pattern differences on their bodies and Tails.

In this research we observed that the auricular tufts of the free range marmosets do not match to the description of any species of the genus but are dark brown, dark gray or light gray with mixed strands, arranged anterior or around the ear and broken in some parts, forming a mosaic, suggesting the animals are hybrids and also they are probably outcomes of more than one generation of hybrids. During this research, no typical *C. jacchus* or *C. penicillata* were observed.

Molecular data

We successfully amplified and sequenced PCR products of ≈ 180bp of COI from all the samples, including the ones used from standards, and after alignment, all the sequences showed the same pattern (100% of identity). Besides, 795bp of D-loop mtDNA from 10 samples, being 4 obtained from captive marmosets (3 of *C. penicillata* from Sagui Legal and 1 of *C. jacchus* from CEMPAS) and the other 6 from wild *Callithrix* sp. (from MBGB). Nucleotide D-loop sequencing allowed us to determine 7 haplotypes, being 1 of the 3 *C. penicillata*, 1 from the only *C. jacchus* and 5 from the 6 *Callithrix* sp. When compared to a data bank using the blast tool, all the sequences corresponded to the species described or the hybrids, in the case of the samples from MBGB. The haplotypes also resulted in 28 polymorphic sites (Table 2), haplotypic diversity (h) of 0,911 and nucleotidic diversity (π) of 0,017. The haplotype 1 (H1) is shared by 2 individuals of MBGB, the haplotype 7 (H7) is shared by 3 individuals of the same species (*C. penicillata*) and location, while the other haplotypes are not shared (Figure 4), e.g. H6, what belongs to *C. jacchus*.

Table 2 Variable sites found in 7 haplotypes (H) of *Callithrix* sp. The first 6 sequences belong to hybrids (from MBGB and Cempas), the others belong to 1 *Callithrix jacchus* (from CEMPAS) and 3 *Callithrix penicillata* (from Sagui Legal) respectively

Samples	Polymorphic sites													
	292	**295**	**304**	**329**	**379**	**423**	**435**	**436**	**441**	**443**	**447**	**452**	**453**	**454**
CI	C	C	T	T	T	T	G	A	A	C	T	C	C	C
C3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C4	T	.	.	.	C	.	.	.	.	.	.	.	.	.
C7	T	T	.	.	C	.	.	.	.	.	.	.	.	.
C8	T	.	.	.	C	.	.	.	.	.	.	.	.	.
CH195	.	.	.	.	C	.	.	.	.	.	.	.	.	.
CJ156	T	.	.	.	C	.	.	.	.	.	.	.	.	.
M108	T	T	C	C	.	C	A	G	G	T	C	T	A	T
M177	T	T	C	C	.	C	A	G	G	T	C	T	A	T
M99	T	T	C	C	.	C	A	G	G	T	C	T	A	T
Samples	**Polymorphic sites**													
	459	**460**	**472**	**473**	**475**	**4SO**	**4S3**	**499**	**507**	**50S**	**519**	**522**	**526**	**535**
CI	C	A	C	C	C	C	C	A	T	T	C	T	T	C
C3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C4	.	G	A	T	.	T	T	C	C	.	.	.	.	.
C7	T	G	A	T	.	T	T	C	C	.	.	.	.	.
C8	T	G	A	T	.	T	T	C	C	.	.	.	.	.
CH195	.	.	.	.	T	.	.	.	.	.	.	.	.	.
CJ156	T	G	A	T	.	T	T	C	C	.	T	C	.	.
M108	.	.	.	.	T	T	.	.	.	C	T	C	C	T
M177	.	.	.	.	T	T	.	.	.	C	T	C	C	T
M99	.	.	.	.	T	T	.	.	.	C	T	C	C	T

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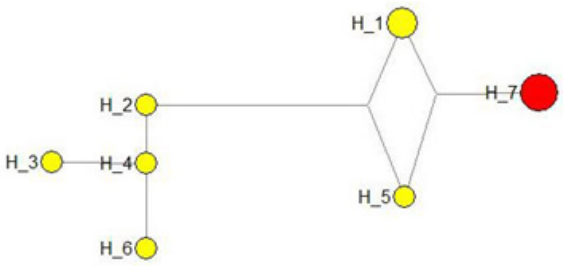

Figure 4 Haplotype (H) network for *Callithrix* sp. based on a 795 base pair (bp) sequence of the mitochondrial DNA control region (D-loop). Circle size is proportional to haplotype frequency. The haplotypes correspond to H1 (C1 and C3), H2 (C4), H3 (C7), H4 (C8), H5 (CH195), H6 (CJ156 - *C. jacchus* standard), and H7 (M99, M108, and M177 - *C. penicillata* standards), respectively.

