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Short title

Titi monkey decline after YF outbreak

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Abstract

Platyrrhini are highly vulnerable to the yellow fever (YF) virus. From 2016 to 2018, the Atlantic Forest of southeast Brazil faced its worst sylvatic YF outbreak in about a century, thought to have killed thousands of primates. It is essential to assess the impact of this epidemic on threatened primate assemblages to design effective conservation strategies. In this study, we assessed the impact of the 2016-2018 YF outbreak on a geographically isolated population of Near Threatened black-fronted titi monkeys (*Callicebus nigrifrons*) in two Atlantic Forest patches of the Santuário do Caraça, MG, Brazil. Extensive pre-outbreak monitoring, conducted between 2008 and 2016, revealed that the home range and group sizes of the population remained stable. In 2016, the population size was estimated at 53-57 individuals in 11-12 groups. We conducted monitoring and playback surveys in 2019 and found that the population had decreased by 68% in one forest patch and completely vanished in the other, resulting in a combined decline of 80%. We discuss this severe loss of a previously stable population and conclude that it was highly likely caused by the YF outbreak. The remaining

population is at risk of disappearing completely because of its small size and geographic isolation. A systematic population surveys of *C. nigrifrons*, along other sensible Platyrrhini species, is needed to re-evaluate their current conservation status.

Keywords

Callicebus nigrifrons, Atlantic Forest, demographic changes, playback survey, monitoring, epizootic

Research highlights

- Brazil faced one of its worst yellow fever outbreaks from 2016 to 2018, but systematic data on the impact on local primate populations are lacking
- We show that a geographically isolated and partially habituated population of black-fronted titi monkeys *Callicebus nigrifrons* have declined by about 80% after the outbreak
- Natural demographic fluctuations or ecological changes do not appear to account for the decline, making the YF outbreak the most likely cause
- A systematic Platyrrhini survey is needed to re-evaluate the species' current conservation statuses

Graphical abstract

A juvenile black-fronted titi monkey photographed shortly after the 2016-2018 sylvatic yellow fever outbreak in the Santuário do Caraça, MG, Brazil

Introduction

Yellow fever (hereafter YF) is an infectious disease caused by a *Flavivirus* arbovirus that originated in Africa in the last 1,500 years. The virus was probably introduced from Africa to the Americas during the slave trade period about 300-400 years ago (Bryant, Holmes, & Barrett, 2007). In America, the virus is maintained by a sylvatic cycle between Culicidae hematophagous mosquitoes (*Haemagogus* and *Sabethes*) and nonhuman primates (Possas et al., 2018), mostly restricted to the Amazon, Araguaia, and Orinoco river basins (Bryant et al., 2007).

These areas are characterized by low altitude and high rainfall, air humidity, ambient temperature and nonhuman primate diversity and density, which create optimal breeding conditions for mosquitoes and explain the regular emergence of YF outbreaks (Almeida et al., 2019b; Childs, Nova, Colvin, & Mordecai, 2019; Hamrick et al., 2017).

Nonhuman primates are the main sources of blood for canopy-inhabiting mosquitoes whose activity peaks during the hottest hours of the day, when primates usually rest (Silva et al., 2020). Unlike African primates, which have long been exposed to the virus and are resistant to the disease (Gould, Lamballerie, Zanotto, & Holmes, 2003), Platyrrhini primates experimentally infected by YF-virus showed high, genera-varying susceptibility (Bugher, 1951; Vasconcelos, 2003). Infected Platyrrhini either die rapidly (3-7 days after infection) or develop immunity, suggesting that they can act as virus amplifiers only during short periods (Bicca-Marques & Freitas, 2010; Dietz et al., 2019). When a population is infected, it rapidly declines and the virus disappears from the area (Abreu et al., 2019a; Moreno et al., 2013; Vasconcelos, 2010). This cycle normally resumes when the virus returns to the area, carried by infected vectors or hosts, and finds a renewed, susceptible monkey population. As a result, outbreaks have occurred in the Brazilian endemic areas, particularly in the Amazon, every 7 to 14 years (Câmara, Gomes, Carvalho, & Castello, 2011).

Despite their central role in the sylvatic cycle, Platyrrhini are not responsible for the spread of the virus to non-infected areas in fragmented landscapes, as they usually live in restricted home ranges and rarely travel on the ground between habitat patches (Bicca-Marques & Freitas, 2010; Possas et al., 2018; Souza-Alves et al., 2019b). Wind, on the other hand, can carry infected mosquitoes over long distances, potentially spreading the disease (Almeida et al., 2019b; Paiva et al., 2019). Finally, human factors are also responsible for the expansion of outbreaks. Humans become accidental hosts when bitten by infected mosquitoes (Consoli & Oliveira, 1994), which occurrence increases along with the increasing human activity in forest areas. In this respect, habitat fragmentation increases nonhuman primate density in forest patches and proximity

between human and wildlife, potentially boosting the transmission rates of the virus (Kaul, Evans, Murdock, & Drake, 2018; Possas et al., 2018). The YF vaccine provides life-long immunity (World Health Organization, 2019), but it only prevents the dissemination of the virus if the population coverage is above 80%, which is rarely the case in Latin America (Shearer et al., 2017). Moreover, most infected humans are asymptomatic or develop mild symptoms (Vasconcelos, 2003). In sum, the spread of the virus is favored by high human population densities, low vaccination coverage and movement of infected people (Childs et al., 2019; Possas et al., 2018).

The highly populated regions of southern and southeastern Brazil remained YF-free for decades until near the end of the 20th century, which have led to a vaccine coverage of less than 80% (Shearer et al., 2017). Between 1998 and 2009, YF outbreaks in these regions caused the death of hundreds of humans and thousands of nonhuman primates, especially howler monkeys (Almeida et al., 2012; Bicca-Marques et al., 2017; Freitas & Bicca-Marques, 2011; Holzmann et al., 2010; Moreno et al., 2013; Romano et al., 2014; Souza et al., 2019; Vasconcelos, Rosa, Monteiro, & Cruz, 2001).

From 2016 to 2018, Brazil has faced one of its worst YF outbreaks in nearly 80 years, with 2,153 confirmed human cases including 744 deaths (2016-2017: 777 confirmed cases and 261 deaths; 2017-2018: 1,376 confirmed cases and 483 deaths; Ministério da Saúde 2017, 2018). The outbreak continued in winter 2018-2019 in a moderate form (75 human cases, 17 deaths; World Health Organization, 2019) and, at the time of this writing (Feb 2021), it is emerging in the state of Rio Grande do Sul (G1 RS, 2021). The 2016-2018 YF outbreak extended over 2,000 km and comprised multiple parallel sylvatic cycles (Moreira-Soto et al., 2018) with *Haemagogus janthinomys* and *H. leucocelaenus* as main vectors (Abreu et al., 2019b).

