Dramatic decline in a titi monkey population after the 2016-2018 sylvatic yellow fever outbreak in Brazil

Short title

Titi monkey decline after YF outbreak

Authors and affiliations

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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

85 primates are the main sources of blood for canopy-inhabiting mosquitoes whose activity
86 peaks during the hottest hours of the day, when primates usually rest (Silva et al., 2020).
87 Unlike African primates, which have long been exposed to the virus and are resistant to
88 the disease (Gould, Lamballerie, Zanotto, & Holmes, 2003), Platyrrhini primates
89 experimentally infected by YF-virus showed high, genera-varying susceptibility (Bugher,
90 1951; Vasconcelos, 2003). Infected Platyrrhini either die rapidly (3-7 days after infection)
91 or develop immunity, suggesting that they can act as virus amplifiers only during short
92 periods (Bicca-Marques & Freitas, 2010; Dietz et al., 2019). When a population is
93 infected, it rapidly declines and the virus disappears from the area (Abreu et al., 2019a;
94 Moreno et al., 2013; Vasconcelos, 2010). This cycle normally resumes when the virus
95 returns to the area, carried by infected vectors or hosts, and finds a renewed, susceptible
96 monkey population. As a result, outbreaks have occurred in the Brazilian endemic areas,
97 particularly in the Amazon, every 7 to 14 years (Câmara, Gomes, Carvalho, & Castello,
98 2011).
99 Despite their central role in the sylvatic cycle, Platyrrhini are not responsible for the
100 spread of the virus to non-infected areas in fragmented landscapes, as they usually live in
101 restricted home ranges and rarely travel on the ground between habitat patches (Bicca-
102 Marques & Freitas, 2010; Possas et al., 2018; Souza-Alves et al., 2019b). Wind, on the
103 other hand, can carry infected mosquitoes over long distances, potentially spreading the
104 disease (Almeida et al., 2019b; Paiva et al., 2019). Finally, human factors are also
105 responsible for the expansion of outbreaks. Humans become accidental hosts when bitten
106 by infected mosquitoes (Consoli & Oliveira, 1994), which occurrence increases along
107 with the increasing human activity in forest areas. In this respect, habitat fragmentation
108 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).

133 Atlantic Forest primates were extensively infected during the 2016-2018 outbreak
134 according to governmental authorities. A total of 2,276 epizootics involving mostly
135 *Callithrix*, *Alouatta*, *Sapajus* and *Callicebus* were reported (2016-2017: N = 1,412 cases;
136 2017-2018: 864 cases; Ministério da Saúde, 2017, 2018). Real rates of epizootics were
137 likely much higher, as only 5% of dead monkeys are estimated to be found and registered
138 (Duchiade, 2018). Systematic analysis of the carcasses showed that *Alouatta* and
139 *Callicebus* are highly sensitive to the YF virus (Sacchetto et al., 2020).
140 In the State of Minas Gerais (MG), 80-90% of the Vulnerable *Alouatta guariba clamitans*,
141 10% of the Critically Endangered *Brachyteles hypoxanthus*, 90% of the Critically
142 Endangered *Callithrix flaviceps* and 40-50% of the Near Threatened *Sapajus nigritus*
143 populations of the Reserva Particular do Patrimônio Natural (RPPN) Feliciano Miguel
144 Abdala (also known as "Caratinga") vanished during the 2016-2017 outbreak, as well as
145 26% of the *B. hypoxanthus* population of the RPPN Mata do Sossego (Lopes, 2017;
146 Possamai, Mendes, & Strier, 2019; Strier et al., 2019). In the neighboring State of Espírito
147 Santo (ES), the disease caused a population decline of 82% for *A. guariba clamitans*, 49%
148 for *C. flaviceps* and the Least Concern *Callithrix geoffroyi*, 25% for the Vulnerable
149 *Callicebus personatus*, 23% for *S. nigritus* and 10-26% for *B. hypoxanthus* (Gontijo,
150 2019; Strier et al., 2019). Finally, 30% of the Endangered *Leontopithecus rosalia*
151 population from the São João river basin (State of Rio de Janeiro [RJ]) disappeared after
152 the outbreak (Dietz et al., 2019). In all these reports, the evidence for virus-caused decline
153 is indirect, as population reductions coincided with the presence of the virus in the regions
154 (Dietz et al., 2019; Lopes, 2017; Strier et al., 2019). Population declines at such rates pose
155 a serious threat to species survival with considerable implications for conservation.

