NEW RECORDS FOR *CALLITHRIX AURITA* AND *CALLITHRIX* HYBRIDS IN THE REGION OF VIÇOSA, MINAS GERAIS, BRAZIL

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Abstract

The genus *Callithrix* includes six species distributed across eastern Brazil in the Atlantic Rainforest, Caatinga, and Cerrado biomes. The buffy-tufted-ear marmoset (*Callithrix aurita*) is endemic to the Atlantic Rainforest and occurs in the states of Minas Gerais, Rio de Janeiro, and São Paulo. This species is considered 'Endangered' and listed among the world's 25 most threatened primates. The release into the wild of marmosets kept as pets has led to numerous invasive populations in southeastern Brazil, which hybridize with native congeneric species. This, combined with habitat loss and fragmentation, has caused a dramatic decline in *C. aurita* populations, and threatens them with extinction. The city of Viçosa, in Minas Gerais, has an almost 50-year history of introduced *Callithrix* species. *C. aurita*, the native species, was last recorded in the region in 1995. With the aim of updating knowledge on the current situation of *Callithrix* in the region, we surveyed 43 fragments of the Atlantic Forest in Viçosa and nearby cities between 2017 and 2019. We recorded 22 groups of hybrid forms, three solitary individuals of hybrid marmosets, two mixed groups of *C. aurita* with hybrids, and a single group of *C. aurita*. This record of *C. aurita* was the first of a pure group of the species in the region in 20 years. These results highlight one of the most important threats to *C. aurita*, and management actions are urgently needed to control invasive and hybrid populations, to allow the endangered buffy-tufted-ear marmoset to recover.

Keywords: Survey, playback, fragmentation, hybridization, primates, Atlantic Forest.

Resumo

O gênero *Callithrix* corresponde a seis espécies de primatas cuja distribuição abrange o leste brasileiro. O sagui-da-serra-escuro (*Callithrix aurita*) é endêmico da Mata Atlântica e ocorre em Minas Gerais, Rio de Janeiro e São Paulo. A espécie encontra-se 'Em Perigo' de extinção e consta entre os 25 primatas mais ameaçados do mundo. O tráfico ilegal de espécies do gênero *Callithrix* ocasionou a liberação de inúmeros saguis alóctones no sudeste brasileiro, resultando em populações invasoras que hibridam com as espécies nativas. Isto, aliado à fragmentação de habitats, fez com que as populações de *C. aurita* diminuíssem ao longo dos anos, resultando no seu atual risco de extinção. A cidade de Viçosa, em Minas Gerais, possui um histórico de introdução de espécies alóctones do gênero *Callithrix* de quase 50 anos, sendo que a espécie nativa, *C. aurita*, foi registrada pela última vez na região em 1995. A fim de atualizar a situação dessas espécies na região, entre 2017 e 2019, foi realizado um levantamento populacional para o gênero *Callithrix*, amostrando 43 fragmentos em Viçosa e região. Foram registrados 22 grupos de formas híbridas do gênero *Callithrix*, três indivíduos híbridos solitários, dois grupos mistos de *C. aurita* com híbridos e um único grupo de *C. aurita*. Estas informações confirmam a grave situação de *C. aurita* nessa região e indicam a necessidade de realizar ações de manejo urgentes para controlar as populações invasoras e híbridas e para conservar o ameaçado sagui-da-serra-escuro.

Palavras-chave: Levantamento populacional, playback, fragmentação, hibridação, primatas, Mata Atlântica

Introduction

The genus *Callithrix* is endemic to Brazil and includes the six non-Amazonian marmosets (*Callithrix aurita, C. flaviceps, C. geoffroyi, C. jacchus, C. kuhlii, and C. penicillata*). These species are widely distributed in eastern Brazil, with parapatric distributions across the Atlantic Forest, Caatinga and Cerrado (Rylands *et al.*, 2000; Rylands; Coimbra-Filho and Mittermeier, 2009). The genus has some unique characteristics, such as twin births and dental adaptations to gummivory (Coimbra-Filho and Mittermeier, 1977; Sussman and Kinzey, 1984). Due to

their relatively recent genetic divergence, *Callithrix* species can produce fertile hybrid offspring through interspecific mating (Malukiewicz, 2014).

The buffy-tufted-ear marmoset, *Callithrix aurita*, is endemic to the Atlantic Forest, in the states of Rio de Janeiro, São Paulo and Minas Gerais (Muskin, 1984; Rylands, Coimbra-Filho and Mittermeier, 2009). The species is categorized as Endangered (Melo *et al.*, 2018, 2020) due to an estimated population reduction of ~50% in the last 18 years (Melo *et al.*, 2020) and the remaining subpopulations being isolated in forest fragments (Bechara, 2012; Bernardo and Galetti, 2004). Additionally, the species competes with invasive congeneric species (*C. geoffroyi, C. jacchus* and *C. penicillata*) and their hybrid forms, which have been introduced into *C. aurita* distributional range (Bechara, 2012; Melo et al, 2015; Carvalho *et al.* 2018).