Atlantic Forest primates were extensively infected during the 2016-2018 outbreak according to governmental authorities. A total of 2,276 epizootics involving mostly *Callithrix*, *Alouatta*, *Sapajus* and *Callicebus* were reported (2016-2017: N = 1,412 cases; 2017-2018: 864 cases; Ministério da Saúde, 2017, 2018). Real rates of epizootics were likely much higher, as only 5% of dead monkeys are estimated to be found and registered (Duchiade, 2018). Systematic analysis of the carcasses showed that *Alouatta* and *Callicebus* are highly sensitive to the YF virus (Sacchetto et al., 2020).

In the State of Minas Gerais (MG), 80-90% of the Vulnerable *Alouatta guariba clamitans*, 10% of the Critically Endangered *Brachyteles hypoxanthus*, 90% of the Critically Endangered *Callithrix flaviceps* and 40-50% of the Near Threatened *Sapajus nigritus* populations of the Reserva Particular do Patrimônio Natural (RPPN) Feliciano Miguel Abdala (also known as "Caratinga") vanished during the 2016-2017 outbreak, as well as 26% of the *B. hypoxanthus* population of the RPPN Mata do Sossego (Lopes, 2017; Possamai, Mendes, & Strier, 2019; Strier et al., 2019). In the neighboring State of Espírito Santo (ES), the disease caused a population decline of 82% for *A. guariba clamitans*, 49% for *C. flaviceps* and the Least Concern *Callithrix geoffroyi*, 25% for the Vulnerable *Callicebus personatus*, 23% for *S. nigritus* and 10-26% for *B. hypoxanthus* (Gontijo, 2019; Strier et al., 2019). Finally, 30% of the Endangered *Leontopithecus rosalia* population from the São João river basin (State of Rio de Janeiro [RJ]) disappeared after the outbreak (Dietz et al., 2019). In all these reports, the evidence for virus-caused decline is indirect, as population reductions coincided with the presence of the virus in the regions (Dietz et al., 2019; Lopes, 2017; Strier et al., 2019). Population declines at such rates pose a serious threat to species survival with considerable implications for conservation.

The purpose of the study is to assess the state of a geographically isolated and partially habituated population of black-fronted titi monkeys (*Callicebus nigrifrons*) in two small Atlantic Forest patches in Brazil before and after the 2016-2018 YF outbreak, and to evaluate the potential impact of the outbreak on population demography. The study population lives in the RPPN Santuário do Caraça, a reserve located in the upper Rio Doce basin, a highly fragmented Atlantic Forest region in MG (Machado & Fonseca, 2000). MG was the epicenter of the 2016-2018 YF outbreak, accounting for 46% of the confirmed human cases and 23% of the confirmed nonhuman primate epizootics (Figueiredo et al., 2018; Ministério da Saúde, 2017, 2018). Three of the closest municipalities to the reserve (namely, Santa Bárbara, Catas Altas and Barão de Cocais) recorded at least 21 confirmed human cases and several suspected cases (Secretaria de Estado de Saúde de Minas Gerais, 2018). These three municipalities also reported confirmed and suspected nonhuman primate cases (Sacchetto et al., 2020; Secretaria de Estado de Saúde de Minas Gerais, 2018). The study population comprised the largest habituated population of *C. nigrifrons* (6 groups and ca. 35 individuals, end of 2016) as well as their neighboring non-habituated groups. Although no primate carcass was recovered, local employees reported fewer cues of black-fronted titi monkeys presence after 2016 (choruses, visual encounters), suggesting that groups were affected by the 2016-2018 YF outbreak (Duchiade, 2018).

To quantify the potential impact of the YF outbreak on the study population, we assessed the stability of the population from 2008 to 2016 and estimated the demographic changes between 2016 and 2019. Given the lack of extreme climatic events and any noticeable change in habitat quality since 2016, we assumed that if the population was stable from 2008 to 2016, any population reduction after 2016 could be

reliably assigned to the YF outbreak. However, if the population had been unstable between 2008 and 2016, it would not be possible to infer the YF as the cause of recent population changes.

Methods

Study site

We conducted the study at the RPPN Santuário do Caraça ("Santuário do Caraça"), a private natural heritage reserve of 11,000 ha in the Serra do Espinhaço, MG, Brazil (20°05'S, 43°29'W) ranging from 730 to 2,072 m above sea level. The reserve is composed of transition zones between the Atlantic Forest and the Cerrado (Brazilian savanna) biomes (Brandt & Motta, 2002; Paz, 1998; Talamoni, Amaro, Cordeiro-Júnior, & Maciel, 2014). Specifically, three main floristic formations structure the reserve's landscape: the grasslands (*campo limpo*), outcrop fields (*campo rupestre*) and the forests (riverine forest, riparian forest, cloud forest and hillside forest) (Província Brasileira da Congregação da Missão, 2013). The climate is tropical, characterized by a rainy and hot season (October to March, mean monthly rainfall ± s.d. = 224.6 ± 76.2 mm, mean temperature ± s.d. = 18.2 ± 0.7°C) and a dry and colder season (April to September, mean monthly rainfall ± s.d. = 43.5 ± 27.3 mm, mean temperature ± s.d. = 15.0 ± 1.4°C) under the strong influence of the altitudinal gradient (Fick & Hijmans, 2017; Moreira & Pereira, 2004).

The central part of the reserve (mean elevation: 1,300 m) includes two forest patches of interest for this study, the Tanque Grande forest patch and the Cascatinha forest patch, located 1 km apart from each other (Jarvis, Reuter, Nelson, & Guevara, 2008). Cascatinha is a hillside forest patch of about 32 ha bounded by a river on its southern

205 part and surrounded by grasslands and rocky outcrops on its other parts, preventing any
206 connection to other forested areas. Tanque Grande is a hillside forest patch of about 60
207 ha bounded by human settlements (road, hotel complex) on its northern part, grasslands
208 and a lake on its western part, and surrounded by grasslands and rocky outcrops on its
209 other parts. It can be connected to the core forest of the reserve via a corridor in the
210 south, which can potentially be crossed by titi monkeys, but which does not constitute a
211 suitable habitat for the species because it is a transition zone between grasslands and
212 forests.
213 The Santuário do Caraça is a tourist attraction that receives 60,000-70,000 visitors each
214 year. Human settlements are restricted to an asphalt road, a farm/hotel complex at the
215 entrance of the reserve and a monastery/hotel complex in the core of the reserve, which
216 allow visitors to spend several days on site. Visitors are required to stay on pre-
217 established trails when walking through the natural areas, and to not interact (e.g., feed,
218 touch) with the wildlife, including primates. Hunting is forbidden and there is no recent
219 record of poaching (Província Brasileira da Congregação da Missão, 2013; pers. obs.).
220 From the 28th of November 2018 to the 8th of March 2019, visitors were required to
221 present a valid certificate of YF vaccination to access the reserve.
222 The sanctuary is a conservation hotspot for the local fauna (Talamoni et al., 2014). Five
223 primate species inhabit the reserve: *C. nigrifrons, S. nigritus, A. guariba clamitans,*
224 *Callithrix penicillata and C. geoffroyi* (Berthet, 2018). Gene flow with populations
225 outside of the reserve is restricted because the area is mostly surrounded by high
226 mountains (1,200-2,700 m) with few trees. Fragmentation has recently been aggravated
227 by the intensification of mining activities, land artificialization and intensive forestry on
228 the reserve's border (Província Brasileira da Congregação da Missão, 2013).