156 The purpose of the study is to assess the state of a geographically isolated and partially
157 habituated population of black-fronted titi monkeys (*Callicebus nigrifrons*) in two small
158 Atlantic Forest patches in Brazil before and after the 2016-2018 YF outbreak, and to
159 evaluate the potential impact of the outbreak on population demography. The study
160 population lives in the RPPN Santuário do Caraça, a reserve located in the upper Rio
161 Doce basin, a highly fragmented Atlantic Forest region in MG (Machado & Fonseca,
162 2000). MG was the epicenter of the 2016-2018 YF outbreak, accounting for 46% of the
163 confirmed human cases and 23% of the confirmed nonhuman primate epizootics
164 (Figueiredo et al., 2018; Ministério da Saúde, 2017, 2018). Three of the closest
165 municipalities to the reserve (namely, Santa Bárbara, Catas Altas and Barão de Cocais)
166 recorded at least 21 confirmed human cases and several suspected cases (Secretaria de
167 Estado de Saúde de Minas Gerais, 2018). These three municipalities also reported
168 confirmed and suspected nonhuman primate cases (Sacchetto et al., 2020; Secretaria de
169 Estado de Saúde de Minas Gerais, 2018). The study population comprised the largest
170 habituated population of *C. nigrifrons* (6 groups and ca. 35 individuals, end of 2016) as
171 well as their neighboring non-habituated groups. Although no primate carcass was
172 recovered, local employees reported fewer cues of black-fronted titi monkeys presence
173 after 2016 (choruses, visual encounters), suggesting that groups were affected by the
174 2016-2018 YF outbreak (Duchiade, 2018).
175 To quantify the potential impact of the YF outbreak on the study population, we assessed
176 the stability of the population from 2008 to 2016 and estimated the demographic changes
177 between 2016 and 2019. Given the lack of extreme climatic events and any noticeable
178 change in habitat quality since 2016, we assumed that if the population was stable from
179 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 part and surrounded by grasslands and rocky outcrops on its other parts, preventing any connection

to other forested areas. Tanque Grande is a hillside forest patch of about 60 ha bounded by human settlements (road, hotel complex) on its northern part, grasslands and a lake on its western part, and surrounded by grasslands and rocky outcrops on its other parts. It can be connected to the core forest of the reserve via a corridor in the south, which can potentially be crossed by titi monkeys, but which does not constitute a suitable habitat for the species because it is a transition zone between grasslands and forests.

The Santuário do Caraça is a tourist attraction that receives 60,000-70,000 visitors each year. Human settlements are restricted to an asphalt road, a farm/hotel complex at the entrance of the reserve and a monastery/hotel complex in the core of the reserve, which allow visitors to spend several days on site. Visitors are required to stay on pre-established trails when walking through the natural areas, and to not interact (e.g., feed, touch) with the wildlife, including primates. Hunting is forbidden and there is no recent record of poaching (Província Brasileira da Congregação da Missão, 2013; pers. obs.). From the 28th of November 2018 to the 8th of March 2019, visitors were required to present a valid certificate of YF vaccination to access the reserve.

The sanctuary is a conservation hotspot for the local fauna (Talamoni et al., 2014). Five primate species inhabit the reserve: *C. nigrifrons, S. nigritus*, *A. guariba clamitans*, *Callithrix penicillata and C. geoffroyi* (Berthet, 2018). Gene flow with populations outside of the reserve is restricted because the area is mostly surrounded by high mountains (1,200-2,700 m) with few trees. Fragmentation has recently been aggravated by the intensification of mining activities, land artificialization and intensive forestry on the reserve's border (Província Brasileira da Congregação da Missão, 2013).

Study species

228 Black-fronted titi monkeys are small (1.0-1.5 kg) diurnal primates (Bicca-Marques &
229 Heymann, 2013) endemic to the Atlantic Forest (states of MG, ES, RJ and São Paulo
230 [SP]). They are classified as IUCN Near Threatened because their populations have
231 declined by more than 20% due to habitat loss and forest fragmentation over the past 24
232 years (Jerusalinksy, Melo, Mittermeier, Quadros, & Rylands, 2020). They live in groups
233 of two to six individuals, composed of a life-long monogamous adult pair and their
234 offspring, on a territory of about 20 ha (21-48 ha, Bicca-Marques & Heymann, 2013; 8-
235 28 ha, Caselli, Mennill, Bicca-Marques, & Setz, 2014). The group jointly defends the
236 territorial resources with loud call displays (solos, duets and choruses, Caselli et al.,
237 2014).
238 Black-fronted titi monkeys are mainly frugivorous but also consume insects, seeds and
239 leaves (Bicca-Marques & Heymann, 2013; Caselli & Setz, 2011; Santos, Galvão, &
240 Young, 2012). They are arboreal and spend most of their time in the lower and
241 intermediate canopy of small fruit trees (10-30 m high) to feed or rest during hot hours
242 (Bicca-Marques & Heymann, 2013; Gestich, Caselli, & Setz, 2014; Trevelin, Port-
243 Carvalho, Silveira, & Morell, 2007). They occasionally descend to the forest floor to
244 forage, travel and play (Souza-Alves et al., 2019b).
245 Mated females give birth to one young per year between July and January (Bicca-
246 Marques & Heymann, 2013; Di Bitetti & Janson, 2000; Souza-Alves, Caselli, Gestich, &
247 Nagy-Reis, 2019a; Valeggia, Mendoza, Fernandez-Duque, Mason, & Lasley, 1999).
248 Young adults of both sexes disperse when they reach 3 years of age (Bicca-Marques &
249 Heymann, 2013). The mechanisms involved in the establishment of new territories
250 remain unknown. A pair of collared titi monkeys *Cheracebus torquatus* has been
251 observed shifting their home range to open space for their offspring (Easley & Kinzey,