The *Callithrix* genus has species-specific pelage patterns which can be used as diagnostic features (Vivo, 1991). *Callithrix aurita* has short, white or yellowish ear tufts, a black body color usually dotted with red, and a non-striated dorsum (Fig. 1A). The white-faced marmoset, *C. geoffroyi*, on the other hand, has long black preauricular tufts; face, forehead and throat entirely white; in addition to the striated back with an orange base pelage, ranging from black and gray bands on its final portion (Fig. 1B). *Callithrix jacchus*, known as common marmoset, has white and long ear tufts; brown to grayish-brown sides of the face; medium white spot on the forehead; dark-brown chest and abdomen; third-posterior part of the dorsum striated with the pelage varying between dark-brown and light gray (Fig. 1C). *C. penicillata*, the black-tufted marmoset, has long preauricular tufts ranging from dark brown to black; medium white spot on the forehead; posterior portion of the back striated with the pelage varying between bands of brown or black and gray (Fig. 1D). All these characteristics were described by Vivo (1991).

In the hybridization process, hybrid individuals are expected to show a wider phenotypic variation than the parental populations, that can result in a mix of phenotypic patterns of each parent, making it possible to distinguish the hybrid's parental species in the first generations (Fuzessy *et al.* 2014) but as the hybrid forms reproduce, those patterns fade away, creating a marmoset with a greyish-pattern pelage, a mix of many generations of hybrids and the parental species could be just inferred (Fig. 1E).

Figure 1. Examples of pelage patterns in *Callithrix* genus. (A) *Callithrix jacchus*. Photo: Ronaldo Pereira. (B) *Callithrix penicillata*. Photo: Fabiano Melo. (C) *Callithrix geoffroyi*. Photo: Milene Figuereido. (D) *Callithrix aurita* and (E) hybrid individuals. Photos: Orlando Vital.

The introduction of non-native species is a huge ecological risk for native *Callithrix* in southeast Brazil, caused by the illegal pet trade that began decades ago and is still an ongoing problem (Mittermeier 1982; Carvalho 2018). Those traded marmosets are often released in rural areas or escape from their owners and colonize rainforest fragments. In that process, those individuals occasionally make contact with *C. aurita* populations, creating mixed groups (i. e., *Callithrix* spp.) at first and leading to hybridization in mid-term (Carvalho *et al.* 2018). The hybridization has caused genetic erosion in *C. aurita* populations throughout its distributional range, including within conservation units, despite those being created as safe areas for the species (Muskin, 1984; Rylands, Coimbra-Filho and Mittermeier, 1993; Costa *et al.*, 2005; Bechara, 2012; Carvalho *et al.*, 2013; Aximoff *et al.*, 2016). Those factors, combined with the isolation of *C. aurita* populations due habitat loss, has led the species to be included in the top 25 most threatened primate species list (Carvalho *et al.*, 2019), emphasizing the importance of surveying *C. aurita*, to evaluate the situation throughout its distribution and to identify whether there are still pure populations of native species (Norris *et al.*, 2011; Melo *et al.*, 2020; Carvalho *et al.*, 2018).

We surveyed *Callithrix aurita* populations, in addition to congeneric alien species and their hybrid forms, in forest fragments of Viçosa and nearby municipalities, in Minas Gerais state, Brazil, using pelage patterns to identify native, invasive and hybrid species in the field. *Callithrix aurita* has not been observed in the Viçosa region since 1995 (Pereira *et al.*, 1995), although this is part of its historic distributional range. This survey was proposed as a priority under the National Action Plan for the Conservation of Mammals of the Central Atlantic Forest (Plano de Ação Nacional para a Conservação dos Mamíferos da Mata Atlântica Central - PAN MAMAC) (Escarlate-Tavares *et al.*, 2016), and again in the National Action Plan for the Conservation of the Primates of the Atlantic Forest and the Maned Sloth (Plano de Ação Nacional para a Conservação dos Primatas da Mata Atlântica e da Preguiça-de-Coleira - PAN PPMA) (ICMBio, 2019).