Study species

Black-fronted titi monkeys are small (1.0-1.5 kg) diurnal primates (Bicca-Marques & Heymann, 2013) endemic to the Atlantic Forest (states of MG, ES, RJ and São Paulo [SP]). They are classified as IUCN Near Threatened because their populations have declined by more than 20% due to habitat loss and forest fragmentation over the past 24 years (Jerusalinksy, Melo, Mittermeier, Quadros, & Rylands, 2020). They live in groups of two to six individuals, composed of a life-long monogamous adult pair and their offspring, on a territory of about 20 ha (21-48 ha, Bicca-Marques & Heymann, 2013; 8-28 ha, Caselli, Mennill, Bicca-Marques, & Setz, 2014). The group jointly defends the territorial resources with loud call displays (solos, duets and choruses, Caselli et al., 2014).

Black-fronted titi monkeys are mainly frugivorous but also consume insects, seeds and leaves (Bicca-Marques & Heymann, 2013; Caselli & Setz, 2011; Santos, Galvão, & Young, 2012). They are arboreal and spend most of their time in the lower and intermediate canopy of small fruit trees (10-30 m high) to feed or rest during hot hours (Bicca-Marques & Heymann, 2013; Gestich, Caselli, & Setz, 2014; Trevelin, Port-Carvalho, Silveira, & Morell, 2007). They occasionally descend to the forest floor to forage, travel and play (Souza-Alves et al., 2019b).

Mated females give birth to one young per year between July and January (Bicca-Marques & Heymann, 2013; Di Bitetti & Janson, 2000; Souza-Alves, Caselli, Gestich, & Nagy-Reis, 2019a; Valeggia, Mendoza, Fernandez-Duque, Mason, & Lasley, 1999). Young adults of both sexes disperse when they reach 3 years of age (Bicca-Marques & Heymann, 2013). The mechanisms involved in the establishment of new territories

253 remain unknown. A pair of collared titi monkeys *Cheracebus torquatus* has been
254 observed shifting their home range to open space for their offspring (Easley & Kinzey,
255 1986) and a mated back-fronted titi monkey adult was reported being evicted from its
256 group by a new individual (Cäsar, 2011). Individuals live up to 12 years in captivity
257 (Rowe, 1996).
258 The study population is composed of four habituated groups of black-fronted titi
259 monkeys living in the Tanque Grande forest patch, two habituated groups living in the
260 Cascatinha forest patch, and their neighbors (i.e., the non-habituated groups whose
261 home ranges overlap with those of the habituated groups). We began the habituation
262 process in 2004 (Berthet, 2018; Cäsar, 2011) and monitored the habituated groups
263 extensively between 2008 and 2010 and between 2014 and 2016.
264 There were other non-habituated black-fronted titi monkey groups in the reserve, but we
265 focused on the habituated ones and their neighbors, for which we have reliable long-
266 term data.
267
268 **Demography, density and spatial distribution**
269 *Pre-outbreak monitoring (2008-2010 and 2014-2016)*
270 We monitored five groups (A, D, M, P and R groups) for 1,295 h over 15 months
271 between 2008 and 2010 (July-December 2008, May-October 2009, May-July 2010). We
272 habituated the remaining (S) group in 2014 and monitored all six groups for 1,714 h
273 over 17 months between 2014 and 2016 (October-December 2014, April-June 2015,
274 October 2015-August 2016).
275 We located the groups at dawn (around 06:00 am) by acoustic cues, and monitored them
276 continuously until i) we lost them, ii) they settled in a sleeping tree, iii) we completed a

behavioral experiment, or iv) after 6 h of monitoring (see Berthet, 2018; Cäsar, 2011 for more details). We georeferenced the position of the estimated center of the group every 5 min (2008-2010) or every 10 min (2014-2016) using a handheld Garmin GPSMAP 60CSx GPS. We opportunistically recorded encounters with neighboring non-habituated groups.

We also opportunistically recorded births, deaths and long-lasting disappearances. We considered that disappearances of unmated individuals older than 30 months were most likely due to dispersion, while disappearances of mated adults were most likely due to death (Bicca-Marques & Heymann, 2013; Bossuyt, 2002; Cäsar, 2011; Dolotovskaya, Roos, & Heymann, 2020). Disappearances of individuals younger than 30 months were also most likely due to death, as they are too young to disperse or to survive solitarily (Cäsar, 2011).

We monitored each habituated group during at least two days per month between 2008 and 2010 and during at least four days per month between 2014 and 2016. Individuals were reliably identified and recognized using a combination of physical cues, such as body size, tail features, color variations and stains, scars, and facial features (Fig. S1).

Post-outbreak survey (2019)

We recorded data during four consecutive weeks between August and September 2019. We conducted an intensive monitoring session (about 90 h) throughout the study using a procedure similar to that described earlier. Whenever possible, we identified individuals from the 2016 habituated population using the aforementioned physical features. We also recorded the reactions to the presence of human observers (from less tolerant to most tolerant: flight, display, avoidance, curiosity, ignore), as a cue of the

habituation stage of the individuals (Williamson & Feistner, 2011). Finally, we georeferenced the position of the estimated center of the group every 10 min using a handheld Garmin GPSMAP 60CSx GPS.

Given that we did not monitor the groups between 2016 and 2019 and that some individuals were not habituated, contact time did not exceed 4 h per day for two main reasons. First, most non-habituated individuals regularly fled, and it was not always possible to find them back. Second, the goal of our study was to survey the remaining groups instead of to (re-)habituate them: we avoided to follow groups containing non-habituated individuals (i.e., individuals displaying avoidance, flight behaviors; Williamson & Feistner, 2011) for long periods of time to minimize unnecessary harmful levels of stress (Fedigan, 2010).

Post-outbreak playback experiments (2019)

We applied a playback method (Gestich, Caselli, Nagy-Reis, Setz, & Cunha, 2016) to locate black-fronted titi monkey groups. This method relies on the territorial behavior of titi monkeys: broadcasted duets of unknown individuals simulate the presence of potential competitors in or close to the territory. Resident groups respond to the playbacks with duets (usually with the participation of the mated pair) or choruses (the adult pair and/or older offspring) to defend their territory's resources against potential intruders (Caselli, Mennill, Gestich, Setz, & Bicca-Marques, 2015). Estimating population densities using playback methods has been shown to be extremely reliable for back-fronted titi monkeys, with a rate of group detection close to 100% (Gestich et al., 2016).