Furthermore, when comparing the haplotypes of hybrids with haplotypes collected for *C. jacchus* and *C. penicillata*, all haplotypes have a bigger similarity to *C. jacchus*, above 57% while the similarity to *C. penicillata* is up to 28.6%.

Tests of neutrality performed on mitochondrial DNA control region sequences for all the individuals showed positive and non-significant values ($P<0.05$). The value obtained for Tajima D was 1.8983 ($p < 0.05$) and the value obtained for the Fu's Fs statistic was 1.64 ($p=0.404$). Our results indicate that demographic parameters are not influencing the groups sampled and that there is no sign of population expansion.

Discussion

The body weight and body length of adults correspond to the expected for the genus, specially to *C. jacchus* and *C. penicillata* species.[6] The other measures are not allowed to be compared due to the lack of information available in literature. According to De Vivo[1] and Diniz,[32] *C. penicillata* has long, narrow and black pre-auricular tufts, with a median white spot on the forehead and menton region, with a face color that is black or dark brown to light-gray brown, striated dorso and ringed tail. While *C. jacchus* has wide and white circum auricular tufts (tufts around the ears), with a median white spot on the forehead, striated back and ringed tail. Both species are much similar and differ basically in tuft colors and disposition (as they can be distinguished from the other species of the genus). This result is similar, in parts, with the research done by Cezar[33] in natural and anthropogenic areas but different from the phenotypes found in a natural hybridization area, where the hybrids maintained tufts similar to their parental, being totally dark or totally light.[34] The fragment sequenced of COI for all the individuals was not long enough to differentiate the individuals or to compare them with pure species, however it was expected once only 1bp was discriminated within Loiola et al.[25] paper.

The comparisons among haplotypes in percentage, indicate that most D-loop is similar to *C. jacchus*, what can indicate *C. jacchus* females as more susceptible to mate with *C. penicillata* males than the opposite. The haplotypic diversity (h) and nucleotidic diversity (π) for the *C. penicillata* from the breeding are null, what can indicate endogamy. Elseways, the haplotypic diversity (h) in the MBGB is high and similar to the ones found by Malukiewicz et al[8] and Faulkes et al.[35] for populations of *C. jacchus*, *C. penicillata* and hybrids of both species in a natural hybridization zone, which probably indicates that the animals from the studied area have parental generations from areas that continue receiving gene flow. For the same research, the value found for areas with anthropogenic hybridization is low. Otherwise, nucleotidic diversity for MBGB is low, similar to the values found for *C. jacchus* and *C. penicillata* pure populations.[8] According to Malukiewicz,[8] populations of *Callithrix* sp. with low value of π, are expected to be in expansion and it also indicates a founder effect. The founder effect corresponds to the origin of these animals in Bauru, once they are not endemic and were probably introduced in the area through illegal traffic of animals.[20] Thus, the hybrids are an unnatural part of this process caused anthropogenically. Although π presumably indicates that the population is in expansion, the values of Fu's Fs statistic and Tajima D suggest that the population is in balance and not expanding. The two results are diverging probably in reason of the restrict number of samples. Either in expansion or in balance, the exotic *Callithrix* sp. population at MBGB are hybridizing and maintaining themselves for a huge period,[20] what comes with consequences for the local ecology. Besides, Bauru is 45 km near from Lençois Paulista (SP), where a population of an endangered species (*Leontopithecus chrysopygus* - Black Lion Tamarin) of primate is resident[6] and has a similar behavior and ecology to the marmosets, what lead us to presume that if they get in the same location, the spends for the fauna and flora can be worse. All the exotic species in UCs are recommended by Ministério do Meio Ambiente (MMA) to be monitored and eliminated as soon as the problem is detected. The "elimination" of the exotic animals of the nature has different scenarios, and the most indicated are the ones that are less invasive as translocating animals to their original range or vasectomize the males. Thus, it is essential that the local authorities provide making a survey of the number of marmosets and repeat this survey year by year, while the other monitoring are made.