Methods

Study area

The study was carried out in 43 fragments in nine municipalities in the region of Viçosa, in the southeast of Minas Gerais state, Brazil (Fig. 2). The region was once covered in Atlantic Forest, but severe anthropic disturbance in the last two centuries has reduced the area of native vegetation to numerous fragments of variable sizes (Valverde, 1958; Dean, 1996). The region is a complex matrix of small and medium-sized cities, pastures, silviculture (eucalyptus), and crops such as coffee and corn (Melo, 2006). The altitude in this region varies from 565 to 1,075 m (Alvares *et al.*, 2013) and, following the Köppen classification, the climate is Subtropical Humid (Cwa), with hot summers and dry winters (Alvares *et al.*, 2013).

Data Collection

We used an adapted method of linear transects combined with playback (Gestich *et al.*, 2016). An JWL® WMA - 7110 Amplifier was used to reproduce long-calls of *Callithrix aurita*, useful for attracting its congeners and hybrids as well (FRM, *pers. comm.*). Callitrichids use long-calls to attract mates, for group location, and in territorial defense (Snowdon, 1993).

The data were collected in two main campaigns: June-October 2017; and August-December 2018. Additionally, a fragment was sampled in May 2019. All fragments were sampled only once, with the exception of the fragment where the pure *Callithrix aurita* group was found. Since that group was found at the beginning of the first campaign, it was monitored through the following years, and more groups were found there, as described in the results.

Surveys were conducted in the morning (07:00 - 12:00) and afternoon (14:00 - 18:00), always by at least two researchers. Transect walks began at the fragment edge, surveying pre-existing trails to the interior of the patch, or along its edges when trails were not available. We played vocalization recordings for two minutes, then waited for the group's response for two minutes in silence; this process was repeated three times for each location point. The points were separated by 100 m minimum distance along the transect. The number of transect points varied with fragment size and especially by degree of difficulty to access the entire fragment area. When animals responded to the recordings, we continued playback until the group approached, allowing us to directly observe and photograph the group members for further identification by pelage patterns.

Results

We recorded *Callithrix* in 25 out of the 43 fragments (Appendix 1). A total of 28 different groups were observed, including: one group of *C. aurita*; two mixed groups, composed of *C. aurita* and hybrids; three solitary (i.e., the rest of a possible group were not identified) hybrid individuals; and 22 groups composed only of hybrids (Fig. 2). We confirmed the presence of *C. aurita* in only two fragments.

In a fragment next to Viçosa´s rural area, we found a group of 6-8 *Callithrix aurita* individuals in July 2017. Due to the rarity of the species in the region, we made subsequent campaigns to that fragment to monitor the *C. aurita* group, and it was last seen in March 2018. In the next year we found a mixed group composed of two *C. aurita* individuals and two hybrid forms, and later a

group formed by only hybrids in the same fragment; the group composed of only *C. aurita* was never seen again in that area. In the area around Teixeiras we found a single *C. aurita* individual living in a group composed of three hybrid individuals.

Hybrid forms were found in 25 fragments comprising the great majority of occurrences, with pelage traits indicating ancestry from up to three invasive species, *Callithrix geoffroyi, C. penicillata* and *C. jacchus*. Out of the 22 hybrid groups found, eight groups included individuals with *C. aurita* pelage characteristics.

