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Article

Concrete survivors: the herpetofauna of an urban green area over 100 years of increasing urbanization

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Abstract: Low biodiversity in urban areas is associated with habitat loss. However, the effects of urbanization on biodiversity should also consider the historical background of land-use, explored herein. Our goal was to evaluate changes in the assemblage of reptiles in an urban habitat over 100 years, aiming to identify which ecological attributes allowed the persistence of species that can be found in the area today. We accessed historical records in scientific collections and carried out fieldwork to access reptile assemblage in an urban green area, in São Paulo, Brazil. Considering land-use changes in the area, we defined three-time intervals between 1901 and 2020. We established species richness for each time interval, categorizing them into three ecological attributes: habitat preference, substrate use, and food habits. We recorded 27 reptile species from 1901 until 2020, 14 resulting from historical data, eight from both historical and fieldwork, and five species exclusively in fieldwork. Amphibians were also sampled during fieldwork, but not used in historical comparison. Reptile's species richness decreased 59% regardless of ecological attributes, and snakes were the group with most species' loss. Fossorial reptiles were the least affected group. We concluded that habitat loss culminated in a species richness decline, and the reptiles that remain until today were likely present since the fragment isolation. Ecological attributes of the remaining taxa include species that use terrestrial substrates and feed on prey commonly found in urban environments.

Keywords: *urban biodiversity, landscape history, reptiles, amphibians, urban ecology.*

Sobreviventes do concreto: a herpetofauna de uma áreaverde urbana ao longo de 100 anos de urbanização

Resumo: A baixa biodiversidade em áreas urbanas está associada à perda de habitat. No entanto, os efeitos da urbanização sobre a biodiversidade também devem considerar o histórico de uso da terra, explorado aqui. Nosso objetivo foi avaliar as mudanças na comunidade de répteis em um habitat urbano ao longo de 100 anos, visando identificar quais atributos ecológicos permitiram a persistência de espécies que podem ser encontradas na área hoje. Acessamos registros históricos em coleções científicas e realizamos trabalho de campo para levantar as espécies de répteis em uma área verde urbana, em São Paulo, Brasil. Considerando as mudanças de uso da terra na área amostrada, definimos três intervalos de tempo entre 1901 e 2020. Estabelecemos a riqueza de espécies para cada intervalo de tempo, categorizando-as em três atributos ecológicos: preferência de habitat, uso de substrato e hábitos alimentares. Registramos 27 espécies de répteis de 1901 até 2020, sendo 14 resultantes de dados históricos, oito de dados históricos e de campo e cinco espécies amostradas exclusivamente de campo. Anfíbios também foram amostrados durante o trabalho de campo, mas não foram usados na comparação histórica. A riqueza de espécies de répteis diminuiu 59% independentemente dos atributos ecológicos, e serpente foi o grupo com maior perda de espécies. Os répteis fossoriais foram o grupo menos afetado. Concluímos que a perda de habitat culminou em um declínio da riqueza de espécies, e os répteis que permanecem até hoje provavelmente estavam presentes desde o isolamento do fragmento. Os atributos ecológicos dos táxons remanescentes incluem espécies que utilizam substratos terrestres e se alimentam de presas comumente encontradas em ambientes urbanos.

Palavras-chave: *biodiversidade urbana, histórico da paisagem, répteis, anfíbios, ecologia urbana.*

Introduction

Urban green areas (UGA) are all vegetation patches within and around cities (Miller 1997, Cilliers et al. 2013). These areas are important as refuge for varied species (Turner and Corlett 1996, Ives et al. 2015, Barbosa et al. 2020). Most studies aiming to evaluate biodiversity in UGA show that species richness in these areas is lower than natural habitats (e.g., Nielsen et al. 2014, Concepción et al. 2016, Lourenço-de-Moraes et al. 2018, Ganci et al. 2020). The species-area model is one possible process that explains this pattern, once it predicts that the replacement of vegetation by impervious surfaces reduces biodiversity by losing habitable area (McKinney 2008). However, biodiversity patterns emerging from interactions between different taxonomic groups and urban environments are not straightforward. The biodiversity in UGA varies according to several factors, including landscape composition (Aronson et al. 2017) and sensitivity of individual species to urbanization (Pena et al. 2017). Therefore, the information about what landscape characteristics and species life-history traits drive the success of biodiversity in urban habitats is essential for conservation in an increasingly urban world (Schochat et al. 2006, Aronson et al. 2017, Lepczyk et al. 2017).