252 1986) and a mated back-fronted titi monkey adult was reported being evicted from its
253 group by a new individual (Cäsar, 2011). Individuals live up to 12 years in captivity
254 (Rowe, 1996).
255 The study population is composed of four habituated groups of black-fronted titi monkeys
256 living in the Tanque Grande forest patch, two habituated groups living in the Cascatinha
257 forest patch, and their neighbors (i.e., the non-habituated groups whose home ranges
258 overlap with those of the habituated groups). We began the habituation process in 2004
259 (Berthet, 2018; Cäsar, 2011) and monitored the habituated groups extensively between
260 2008 and 2010 and between 2014 and 2016.
261 There were other non-habituated black-fronted titi monkey groups in the reserve, but we
262 focused on the habituated ones and their neighbors, for which we have reliable long-term
263 data.
264
265 **Demography, density and spatial distribution**
266 *Pre-outbreak monitoring (2008-2010 and 2014-2016)*
267 We monitored five groups (A, D, M, P and R groups) for 1,295 h over 15 months between
268 2008 and 2010 (July-December 2008, May-October 2009, May-July 2010). We
269 habituated the remaining (S) group in 2014 and monitored all six groups for 1,714 h over
270 17 months between 2014 and 2016 (October-December 2014, April-June 2015, October
271 2015-August 2016).
272 We located the groups at dawn (around 06:00 am) by acoustic cues, and monitored them
273 continuously until i) we lost them, ii) they settled in a sleeping tree, iii) we completed a
274 behavioral experiment, or iv) after 6 h of monitoring (see Berthet, 2018; Cäsar, 2011 for
275 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

299 the estimated center of the group every 10 min using a handheld Garmin GPSMAP 60CSx
300 GPS.
301 Given that we did not monitor the groups between 2016 and 2019 and that some
302 individuals were not habituated, contact time did not exceed 4 h per day for two main
303 reasons. First, most non-habituated individuals regularly fled, and it was not always
304 possible to find them back. Second, the goal of our study was to survey the remaining
305 groups instead of to (re-)habituate them: we avoided to follow groups containing non-
306 habituated individuals (i.e., individuals displaying avoidance, flight behaviors;
307 Williamson & Feistner, 2011) for long periods of time to minimize unnecessary harmful
308 levels of stress (Fedigan, 2010).
309
310 *Post-outbreak playback experiments (2019)*
311 We applied a playback method (Gestich, Caselli, Nagy-Reis, Setz, & Cunha, 2016) to
312 locate black-fronted titi monkey groups. This method relies on the territorial behavior of
313 titi monkeys: broadcasted duets of unknown individuals simulate the presence of potential
314 competitors in or close to the territory. Resident groups respond to the playbacks with
315 duets (usually with the participation of the mated pair) or choruses (the adult pair and/or
316 older offspring) to defend their territory's resources against potential intruders (Caselli,
317 Mennill, Gestich, Setz, & Bicca-Marques, 2015). Estimating population densities using
318 playback methods has been shown to be extremely reliable for back-fronted titi monkeys,
319 with a rate of group detection close to 100% (Gestich et al., 2016).
320 We broadcasted duets from one resident group of each forest patch into the other forest
321 patch to stimulate an intrusion by an unknown couple and trigger vocal responses by
322 residents (Caselli et al., 2015). We used 1-min-long samples extracted from four duets

323 from P and S groups recorded in 2016 in which both mates were calling. We normalized
324 sequences at -1 dB and broadcasted them using an Anchor An-30 (Anchor, Carlsbad, CA)
325 loudspeaker (frequency response range: 100–15,000 Hz, output power: 30 W, Maximum
326 SPL at 1 m: 100 dB), which covers the frequency spectrum of black-fronted titi monkeys'
327 vocalizations and reaches the same levels of the natural emissions of duets. We held the
328 speaker at a height of 2 m and directed it to four directions separated by an angle of 90°
329 for 15 s each to cover a circular area in 1 min.
330 We determined a 200-m playback circumference (i.e., the distance at which the
331 broadcasted duets could be heard) during pilot trials in a forest patch that was not
332 occupied by titi monkeys. We conducted the playback trials in the maximum area
333 occupied by the habituated groups, i.e., the sum of the area occupied by each group from
334 2008 to 2010 and from 2014 to 2016. We conducted 14 playback trials at 180-m intervals
335 to fully cover the area of interest (Fig. 1).
336 We played two sequences at 5-min intervals per trial in the morning (Gestich et al., 2016)
337 and alternated recordings to avoid habituation to the stimuli. We registered the responses
338 of neighboring groups during the first 5 min after each playback sequence. A trial lasted
339 12 min (1 min stimulus followed by 5-min waiting period, followed by 1 min stimulus
340 then 5-min waiting period). We estimated the distance of all vocal responses to the
341 playback stimuli and registered their direction in relation to the location of the playback
342 with a compass. When a responding group approached the speaker and was in sight, we
343 did not play the second stimulus to avoid a reduction in responsiveness in future trials.
344 We conducted playback experiments for four days (two consecutive days in the
345 Cascatinha forest patch and two consecutive days in the Tanque Grande forest patch) for
346 at most 4 h per day (Gestich et al., 2016) (See Supplementary Material).