We broadcasted duets from one resident group of each forest patch into the other forest patch to stimulate an intrusion by an unknown couple and trigger vocal responses by residents (Caselli et al., 2015). We used 1-min-long samples extracted from four duets from P and S groups recorded in 2016 in which both mates were calling. We normalized sequences at -1 dB and broadcasted them using an Anchor An-30 (Anchor, Carlsbad, CA) loudspeaker (frequency response range: 100–15,000 Hz, output power: 30 W, Maximum SPL at 1 m: 100 dB), which covers the frequency spectrum of black-fronted titi monkeys' vocalizations and reaches the same levels of the natural emissions of duets. We held the speaker at a height of 2 m and directed it to four directions separated by an angle of 90° for 15 s each to cover a circular area in 1 min.

We determined a 200-m playback circumference (i.e., the distance at which the broadcasted duets could be heard) during pilot trials in a forest patch that was not occupied by titi monkeys. We conducted the playback trials in the maximum area occupied by the habituated groups, i.e., the sum of the area occupied by each group from 2008 to 2010 and from 2014 to 2016. We conducted 14 playback trials at 180-m intervals to fully cover the area of interest (Fig. 1).

We played two sequences at 5-min intervals per trial in the morning (Gestich et al., 2016) and alternated recordings to avoid habituation to the stimuli. We registered the responses of neighboring groups during the first 5 min after each playback sequence. A trial lasted 12 min (1 min stimulus followed by 5-min waiting period, followed by 1 min stimulus then 5-min waiting period). We estimated the distance of all vocal responses to the playback stimuli and registered their direction in relation to the location of the playback with a compass. When a responding group approached the speaker and was in sight, we did not play the second stimulus to avoid a reduction in responsiveness in

348 future trials. We conducted playback experiments for four days (two consecutive days
349 in the Cascatinha forest patch and two consecutive days in the Tanque Grande forest
350 patch) for at most 4 h per day (Gestich et al., 2016) (See Supplementary Material).
351 We later plotted all location records on the home range map and clustered vocal
352 responses according to the spatial and temporal distance between the responses. We
353 registered clusters as belonging to the same group unless we had evidence that they
354 were distinct groups (Gestich et al., 2016).
355
356 **Estimation of population changes**
357 *Demography*
358 To estimate the population stability between 2008 and 2016, the size of each habituated
359 group in summer (between July and October, depending on data availability) was
360 extracted for the two pre-outbreak monitoring periods. We calculated each group's
361 2008-2010 and 2014-2016 mean size and we used a two-tailed Wilcoxon paired signed-
362 rank test to test whether the mean group sizes varied between the two monitoring
363 periods.
364 To estimate the population changes between 2016 and 2019, we assessed the size of the
365 habituated groups in 2016 and in 2019 based on the monitoring, survey and playback
366 data. We assessed the presence and location of neighboring groups based on anecdotal
367 encounters and playback results. Since we did not know the exact composition of the
368 neighboring groups, we assigned them a hypothetical size of four individuals because
369 black-fronted titi monkey groups are usually composed of one mated pair and one to
370 three offspring (Bicca-Marques & Heymann, 2013).
371

Home ranges

We used the estimated home ranges as another proxy of the changes in the black-fronted titi monkey population. Given the stability and high territorialism of titi monkey groups (Bicca-Marques & Heymann, 2013; Caselli et al., 2014), home ranges usually remain constant over the years. To estimate the stability of the groups before 2016, we compared the home range size and location of each habituated group between 2008-2010 and 2014-2016: if the population was stable (i.e., well established home ranges, no disappearance of a group or establishment of a new one) then home ranges of the habituated groups should remain constant between the two monitoring periods prior to 2016.

To this end, we georeferenced the home ranges of the habituated groups using GPS data collected in 2008-2010 and 2014-2016. Due to logistic issues, some of the 2008-2010 GPS data were lost, so associated home ranges were drawn using the remaining GPS data, which probably underestimated their real size (see Table 1). We mapped the borders using characteristic hull polygons (Downs & Horner, 2009). While the home range is usually measured as the smallest area in which animals spend 95% of their time, we decided to use 100% of the collected GPS points to remain conservative. We estimated the size of each home range in 2008-2010 and in 2014-2016, and compared them using a two-tailed Wilcoxon paired signed-rank test. We also calculated the proportion of overlap between the two periods (i.e., the proportion of the 2008-2010 home range that was still used by the same group in 2014-2016). Finally, we used opportunistic encounters with neighbors to identify the home range borders shared with non-habituated groups in 2008-2010 and 2014-2016.

We also used the home range data to estimate changes in the population between 2016 and 2019. We hypothesized that if, in 2019, a black-fronted titi monkey group occupied an area located in the 2016 home range of another group, then the latter had probably disappeared from the area between 2016 and 2019.

Mapping and calculations were conducted in QGIS 3.8.2 (QGIS Development Team, 2009) with the concave hull add-on (Detlev, 2019) and statistical analyses were conducted in R 4.0.0 (R Core Team, 2020).

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Ethics statement

The research reported in this article was conducted in compliance with all relevant local and international laws. The 2008-2010 data collection was approved by the University of St Andrews Psychology Ethics Board, the 2014-2016 data collection was approved by the ethical committee CEUA/UNIFAL (number 665/2015) and the 2019 data collection was approved by the CEUA/PUCRS (number 9438).

Results

Pre-outbreak monitoring (2008-2010 and 2014-2016)

Demography

The size of habituated groups was stable prior to the outbreak (Fig. S2): mean group size did not significantly vary between 2008-2010 and 2014-2016 (W = 1, p-value =

0.125) (Table 1). In August 2016, the habituated population comprised six groups (33 individuals): four groups in Tanque Grande (21 individuals) and two groups in Cascatinha (12 individuals). They were neighbors of five or six non-habituated groups: four groups in Tanque Grande and one or two groups in Cascatinha (Fig. 2). Overall, we found that 11 to 12 groups inhabited the two studied forest patches by the end of 2016.

The characteristics of the demographic events confirm that the dispersal of young adults and the death of young individuals were the main causes of disappearance, while the disappearance of mated adults was rare (one observation, i.e., 7% of the total disappearances, see Table 2).

Home ranges

The size of the habituated groups' estimated home ranges tended to increase between the two pre-outbreak monitoring periods, although the difference was not significant: home ranges varied from 4.5-6.2 ha in 2008-2010 (mean ± s.d. = 5.1 ± 0.8 ha) to 6.3-7.9 ha in 2014-2016 (mean ± s.d. = 7.1 ± 0.7 ha; V = 0, p-value = 0.058) (Table 1). Each group's home range in 2008-2010 was still mostly occupied by the same group in 2014-2016 (overlap = 82-97 %, mean ± s.d. = 89 ± 6 %, Table 1, Fig. 2).