Biodiversity patterns in UGA are usually assessed by comparing patches in urban or urban-rural environments; on the other hand, temporal comparisons are less common in the literature (but see Tait et al. 2005, Hahs et al. 2009, Marques et al. 2009). However, historical backgrounds in cities differ in several ways, such as cities' age or the presence of natural landscapes (von der Lippe et al. 2020). Therefore, comparing historical data with current information from the same locality allows to evaluate changes in community composition throughout the years (Acosta et al. 2005) in order to answer how anthropic impacts affects the activity and survival of the remaining taxa (Pearman 1997, Schlaepfer and Gavin 2001, Loehle et al. 2005).

Birds, arthropods, and plants communities are often assessed in UGA studies (e.g., Saari et al. 2016, Palacio et al. 2018), but the effect of urbanization on communities of reptiles and amphibians are less understood (Almeida-Corrêa et al. 2020). In a recent review, French et al. (2018) suggests a general negative impact of urbanization on reptiles. In Brazil, recent empirical studies are suggesting a pattern of decline in species richness (e.g., de Andrade et al. 2019, Avila-Pires et al. 2018), and possible trophic cascades consequences (Siqueira and Marques 2018). São Paulo is the largest city in South America and its forests support a high diversity of reptiles and amphibians (e.g., Malagoli 2008, Marques et al. 2009, Barbo et al. 2011). A recent study carried out in an UGA in São Paulo city recorded an impressive amount of anuran and reptiles, representing respectively 10 and 12% of the total richness known for these taxa in the Atlantic Forest in São Paulo state (Lisboa et al. 2021). The authors highlight the importance of studies that assess the ecology of extant species to better understand their ability to thrive in urban environments.

Here, we characterized the herpetofauna community in an UGA within the city of São Paulo, Brazil, aiming to evaluate the temporal changes in species richness and community composition from 1901 until 2020, identifying the ecological attributes that allowed the resistance of herpetofauna in this urbanized environment. With two important scientific collections in the city of São Paulo, data on fauna diversity has been recorded since early 20th century, allowing us to access knowledge from the past and integrate it in new discoveries.

Material and Methods

1. Study site

The study was conducted in the UGA of Instituto Butantan (IBu) (Figure 1), a research institute located amidst 80 hectares of green area, in São Paulo, Brazil (23°34'03.96"S, 46°43'06.16"W), one of the most populous cities in the world (Instituto Brasileiro de Geografia e Estatistica [IBGE], 2018). Historically, the city of São Paulo is part of the range of the Ombrophile Semidecidual Atlantic Forest interspersed with open grasslands that were either part of the Brazilian Cerrado biome or the result of the action of pre-European human settlements (Usteri 1911, Joly 1950, Ab'Saber 1963). Currently, the urban matrix of São Paulo city still contains numerous small parks and is surrounded by two large patches of Atlantic Forest, the Serra da Cantareira to the northwest and the Serra do Mar to the southwest. The rainfall and hottest months predominate from November to March.

In 1901, the IBu was a 400-hectare farm that comprised a flooded area, formed mainly by crops, pasture, Eucalyptus grove, and its limits extended over rivers that came from inland to the city of São Paulo (Joly 1950). During 50 years of infrastructure adequacy, most of the rivers were rectified, and, consequently, IBu lost its flooded boundaries (Diasas 2019). Meanwhile, the city of São Paulo grew exponentially, and urbanization reached the western region of the town, modifying the urban matrix surrounding IBu. A significant urban development occurred during 1951 and 1990, with new buildings, pavements, and the rivers' silting. At the same time, more than 600 seedlings of Atlantic Forest native trees were intentionally planted to replace the former pasture area. Today, besides being a worldwide reference in the study of venomous animals and public health, the IBu is a significant UGA in the western zone of São Paulo. With 80 hectares of green area in an urban environment, only a quarter of its original area 120 years ago, 62% of its current area is covered by native and exotic vegetation within an urban environment (Teixeira-Costa et al. 2014, Secretaria do Verde e Meio Ambiente [SVMA], 2020). It is possible to find small and shallow water bodies inside the forest that remain full throughout the year, although dryer between May and September.