We later plotted all location records on the home range map and clustered vocal responses according to the spatial and temporal distance between the responses. We registered clusters as belonging to the same group unless we had evidence that they were distinct groups (Gestich et al., 2016).

Estimation of population changes

Demography

To estimate the population stability between 2008 and 2016, the size of each habituated group in summer (between July and October, depending on data availability) was extracted for the two pre-outbreak monitoring periods. We calculated each group's 2008-2010 and 2014-2016 mean size and we used a two-tailed Wilcoxon paired signed-rank test to test whether the mean group sizes varied between the two monitoring periods.

To estimate the population changes between 2016 and 2019, we assessed the size of the habituated groups in 2016 and in 2019 based on the monitoring, survey and playback data. We assessed the presence and location of neighboring groups based on anecdotal encounters and playback results. Since we did not know the exact composition of the neighboring groups, we assigned them a hypothetical size of four individuals because black-fronted titi monkey groups are usually composed of one mated pair and one to three offspring (Bicca-Marques & Heymann, 2013).

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

371 constant over the years. To estimate the stability of the groups before 2016, we compared
372 the home range size and location of each habituated group between 2008-2010 and 2014-
373 2016: if the population was stable (i.e., well established home ranges, no disappearance
374 of a group or establishment of a new one) then home ranges of the habituated groups
375 should remain constant between the two monitoring periods prior to 2016.
376 To this end, we georeferenced the home ranges of the habituated groups using GPS data
377 collected in 2008-2010 and 2014-2016. Due to logistic issues, some of the 2008-2010
378 GPS data were lost, so associated home ranges were drawn using the remaining GPS data,
379 which probably underestimated their real size (see Table 1). We mapped the borders using
380 characteristic hull polygons (Downs & Horner, 2009). While the home range is usually
381 measured as the smallest area in which animals spend 95% of their time, we decided to
382 use 100% of the collected GPS points to remain conservative. We estimated the size of
383 each home range in 2008-2010 and in 2014-2016, and compared them using a two-tailed
384 Wilcoxon paired signed-rank test. We also calculated the proportion of overlap between
385 the two periods (i.e., the proportion of the 2008-2010 home range that was still used by
386 the same group in 2014-2016). Finally, we used opportunistic encounters with neighbors
387 to identify the home range borders shared with non-habituated groups in 2008-2010 and
388 2014-2016.
389 We also used the home range data to estimate changes in the population between 2016
390 and 2019. We hypothesized that if, in 2019, a black-fronted titi monkey group occupied
391 an area located in the 2016 home range of another group, then the latter had probably
392 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 least two other groups, both located outside of the home ranges of the 2016 habituated groups (i.e., two non-habituated groups).

439 The group that we encountered (later referred to as the 'partially habituated group') was
440 composed of three individuals. The mated male was the resident adult male of the R group
441 from 2008 to 2016, easily recognizable by its specific physical features. Moreover, this
442 individual ignored our presence in 2019, which is congruent with the fact that the adult
443 male of the R group was one of the most habituated individuals of the 2016 titi population.
444 The mated female was born in the A group in 2014, also easily recognizable by her
445 physical traits. This individual exhibited intermediate-tolerance behaviors (avoidance,
446 curiosity) in our presence, suggesting that she was still in the habituation process, a
447 conclusion congruent with the fact that she was only monitored for two years before 2016.
448 The last individual was a juvenile estimated to have been born by the end of 2017 based
449 on observations of its size and behavior (e.g., play, exploration, no participation in
450 territorial defense). The juvenile was not habituated to human presence (flight, avoidance,
451 curiosity). The group ranged in an area previously occupied by the A, D, R and S groups
452 (Fig. 3).
453
454 *Playback experiments*
455 We recorded no response to the five playback trials conducted in the Cascatinha forest
456 patch, but recorded 20 vocal responses to the nine playback trials conducted in the Tanque
457 Grande forest patch (Table S1). The responding individuals were the partially habituated
458 group monitored during the survey, two non-habituated groups whose duets were heard
459 during the monitoring, and a supposedly solitary individual who emitted solos (Fig. 4).
460 One of the non-habituated groups was sighted once and immediately lost, while the other
461 non-habituated group and the solitary individual were never sighted. We did not find any