Post-outbreak period: 2019

Survey

During the 90-h survey, we did not find any sign of the presence of titi monkey groups in the Cascatinha forest patch (no encounter, no duet). In the Tanque Grande forest, we found evidence of the presence of at least three groups. We encountered one of these groups on several occasions, and we heard several duets emitted by this group and at

443 least two other groups, both located outside of the home ranges of the 2016 habituated
444 groups (i.e., two non-habituated groups).
445 The group that we encountered (later referred to as the 'partially habituated group') was
446 composed of three individuals. The mated male was the resident adult male of the R
447 group from 2008 to 2016, easily recognizable by its specific physical features.
448 Moreover, this individual ignored our presence in 2019, which is congruent with the
449 fact that the adult male of the R group was one of the most habituated individuals of the
450 2016 titi population. The mated female was born in the A group in 2014, also easily
451 recognizable by her physical traits. This individual exhibited intermediate-tolerance
452 behaviors (avoidance, curiosity) in our presence, suggesting that she was still in the
453 habituation process, a conclusion congruent with the fact that she was only monitored
454 for two years before 2016. The last individual was a juvenile estimated to have been
455 born by the end of 2017 based on observations of its size and behavior (e.g., play,
456 exploration, no participation in territorial defense). The juvenile was not habituated to
457 human presence (flight, avoidance, curiosity). The group ranged in an area previously
458 occupied by the A, D, R and S groups (Fig. 3).
459
460 *Playback experiments*
461 We recorded no response to the five playback trials conducted in the Cascatinha forest
462 patch, but recorded 20 vocal responses to the nine playback trials conducted in the
463 Tanque Grande forest patch (Table S1). The responding individuals were the partially
464 habituated group monitored during the survey, two non-habituated groups whose duets
465 were heard during the monitoring, and a supposedly solitary individual who emitted
466 solos (Fig. 4).

One of the non-habituated groups was sighted once and immediately lost, while the other non-habituated group and the solitary individual were never sighted. We did not find any evidence of the presence of other habituated groups during the 4-week survey (no duets nor direct observations).

Impact of the YF outbreak on the titi monkey population

In 2016, we estimated the size of the Cascatinha population at 16-20 individuals (12 habituated and 4-8 non-habituated individuals) and the 2016 Tanque Grande population at 37 individuals (21 habituated and 16 non-habituated individuals), i.e., a population of 53-57 individuals in the two forest patches. In 2019, we estimated the Cascatinha population at zero, and the Tanque population at 12 individuals (three individuals in the partially habituated groups, one solitary individual and two unknown groups) (Fig. S2). Therefore, we estimate the Cascatinha population to have declined by 100% and the Tanque Grande population to have declined by 68% between 2016 and 2019. Overall, we estimate the black-fronted titi population to have declined by about 80% between 2016 and 2019 in the two forest patches (from a total of 53-57 individuals in 2016 to 12 individuals in 2019).

Discussion

We found that the home ranges and the size of the habituated black-fronted titi monkey groups of the Santuário do Caraça did not markedly vary from 2008 to 2016. The size of the estimated home range tended to increase between 2008-2010 and 2014-2016, and the 2008-2010 home ranges were almost entirely occupied in 2014-2016 by the same groups (mean overlap of around 90%). We believe that the variation trend of the home

ranges' size is better explained by differences in sampling effort (given the loss of some 2008-2010 GPS data, Table 1) rather than true home ranges variations. The stability of the two forest patches' groups size and home ranges is compatible with the conclusion that populations were stable in both forest patches in 2008-2016.

After the 2016-2018 sylvatic YF outbreak, we did not find any black-fronted titi monkey in the Cascatinha forest patch, and we found only three groups and one likely solitary individual in the Tanque Grande forest patch (ca. 12 individuals). One of the remaining groups is composed of the former resident male of the R group, which now occupies an area greatly overlapping the 2008-2016 home range of at least two other groups (D and S group). Given titi monkeys' strong site fidelity and high territoriality (Bicca-Marques & Heymann, 2013; Caselli et al., 2015), the death of D and S group members is the most likely explanation for the changes in home ranges' occupation.

We are confident that our combination of a 90-h survey and playback experiments provided reliable data on the occurrence of these shy, but highly vocal platyrrhines (Bicca-Marques & Heymann, 2013). First, playback surveys have an accuracy close to 100% to estimate the presence of black-fronted titi monkey groups (Gestich et al., 2016). Second, the two researchers conducting the survey had an excellent knowledge of the habituated black-fronted titi monkey groups from 2016 and their behavior and ecology (home range, regular paths, feeding and sleeping sites, activity budget). Moreover, in 2016, all habituated groups were duetting/chorusing almost every day, and up to nine times per day (unpublished data). It is therefore very unlikely that our 90-h effort over a four-week survey (combined with our presence in the forest patches during/around the playback experiments) was insufficient to detect the titi monkeys. Third, the survey results corroborate those from the playback experiments. In

conclusion, it is unlikely that the combination of the two methods failed to detect other titi monkey groups in the Tanque Grande and Cascatinha forest patches. Although we may have missed solitary individuals, which can be argued to be less responsive to intruders' duets than resident groups, the three responses of one solitary to our playback trials (Table S1) does not support this hypothesis. Irrespective of the presence of some undetected solitary individuals, we are certain that most groups disappeared from the forest patches between 2016 and 2019.

It is unlikely that natural demographic oscillations could account for the observed overall decline, given the aforementioned long-term population and home range stability of titi monkey species (Bicca-Marques & Heymann, 2013; Easley & Kinzey, 1986; Gestich et al., 2016; Müller, 1995), which were confirmed by the pre-outbreak monitoring. Although young adults of both sexes disperse when they reach adulthood (Bossuyt, 2002; Dolotovskaya et al., 2020), resident adults rarely disappear from their home ranges (Bicca-Marques & Heymann, 2013, this study).

The hypothesis of major ecological changes either causing the death of the resident groups or forcing them to leave the area is also not supported by the available evidence. No forest fire occurred in the reserve between 2008 and 2019 (INPE, 2011; pers. obs.) and long-term meteorological data do not reveal unusual climatic events (e.g., drought, extreme flooding or extreme temperature variations) between September 2016 and September 2019 (Fig. S3) that could have led to dramatic food shortage. Additionally, black-fronted titi monkeys are not targeted by the illegal pet trade or hunting (Jerusalinksy et al., 2020), and activities detrimental to local wildlife are forbidden within the RPPN Santuário do Caraça by the Sistema Nacional de Unidades de Conservação (SNUC) law, which is locally enforced by forest guards. No logging,