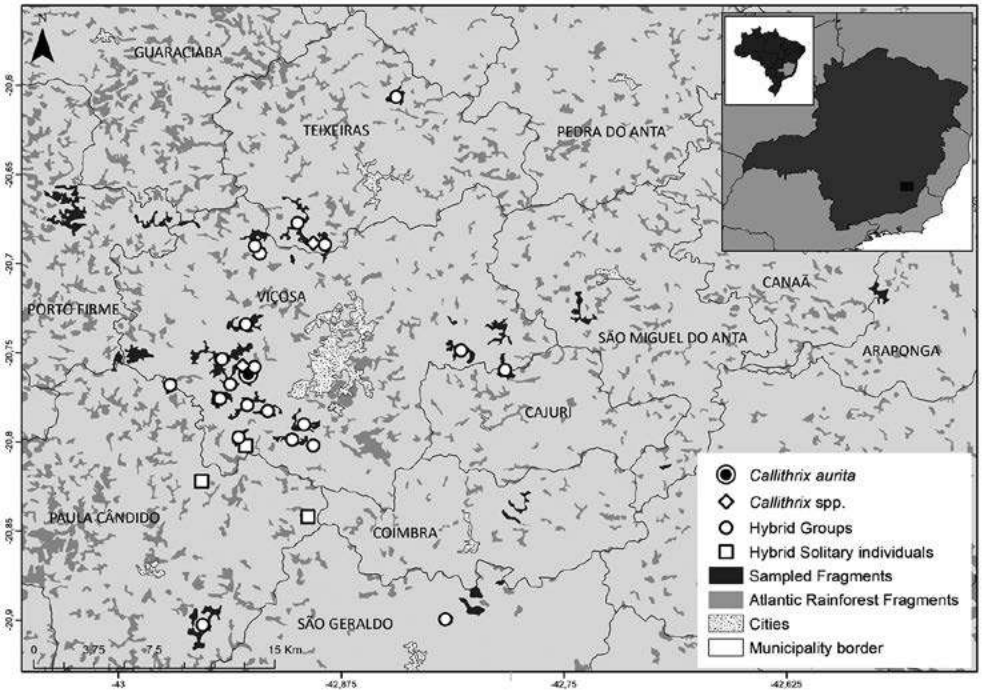

Figure 2. Sampled fragments in the municipality of Viçosa - MG and cities nearby, displaying the location of *Callithrix* groups found. *Callithrix* spp. involves mixed groups formed by individuals of *C. aurita* and hybrids; hybrid groups are formed only by hybrid individuals. Solitary individuals denote marmosets found alone, with the rest of the group not identified in the survey.

Discussion

The rediscovery of *Callithrix aurita* in Viçosa was an unexpected but positive result given the history of marmoset invasion and hybridization, and that the species had not been recorded here since the 1995 (Pereira, 1995; Fuzessy *et al.*, 2014; Pereira, 2012). In addition, we noted a pelage pattern in some individuals indicating hybridization (i. e., darkish pelage, but striated dorsum) with *C. aurita* in eight groups sampled, as well as in the described mixed group, suggesting an ancestry or at least an interaction with the native species a few generations ago. Thus, it seems likely that the former population of *C. aurita* in the Viçosa region was larger than we had expected.

The pure group of *Callithrix aurita* was found in 2017 and continued to be monitored by our research team but it was last seen in March 2018. Also in 2018 we located a mixed group and later a hybrid group living in the same fragment where the *C. aurita* group had been observed. We still do not know what happened with the pure group but it is clearly an example of how fast a fragment can be colonized by hybrid forms, outcompeting and/or mixing with native groups. Hybridization in callitrichids has been observed experimentally in captivity, and in areas of natural and anthropogenic hybridization (Malukiewicz, 2019). This process, with the exception of what occurs naturally in the edges of its parapatric distributional ranges, constitutes a threat to *C. aurita* genetic integrity. In addition, invasive species and hybrids are outcompeting and replacing native populations throughout the distributional range of *C. aurita* (Carvalho *et al.*, 2019).

The large number of hybrid marmosets found in the Viçosa region is worrying but not unexpected, as there have been reports of non-native marmoset introductions since the 1970s (Fuzessy *et al.*, 2014). The high reproductive rates of *Callithrix* have allowed the hybrid populations to rapidly increase, colonizing rural and urban fragments in the decades since the first introductions. The individuals we observed had morphological characteristics from four *Callithrix* species: *C. geoffroyi*, *C. penicillata*, *C. jacchus* and *C. aurita*. These results corroborate what has been found in other *Callithrix* surveys in southeast Brazil, hybridization that mainly involves invasive *C. penicillata* and *C. jacchus* (Aximoff *et al.*, 2016; Detogne *et al.*, 2017; Norris *et al.*, 2011; Pereira *et al.*, 2008; Silva *et al.*, 2018).

Callithrix penicillata and *C. jacchus* are more adapted for gummivory than *C. aurita*, which may mean they are better able to adapt to fragmented habitats where other resources are scarce, making them potential competitors for *C. aurita* (Mendes, Brandão and Igayara, 2016). *C. geoffroyi*, also observed in the study, is native to the Atlantic Rainforest, but not historically sympatric with *C. aurita* (Rylands, Coimbra-Filho and Mittermeier, 2009). The similarity of the niches used by both species leads to competition by *C. aurita* and invasive *C. geoffroyi* groups (Pereira *et al.*, 2008).

The development of effective methods to control the invasive and hybrid forms and implement recovery strategies for native populations are needed. A Captive Management Program has been set up by the National Center for Research and Conservation of Brazilian Primates of the Chico Mendes Institute for Biodiversity Conservation (ICMBio/CPB) and the Brazilian Association of Zoos and Aquaria (AZAB). This program is developing a large captive colony of pure *Callithrix aurita* for future reintroductions in areas where the species has been extirpated. The recently opened Mountain Marmosets Conservation Center of the Universidade Federal de Viçosa will breed and maintain captive groups of *C. aurita* and *C. flaviceps* – another Threatened *Callithrix* species. These projects are part of the Mountain Marmosets Conservation Program, as part of the implementation of the NAP PPMA (ICMBio, 2019).

Even though our results confirmed that isolation and hybridization are widespread in the study region, we also found that there was still a pure group of *C. aurita*. This, together with ongoing research and management actions, provides some hope for the species' future.

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