462 evidence of the presence of other habituated groups during the 4-week survey (no duets
463 nor direct observations).
464
465 *Impact of the YF outbreak on the titi monkey population*
466 In 2016, we estimated the size of the Cascatinha population at 16-20 individuals (12
467 habituated and 4-8 non-habituated individuals) and the 2016 Tanque Grande population
468 at 37 individuals (21 habituated and 16 non-habituated individuals), i.e., a population of
469 53-57 individuals in the two forest patches. In 2019, we estimated the Cascatinha
470 population at zero, and the Tanque population at 12 individuals (three individuals in the
471 partially habituated groups, one solitary individual and two unknown groups) (Fig. S2).
472 Therefore, we estimate the Cascatinha population to have declined by 100% and the
473 Tanque Grande population to have declined by 68% between 2016 and 2019. Overall, we
474 estimate the black-fronted titi population to have declined by about 80% between 2016
475 and 2019 in the two forest patches (from a total of 53-57 individuals in 2016 to 12
476 individuals in 2019).
477
478 **Discussion**
479 We found that the home ranges and the size of the habituated black-fronted titi monkey
480 groups of the Santuário do Caraça did not markedly vary from 2008 to 2016. The size of
481 the estimated home range tended to increase between 2008-2010 and 2014-2016, and the
482 2008-2010 home ranges were almost entirely occupied in 2014-2016 by the same groups
483 (mean overlap of around 90%). We believe that the variation trend of the home ranges'
484 size is better explained by differences in sampling effort (given the loss of some 2008-
485 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, deforestation, or poaching was recorded in the Cascatinha or Tanque Grande forest patches between 2016 and 2019 (Abreu A., pers. com.).

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

558 sensitive or more exposed to the YF virus than the rest of the population. Additionally,
559 Caraça's employees reported lower rates of titi monkey choruses or sightings in other
560 parts of the reserve. Therefore, we suspect that the YF outbreak not only affected the
561 Cascatinha and Tanque Grande's population, but also impacted other groups of black-
562 fronted titi monkeys at the Santuário do Caraça. Further investigation is needed to
563 estimate the current state of the remaining Caraça population.
564 Despite the legal protection provided by the reserve, the surviving black-fronted titi
565 monkey population may disappear in the medium- to long-term. Even if the remaining
566 adult individuals are resistant to the YF virus and can pass this trait to descendants
567 (Almeida et al., 2019a), the population is small and geographically isolated from other
568 populations due to the reserve's topography, habitat fragmentation and the intensive
569 human activities in the surrounding areas. These conditions increase the population's
570 vulnerability to stochastic events, such as genetic drift and inbreeding, random
571 demographic variations, natural catastrophes, other disease outbreaks and climatic events
572 (Costa, Fernandes, Hilário, Gonçalves, & Souza, 2012).
573 This prospect is worrisome at the species level. Black-fronted titi monkey populations
574 have experienced declines over the last decades (more than 20% in the past 24 years,
575 Jerusalinksy et al., 2020), mainly due to the degradation of the Atlantic Forest (Ribeiro,
576 Metzger, Martensen, Ponzoni, & Hirota, 2009). Titi monkeys can live in primary and
577 secondary forests (Trevelin et al., 2007) with high and closed canopy (Sales, Hayward,
578 & Passamani, 2016), which enables them to occur in small forest patches embedded in
579 agricultural landscapes (Ribeiro et al., 2009). The inevitable proximity to humans and
580 domestic animals in these landscapes increases the chances of pathogens transmission.

581 The resurgence of similar deadly outbreaks is a severe threat to the local fauna. Brazil is
582 the world's richest country in primate diversity, but 48% of its primate species have
583 declining populations because of habitat loss and fragmentation, hunting, infectious
584 diseases and climate change (Estrada et al., 2018). This YF outbreak worsened the
585 conservation status of most nonhuman primates of southeastern Brazil. Given the absence
586 of accurate pre-outbreak demographic data for most species, reported figures are likely
587 underestimating the damage. Populations of *A. guariba clamitans*, *B. hypoxantus*, *C.*
588 *personatus*, *S. nigritus*, *C. flaviceps, C. geoffroyi* and *L. rosalia* (Dietz et al., 2019;
589 Gontijo, 2019; Lopes, 2017; Possamai et al., 2019; Strier et al., 2019) in addition to
590 *Callicebus nigrifrons* (this study) have suffered dramatic losses. The risk of the YF virus
591 remaining in the same region for three transmission seasons or longer (Abreu et al.,
592 2019a), re-emerging and causing further population declines is real.
593 In the light of such a demographic decline in the Santuário do Caraça's population, we
594 highlight the emergency of surveying other, less protected populations of black-fronted
595 titi monkeys, but also other Platyrrhini species, to re-evaluate the conservation status of
596 impacted species and take appropriate measures to protect them. At a broader scale, we
597 call for action, and advise local health and environmental authorities to hear scientists
598 (Abreu et al., 2019a; e.g., Bicca-Marques & Freitas, 2010; Cupertino et al., 2019; Gouveia
599 et al., 2016; Kaul et al., 2018; Oliveira Figueiredo et al., 2020; Possas et al., 2018; Possas,
600 Martins, Oliveira, & Homma, 2017) and to adopt sound conservation and sanitary
601 strategies (e.g. continuous active surveillance of wildlife reserves, regular monitoring of
602 key primate populations, extensive vaccination of vulnerable human populations,
603 communication and awareness campaigns, restriction of wildlife reserves to unvaccinated

visitors) to avoid future dramatic outbreaks that can lead to the local or regional extirpation of sensitive species.