539 deforestation, or poaching was recorded in the Cascatinha or Tanque Grande forest
540 patches between 2016 and 2019 (Abreu A., pers. com.).
541 Contrary to these unlikely hypotheses, the short-term disappearance of a large part of
542 the black-fronted titi monkey population during a YF outbreak can be explained by the
543 high vulnerability of *Callicebus* species to the virus (Sacchetto et al., 2020).
544 Furthermore, the mean home ranges size of the habituated groups in 2016 was smaller
545 than in other populations (7 ha vs 20 ha, Bicca-Marques & Heymann, 2013; Caselli et
546 al., 2014), suggesting a high titi monkey density that may have also facilitated the
547 spread of the YF virus (Possas et al., 2018). Therefore, although we do not have
548 uncontestable evidence of the role of YF in the documented dramatic population
549 collapse, this is by far the strongest hypothesis.
550 The fact that no primate carcass was reported by the reserve workers and visitors does
551 not provide a strong argument against the YF hypothesis, as the likelihood of finding a
552 dead small animal is low: it is estimated that only 5% of the monkeys (including species
553 much larger than titi monkeys such as howler monkeys) that die of YF in the interior of
554 forests are recorded (Duchiade, 2018). First, a monkey carcass is quickly eaten by the
555 local scavengers, disappearing in <24 h (pers.obs.). Second, only a small proportion of
556 visitors hike in the forests as the majority remains in the farm/hotel/church complexes
557 or walk on trails that do not cross forested areas. Third, visitors crossing forest patches
558 remain on trails that cover only 0.6% of Cascatinha forest and 0.3% of Tanque Grande.
559 Finally, we cannot exclude the possibility that some visitors encountered a carcass but
560 did not report it to local workers and authorities.
561 We focused our study on a small proportion (11-12 groups) of the Santuário do
562 Caraça's black-fronted titi monkey population because we lacked long-term

563 demographic data for other groups. However, we can likely extrapolate our findings to
564 the whole reserve, as there is no reason to believe that the habituated groups and their
565 neighbors would be more sensitive or more exposed to the YF virus than the rest of the
566 population. Additionally, Caraça's employees reported lower rates of titi monkey
567 choruses or sightings in other parts of the reserve. Therefore, we suspect that the YF
568 outbreak not only affected the Cascatinha and Tanque Grande's population, but also
569 impacted other groups of black-fronted titi monkeys at the Santuário do Caraça. Further
570 investigation is needed to estimate the current state of the remaining Caraça population.
571 Despite the legal protection provided by the reserve, the surviving black-fronted titi
572 monkey population may disappear in the medium- to long-term. Even if the remaining
573 adult individuals are resistant to the YF virus and can pass this trait to descendants
574 (Almeida et al., 2019a), the population is small and geographically isolated from other
575 populations due to the reserve's topography, habitat fragmentation and the intensive
576 human activities in the surrounding areas. These conditions increase the population's
577 vulnerability to stochastic events, such as genetic drift and inbreeding, random
578 demographic variations, natural catastrophes, other disease outbreaks and climatic
579 events (Costa, Fernandes, Hilário, Gonçalves, & Souza, 2012).
580 This prospect is worrisome at the species level. Black-fronted titi monkey populations
581 have experienced declines over the last decades (more than 20% in the past 24 years,
582 Jerusalinksy et al., 2020), mainly due to the degradation of the Atlantic Forest (Ribeiro,
583 Metzger, Martensen, Ponzoni, & Hirota, 2009). Titi monkeys can live in primary and
584 secondary forests (Trevelin et al., 2007) with high and closed canopy (Sales, Hayward,
585 & Passamani, 2016), which enables them to occur in small forest patches embedded in

agricultural landscapes (Ribeiro et al., 2009). The inevitable proximity to humans and domestic animals in these landscapes increases the chances of pathogens transmission.

The resurgence of similar deadly outbreaks is a severe threat to the local fauna. Brazil is the world's richest country in primate diversity, but 48% of its primate species have declining populations because of habitat loss and fragmentation, hunting, infectious diseases and climate change (Estrada et al., 2018). This YF outbreak worsened the conservation status of most nonhuman primates of southeastern Brazil. Given the absence of accurate pre-outbreak demographic data for most species, reported figures are likely underestimating the damage. Populations of *A. guariba clamitans*, *B. hypoxantus*, *C. personatus*, *S. nigritus*, *C. flaviceps*, *C. geoffroyi* and *L. rosalia* (Dietz et al., 2019; Gontijo, 2019; Lopes, 2017; Possamai et al., 2019; Strier et al., 2019) in addition to *Callicebus nigrifrons* (this study) have suffered dramatic losses. The risk of the YF virus remaining in the same region for three transmission seasons or longer (Abreu et al., 2019a), re-emerging and causing further population declines is real.

In the light of such a demographic decline in the Santuário do Caraça's population, we highlight the emergency of surveying other, less protected populations of black-fronted titi monkeys, but also other Platyrrhini species, to re-evaluate the conservation status of impacted species and take appropriate measures to protect them. At a broader scale, we call for action, and advise local health and environmental authorities to hear scientists (Abreu et al., 2019a; e.g., Bicca-Marques & Freitas, 2010; Cupertino et al., 2019; Gouveia et al., 2016; Kaul et al., 2018; Oliveira Figueiredo et al., 2020; Possas et al., 2018; Possas, Martins, Oliveira, & Homma, 2017) and to adopt sound conservation and sanitary strategies (e.g. continuous active surveillance of wildlife reserves, regular monitoring of key primate populations, extensive vaccination of vulnerable human

610 populations, communication and awareness campaigns, restriction of wildlife reserves
611 to unvaccinated visitors) to avoid future dramatic outbreaks that can lead to the local or
612 regional extirpation of sensitive species.
613
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Tables

Table 1. Size and monitoring effort of six habituated titi monkey groups during the 2008-2010 and 2014-2016 surveys. Group mean sizes were calculated using the group sizes recorded in summer (July-October). The 2008-2010 monitoring effort comprises the total monitoring effort (i.e., time spent monitoring the groups) and the monitoring effort from which GPS data were extracted to draw home ranges. Home range sizes are estimated using 100% characteristic hull polygons (Downs & Horner, 2009). Overlaps are calculated as proportion of the 2008-2010 home range that was still occupied by the same group in 2014-2016.

			2008-2010			2014-2016		
Forest	Group	Habituation	# Individuals	Home range size (ha)	Monitoring effort (h) [GPS monitoring (h)]	# Individuals [# remaining from 2010]	Home range size (ha) [overlap in %]	Monitoring effort (h)
Tanque Grande	A	2008	5-7 (mean = 6)	5.7	324 [60]	6 (mean = 5.6) [1]	7.7 [97]	225
	D	2004	2-4 (mean = 2.6)	4.5	322 [42]	4-5 (mean = 4.6) [2]	7.5 [82]	197
	R	2004	2-4 (mean = 3.3)	4.8	347 [60]	4-6 (mean = 5) [1]	6.6 [85]	261
	S	2015	-	-		4-5 (mean = 4.3)	6.5	425
Cascatinha	M	2009	4-5 (mean = 5)	6.2	144 [44]	5-6 (mean = 5.6) [2]	7.9 [89]	269
	P	2008	3-5 (mean = 4)	4.5	158 [60]	4-5 (mean = 5.3) [2]	6.3 [93]	335

950 Table 2. Likely causes of disappearance of individuals and their age-class, during the
951 2008-2010 and 2016-2016 surveys (Berthet, 2018; Cäsar, 2011).