2. Data collection

We searched for reptile species recorded in scientific collections from 1901 to the present day; we also carried out fieldwork throughout 2016 to assess the current herpetological community. The Collections examined were: Herpetological Collection "Alphonse Richard Hoge" (IBSP) and the Museu de Zoologia da Universidade de São Paulo (MZUSP), two centenary scientific collections in the city of São Paulo.

During the fieldwork in 2016, we employed active and passive methods (Auricchio and Salomão 2002): pitfalls traps with drift-fences (Corn 1994, Cechin and Martins 2000), visual surveys (Crump and Scott 1994), and records of occasional encounters from the authors and third parties (Auricchio and Salomão 2002). Five lines of pitfall traps were installed, in 50m long transects, each with five 100-liter plastic buckets buried in the ground. Every bucket was connected to another

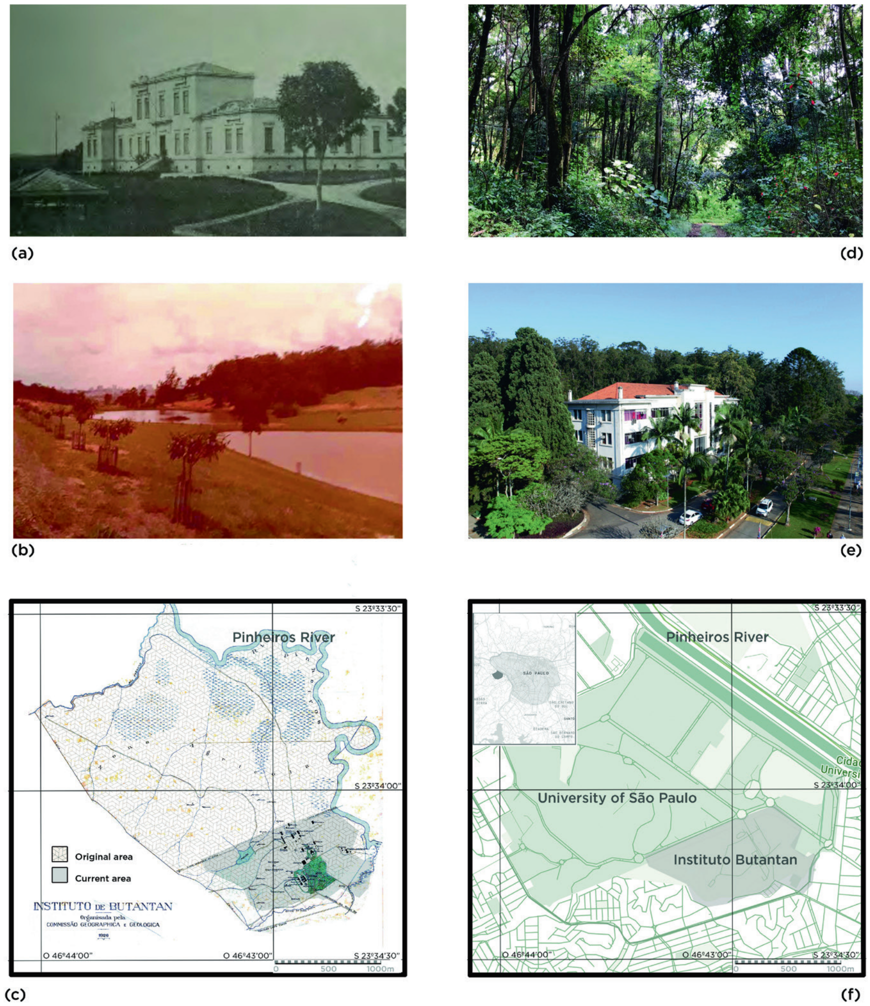

Figure 1. Urban green area of the Instituto Butantan, São Paulo, Brazil. (a) IBu in 1928, showing the main building and surrounding grasses; (b) IBu in 1983, showing artificial lakes, grass, and secondary forest; (c) Manipulated map of IBu in 1926 showing the original area of 400 hectares (dotted area) and the current area of 80 hectares (shaded area). Note the Pinheiros river before rectification; (d) secondary forest of IBu in 2021; (e) aerial view of IBu in 2014, showing a building, pavements, and secondary forest; (f) current area of IBu (shaded area). Note the Pinheiros river after rectification. Photographs (a) and (b): Butantan Institute Archive/ Memory Center; Original map in (c): Cardoso 1926; photograph (d): Bruno Martins da Costa Batista; photograph (e): Camilla Suescun Marques de Carvalho.

by 10m with drift-fences. The fences were 1m high and were buried approximately 20cm into the ground, passing through the center of each pitfall. The visual survey consisted of walking around in search of reptiles and amphibians turning over leaves and shrubs. Animals found outside the collecting periods were recorded as occasional encounters. All collected animals were identified in the level of species. Because the sampled area has homogeneous vegetation and the pitfall lines were not more than 500m distant from each other, the transects were considered replicas. Sampling took place from January to December 2016, lasting seven days per month and totaling 84 days. The sampling effort and the encounter rate were measured in person-hours of visual search (two researchers in an hour daily for 53 days) and considering the 84 days that the pitfall traps remained open (Martins and Oliveira 1998). The collected specimens were deposited in the IBSP. We carried out the fieldwork under the required permits (SISBIO license nº 51345-1, CEUA Instituto Butantan nº 7717221015).

2.1. Habitat preference and ecological attributes