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924

925 **Tables**

926
927 Table 1. Size and monitoring effort of six habituated titi monkey groups during the 2008-
928 2010 and 2014-2016 surveys. Group mean sizes were calculated using the group sizes
929 recorded in summer (July-October). The 2008-2010 monitoring effort comprises the total
930 monitoring effort (i.e., time spent monitoring the groups) and the monitoring effort from
931 which GPS data were extracted to draw home ranges. Home range sizes are estimated
932 using 100% characteristic hull polygons (Downs & Horner, 2009). Overlaps are
933 calculated as proportion of the 2008-2010 home range that was still occupied by the same
934 group in 2014-2016.
935

			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

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937

938 Table 2. Likely causes of disappearance of individuals and their age-class, during the
939 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

940 † The carcass was found by researchers.
941

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.

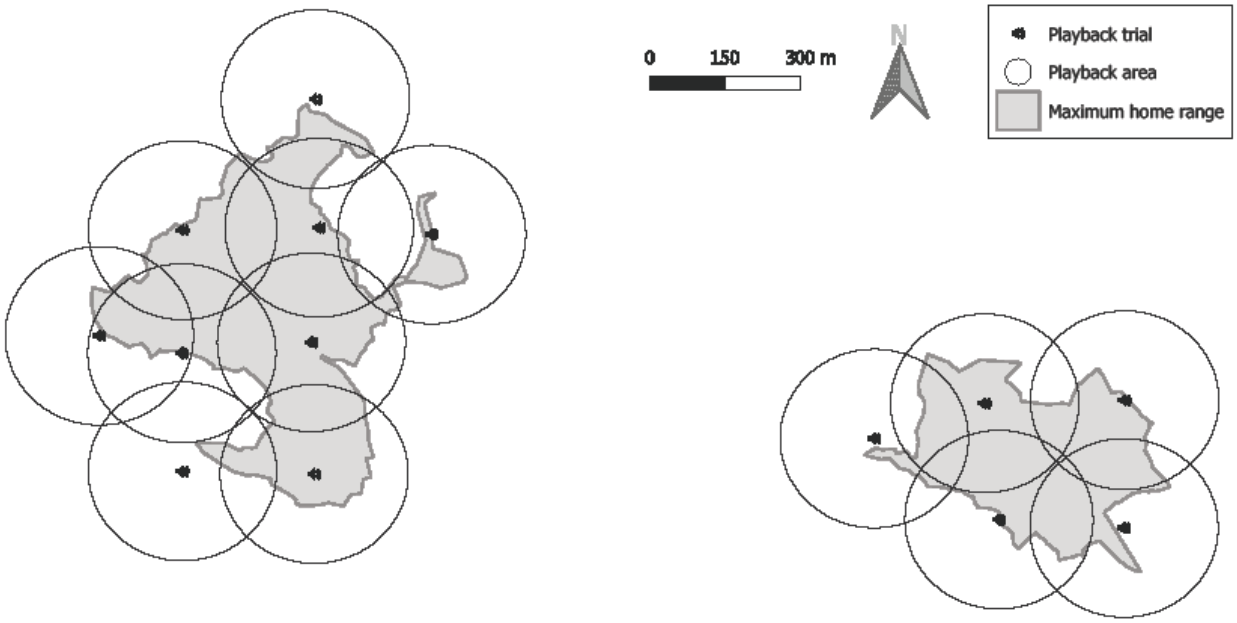
Fig. 2. Home ranges of habituated titi monkey groups from (A) 2008 to 2010 and (B) 2014 to 2016. The S group was habituated in 2014. Home range borders in bold are shared with at least one non-habituated group. In 2016, four habituated groups and four non-habituated groups inhabited the Tanque Grande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

960 **Figures**

961



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964 groups in the Tanque Grande (west) and Cascatinha (east) forest patches.