While monitoring and managing animals in these circumstances are crucial, it is as important or even more to address the problem from its origin. Some actions may include environmental education (e.g., teaching kids and the general population about the harmful outcomes of buying illegal animals, offering food for wild animals, or releasing animals outside their range), forbidding people from feeding wild animals, applying penalties if necessary, and reinforcing the surveillance over illegal traffic. The challenge gets exhausting when one effort diverges from the direction of the others.

Acknowledgments

We would like to thank the financing agency Coordination for the Improvement of Higher Education Personnel (CAPES) for providing a master scholarship to Bruna Mendonça Santos. We are grateful to the Municipal Botanical Garden of Bauru for all the support during this research.

Conflicts of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

1. De Vivo M. *Taxonomia de Callithrix (Erxleben, 1777) (Callitrichidae, Primates)*. Brazil: Belo Horizonte. 1991. Pp. 105.

2. Nakamura EM. Coexistence between marmosets and people: experiences in the Córrego Grande and Entorno Ecological Park, Florianópolis - SC. Completion of course work. Federal University of Santa Catarina, Florianópolis. 2009.

3. Primack RB, E Rodrigues. *Conservation Biology*. Brasil: Editora Planta Londrina. 2001. Pp. 328.

4. Ruiz Miranda CR, A Affonso, M De Morais, et al. Behavioral and ecological interactions between reintroduced golden lion tamarins (Leontopithecus rosalia Linnaeus, 1766) and introduced marmosets (*Callithrix spp* Linnaeus, 1758) in Brazil's Atlantic coast forest fragments. *Braz Arch Biol Techn*. 2006;49:99–109.

5. Ahlborn S, H. Rothe. Food selection of semifree common marmosets (*Callithrix jacchus*): Indications for optimal foraging. *Primates*. 1999;40:479–486.

6. Miranda GHB, DS Faria. Ecological aspects of black-pincelled marmoset (*Callithrix penicillata*) in the cerradão and dense cerrado of the Brazilian Central Plateau. *Brazilian Journal of Biology*. 2001;61(3):397–404**.**

7. IUCN, *The IUCN Red List of Threatened Species*. 2021.

8. Silva DF, EB Silva, AP Terra. Population control of invasive wild species through tubal ligation and vasectomy in *callithrix penicillata* primates. *Veterinária e Zootecnia*. 2018;25:9–15.

9. Malukiewicz JB, Vanner LF, Fuzessy AD, et al. Hybridization effects and genetic diversity of the Common and Black-Tufted Marmoset (*Callithrix jacchus* and *Callithrix penicillata*) mitochondrial control region. *American Journal of Physical Anthropology*. 2014;155(4):522–536.

10. Malukiewicz J. A Review of experimental, natural, and anthropogenic hybridization in *callithrix* marmosets. *International Journal of Primatology*. 2019;40:72–98.

11. Rylands AB, Coimbra Filho AF, Mittermeier RA. *The systematics and distributions of the marmosets (Callithrix, Callibella, Cebuella, and Mico) and Callimico (Callimico) (Callitrichidae, primates)*. In Ford SM, Porter LM, Davis LC (Eds), the smallest anthropoids: The marmoset/ callimico radiation. New York: Springer Science+Business Media. 2009. Pp. 25–61.

12. Brazil, MMA, IBAMA. *Espécies exóticas invasoras: situação brasileira*. Ministério do Meio Ambiente. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Brasília. 2006.

13. Levacov D, Jerusalinsky L, Fialho MS. Survey of primates received in screening centers and their relationship with wildlife trafficking in Brazil. *Primatology in Brazil*. 2011;11:32.

14. Pereira DG, Oliveira MEA, Ruiz Miranda CR, et al. *Impacts of invasive alien species on native species: the case of callitrichids in the Serra dos* Órgãos *National Park, Rio de Janeiro*. In: Simpósio Brasileiro Sobre Espécies Exóticas Invasoras, 2005, Brasília, DF. Sessão de Painéis. 2005.