	Mated adult (>30 months)	Unmated adult (> 30 months)	Subadult (18-30 months)	Infant (< 6 months)
Confirmed death		1†		
Supposed death	1		1	8
Supposed dispersion		4		
Total	1	5	1	8

952 † The carcass was found by researchers.
953

Figure legends

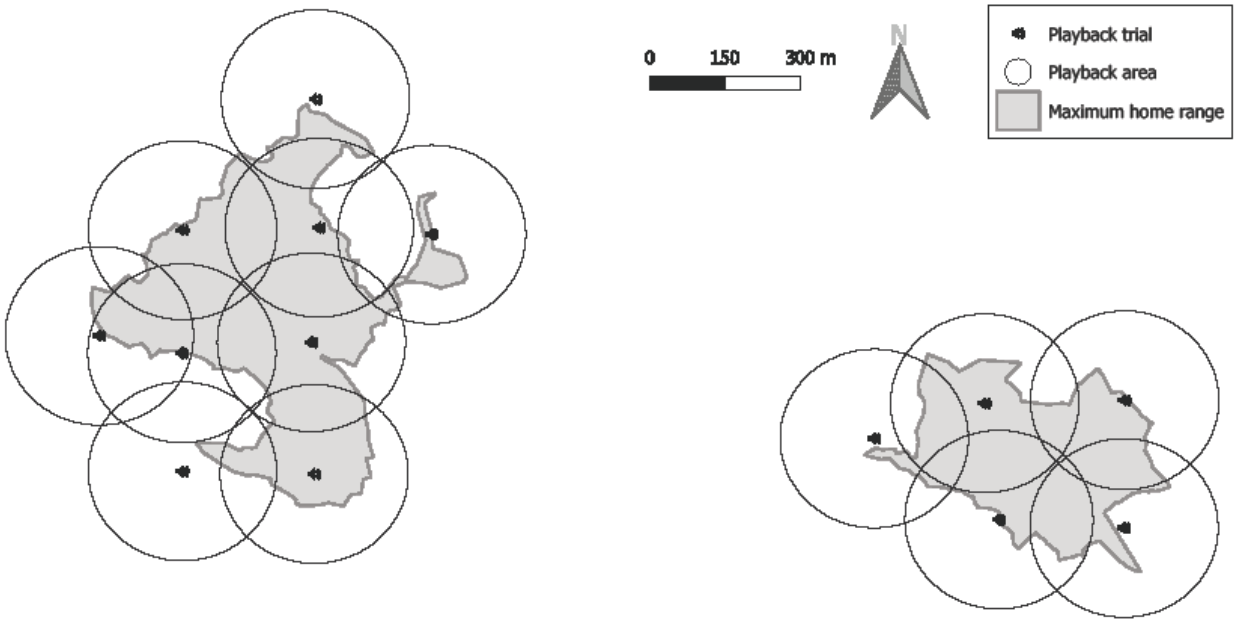
Fig. 1. Distribution of playback trials in the home ranges of the habituated titi monkey groups in the Tanque Grande (west) and Cascatinha (east) forest patches.

Fig. 2. Home ranges of habituated titi monkey groups from (A) 2008 to 2010 and (B) 2014 to 2016. The S group was habituated in 2014. Home range borders in bold are shared with at least one non-habituated group. In 2016, four habituated groups and four non-habituated groups inhabited the Tanque Grande forest patch (west), and two habituated groups and one-two non-habituated groups inhabited the Cascatinha forest patch (east).

Fig. 3. Spatial occupation of the surviving partially habituated group (pink dots) during the 2019 survey. The group used an area occupied by the A, D, R, and S groups in 2008-2016.

Fig. 4. Vocal responses to playbacks by a partially habituated group, a supposedly solitary individual and two non-habituated groups in 2019.

Figures



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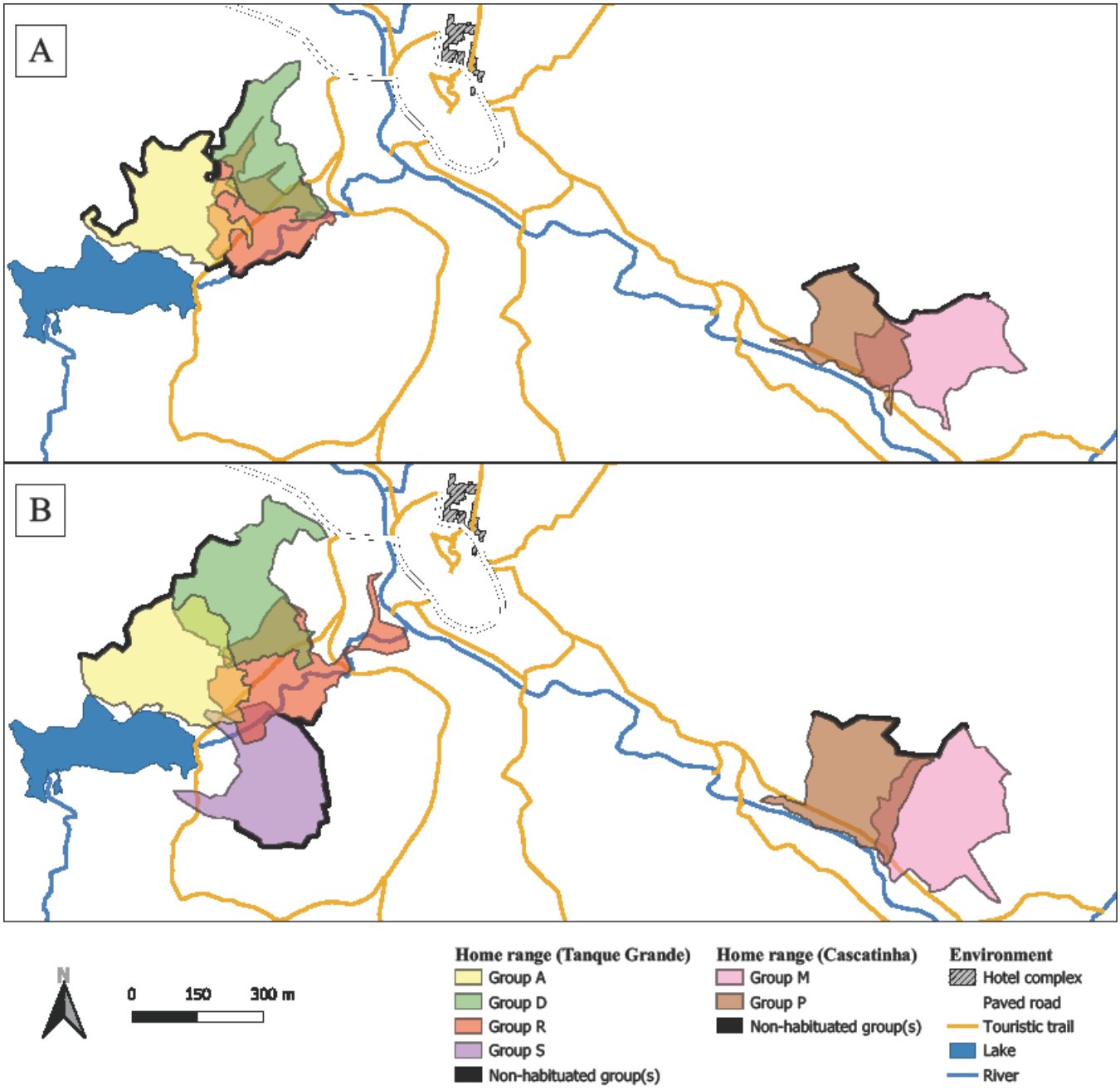