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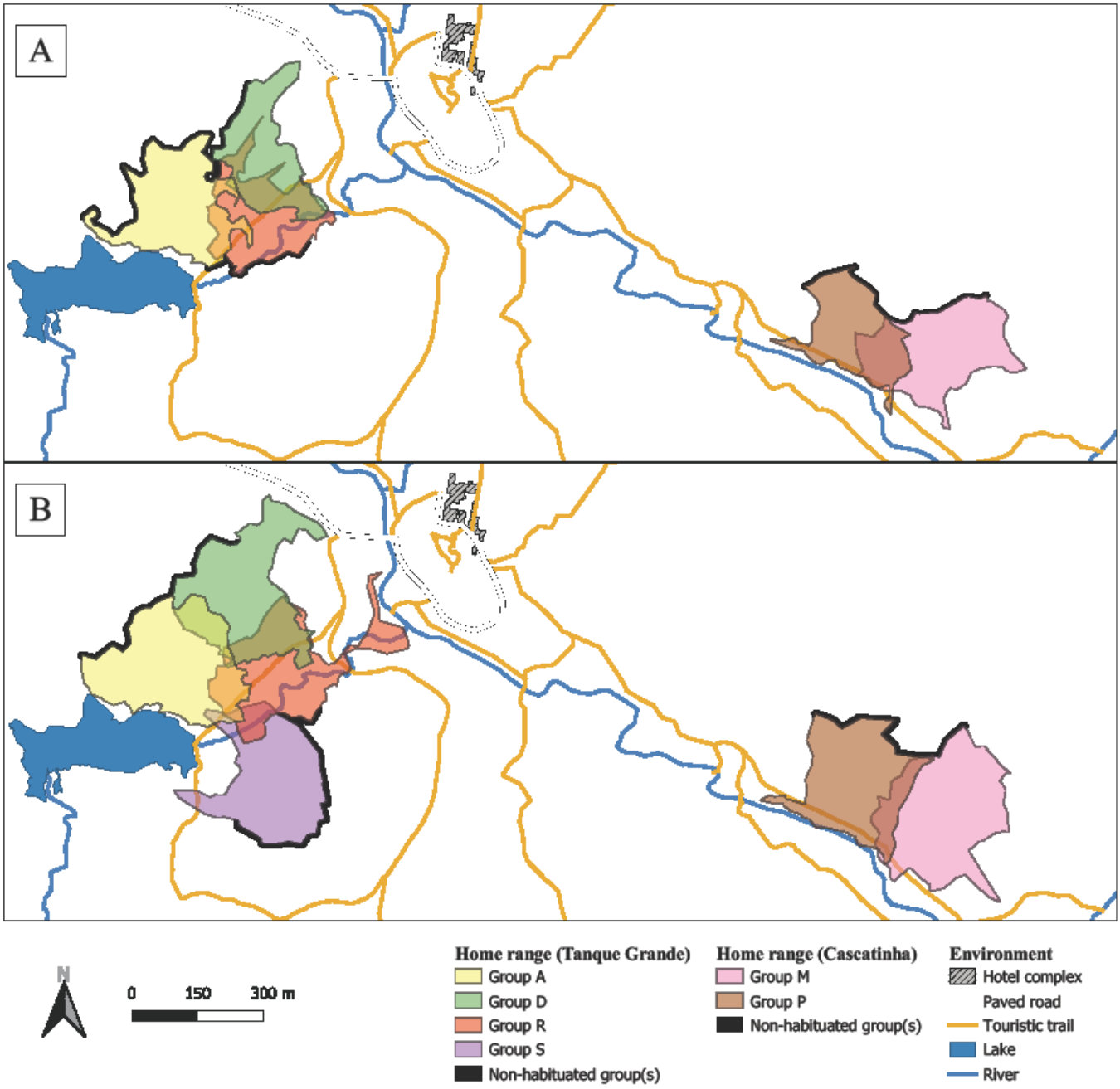

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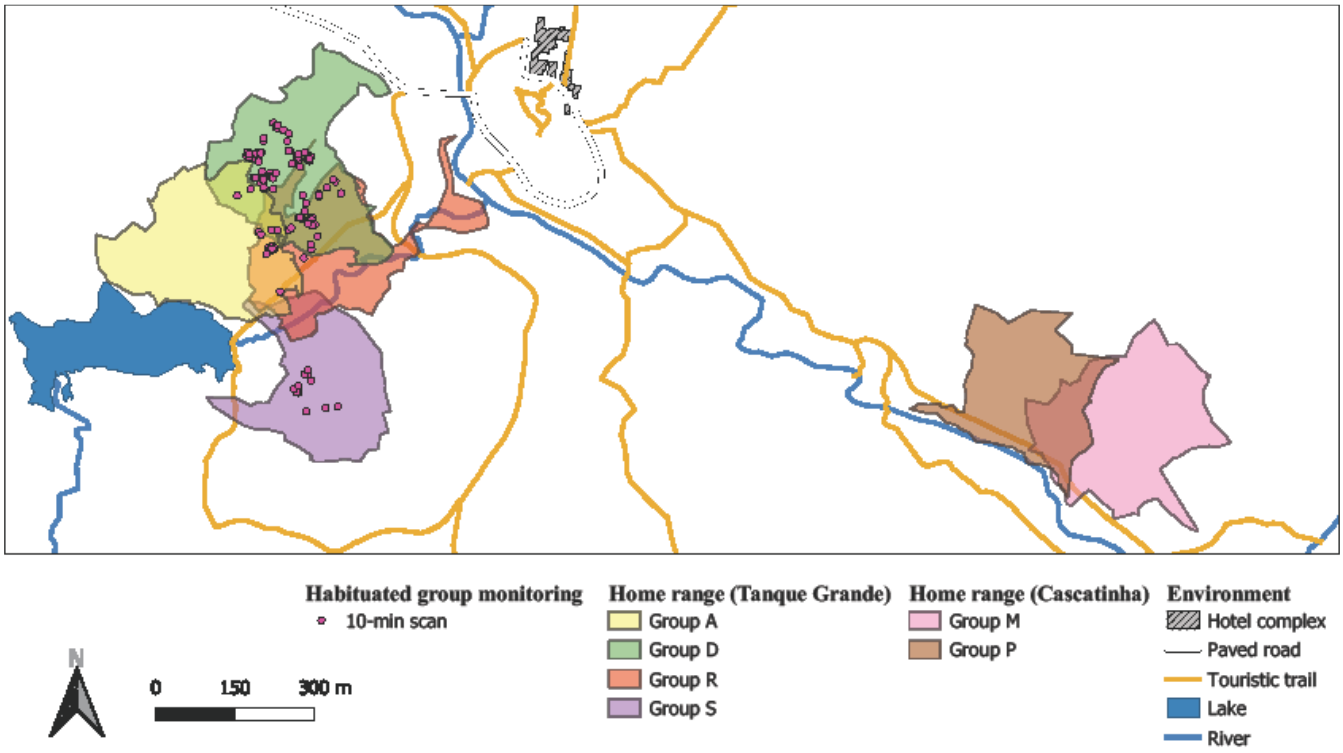