15. Silva GL. *Avaliação dos impactos causados pela introdução do Sagui-de-tufos-brancos (Callithrix jacchus) sobre a comunidade de aves e serpentes nativas do interior de SP*. Anuário da produção de iniciação científica discente. Faculdade Anhanguera de Bauru, Bauru. 2009;12:29–37.

16. Vitousek PM, Aber JD, Howarth RW, et al. Human alteration of the global nitrogen cycle: causes and consequences. *Issues in Ecology*. 1997;1:1–16.

17. Lyra Neves RMD, Oliveira MAB, Telino Júnior WR, et al. Interspecific behaviour between *Callithrix jacchus* (Linnaeus) (Callitrichidae, Primates) and some birds of the Atlantic forest, Pernambuco State, Brazil. *Revista Brasileira de Zoologia*. 2007;24:709–716.

18. Begotti RA, Landesmann LF. Predação de ninhos por um grupo híbrido de sagüis (*Callithrix jacchus*/penicillata) introduzidos em área urbana: implicações para a estrutura da comunidade. *Neotropical Primates*. 2008;15(1):28–29.

19. Cortés Ortiz L, Roos C, Zinner D. Introduction to Special Issue on Primate Hybridization and Hybrid Zones. *International Journal of Primatology*. 2019;40:1–8.

20. Zinner D, ML Arnold, C Roos. The strange blood: Natural hybridization in primates. *Evolutionary Anthropology*. 2011;20(3):96–103.

21. De Paula HMG, RS Távora, MV De Almeida, et al. Estudos Preliminares da Presença de Sagüis no Município de Bauru, São Paulo, Brasil. *Neotropical Primates*. 2005;13(3):6–11.

22. Cardoso VS. O Programa de Educação Ambiental do Jardim Botânico Municipal de Bauru (Bauru-SP): a busca por uma identidade. Dissertação (mestrado), São Paulo State University, Bauru. 2013.

23. Myers N, RA Mittermeier, CG Mittermeier, et al. Biodiversity hotspots for conservation priorities. *Nature*. 2000;403(6772):853–858.

24. Silva MOM. *Análise fenotípica e genética em indivíduos de Callithrix (Callithrichidae: primates) no estado do Rio de Janeiro*. Dissertação (mestrado) Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro. 2018.

25. Sambrook J, DW Russell. *Molecular cloning: a laboratory manual*. 3 edn, New York: Cold Spring Harbor Laboratory Press. 2001.

26. Loiola S, RS Carvalho, HG Bergallo, et al. Barcode analysis using mini-amplicons strategy for museum samples of neotropical primates *Callithrix spp*. *Forensic Science International: Genetics Supplement Series*. 2015;5:e225–e227.

27. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004;32(5):792–1797.

28. Librado P, J Rozas. DnaSP version 5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 2009;25(11):1451–1452.

29. Excofer L, S Schneider. Arlequin version 3.0: an integrated software package for population genetics data analysis. *Evolut Bioinform Online*. 2005;1:47–50.

30. Fu XY. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics.* 1997;147:915–925.

31. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*. 1989;123:585–595.

32. Forster M, P Forster, J Watson. Network version 4.2.0.1: a software for population genetics data analysis. *Fluxus*. 2007.

33. Diniz LSM. *Primates in captivity: management and veterinary problems*. São Paulo: Icon.1997.

34. Cezar AM, LM Pessôa, CR Bonvicino. Morphological and genetic diversity in *Callithrix* hybrids in an anthropogenic area in southeastern Brazil (Primates: Cebidae: Callitrichinae). *Zoologia.* 2017;34:1–9.

35. Valdivino E. MAO Rímoli, J Rímoli, et al. Primates of the Dom Bosco Museum: identification and characterization. *Multitemas*. 2003;45–72.

36. Faulkes CG, MF Arruda, AO M Da Cruz. Genetic structure within and among populations of the common marmoset, *callithrix jacchus*: implications for cooperative breeding. *Mol Ecol*. 2003;12(4):1101–1108.