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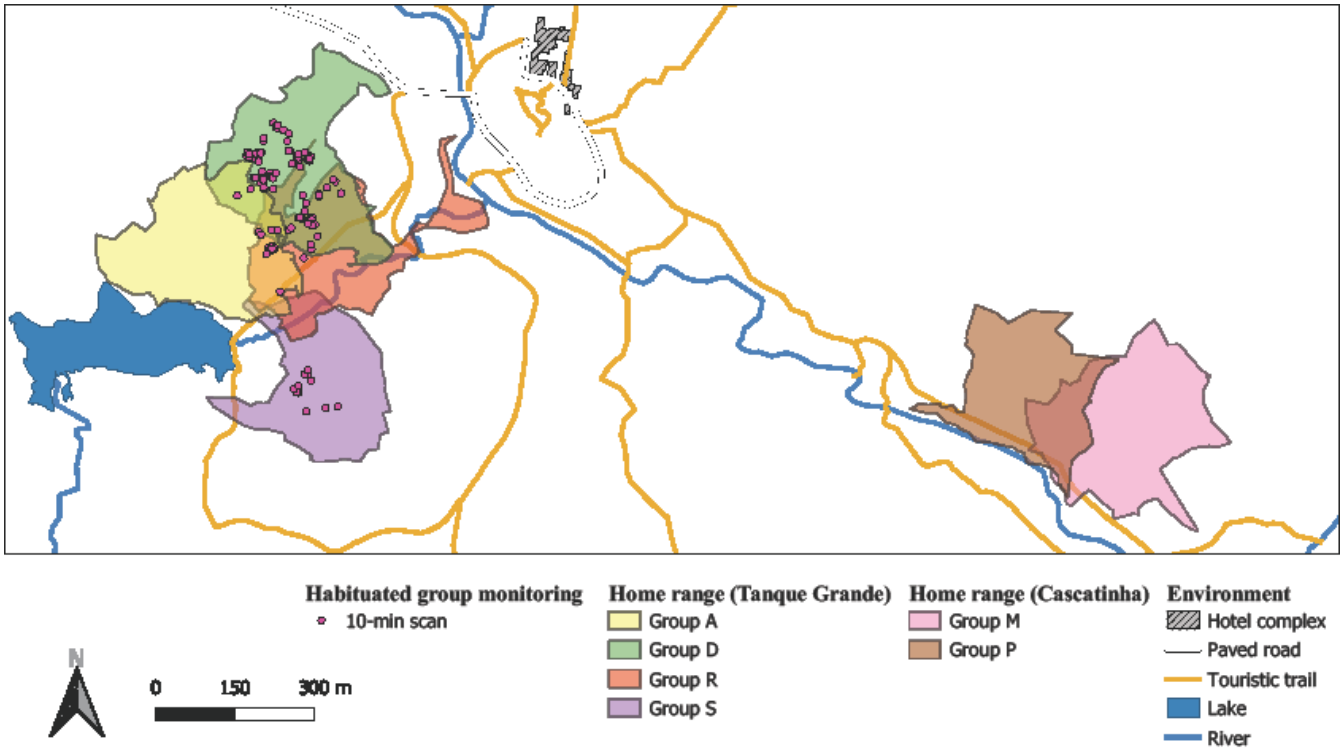

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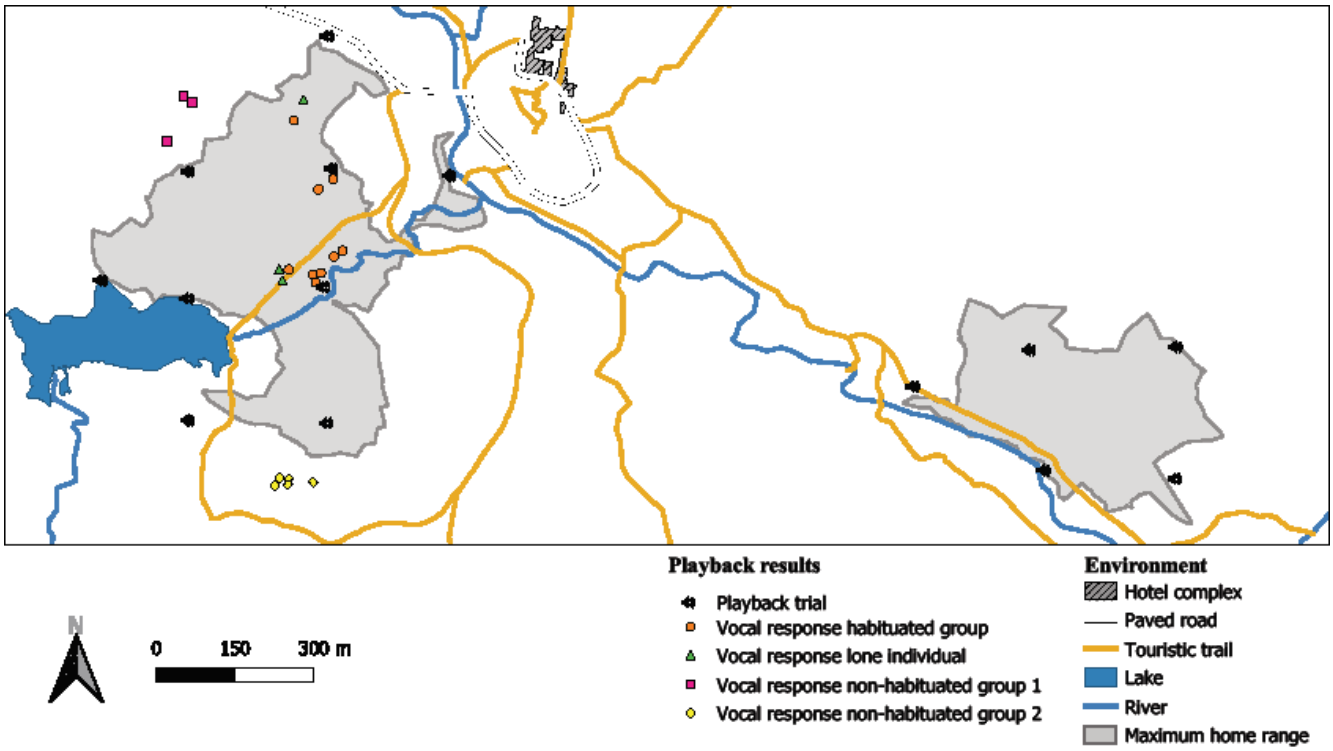

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