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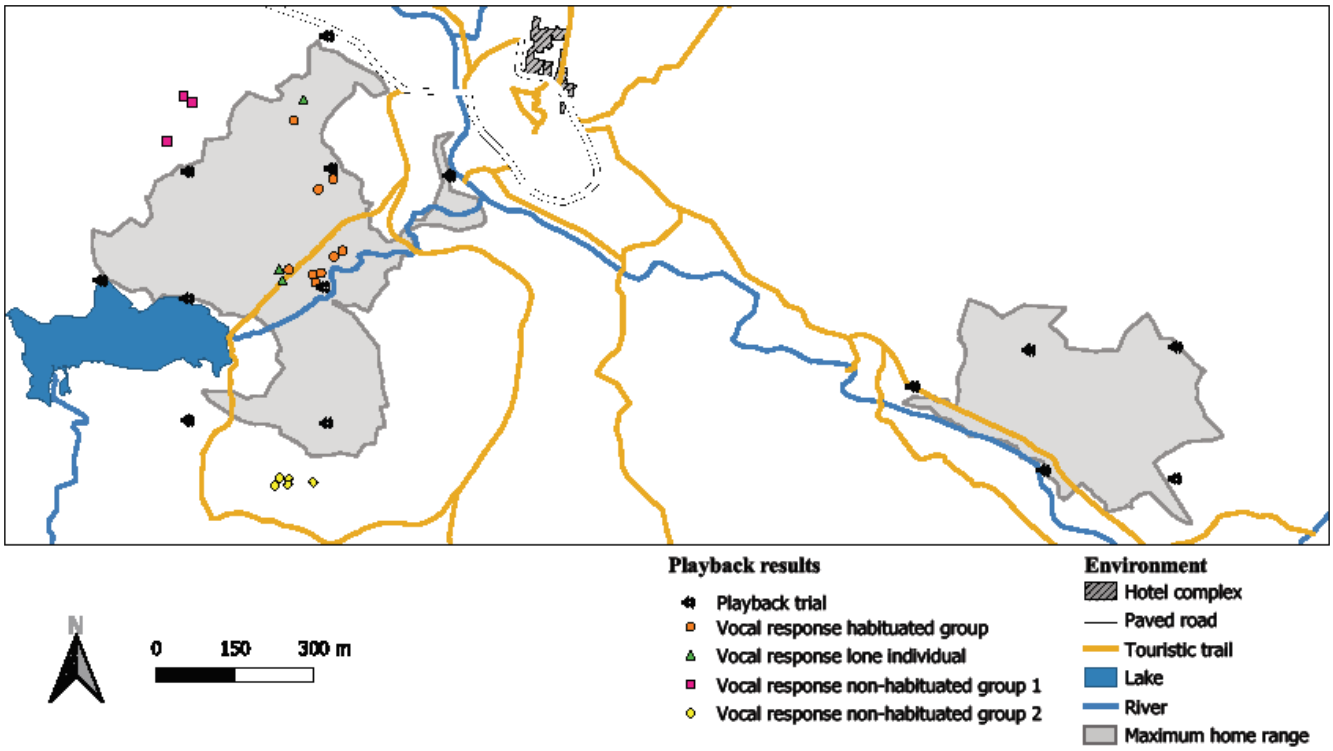

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983 Fig. 4. Vocal responses to playbacks by a partially habituated group, a supposedly solitary

984 individual and two non-habituated groups in 2019.

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