We categorized the recorded species into ecological attributes in order to evaluate which life-history traits allow species to persist in patches of habitat within an urban area. We divided each sampled species by habitat preference or exclusiveness: forest specialist, open-area specialist, or generalist (i.e., no habitat preference or exclusiveness); the main type of substract: terrestrial, arboreal, aquatic, or fossorial; and the feeding habits. The feeding habitats were classified accordingly with the diet preference of each species, and we considered preferences for invertebrates, anurans, snakes, lizards, mammals, mollusks, earthworm, or generalist if the species doesn't show any diet preference. To determine the ecological attributes of each species recorded, we used data available in literature: Fialho et al. 2000, Parpinelli and Marques 2008, Ghizoni-Junior et al. 2009, Hartmann et al. 2009, Araújo et al. 2010, da Silva et al. 2010, and Marques et al. 2019.

3. *Data analysis*

Considering data gathered during fieldwork, the efficiency of the sampling method was evaluated using species rarefaction curves (Colwell and Coddington 1994, Thompson et al. 2003, Trevine et al. 2014), with a 95% confidence interval and 1000 randomizations. We assessed the richness through a non-parametric first-order Jackknife index (Heltsche and Forrester 1983, Hellmann and Fowler 1999). Due to the lack of accurate historical records on species abundance, we choose species richness as a method for comparison in the temporal analysis (Tait et al. 2005). We determined the richness of snakes, lizards and amphisbaenians for each time interval using the total number of species recorded in that period, disregarding the lost specimens. No historical record of amphibian species was available in IBSP or MZUSP. Thus, the amphibian species presented here results only from fieldwork, and we did not use it in the temporal comparison.

To make it possible to compare the reptiles richness at different times, we stipulated three time intervals considering major land-use changes in the IBu area (Figure 1): 1901-1950, which includes the initial area of 400 hectares with wetlands and agricultural activities (Figure 1a-c); 1951-1990, including new buildings, rivers and roads paving, and reforestation (Figure 1a-c); 1991-current days, an area of 80 hectares and a well-established secondary forest (Figure 1d-f).

Species were categorized by "present", "absent", or "data not available - NA" for each time interval. If a species were recorded in earlier years but not in years after that (e.g., record available in 1930 but no record after that) we considered the species "absent" in the next years. If a species were recorded in one year but not in years before that (e.g., record available in 2000 but no record before that), we considered that this species has no available data "NA" for years before (see Table 1). We made this decision based on the fact that sometimes historical data can be missed or not kept. We analyzed the proportion of present species against time (e.g., Tait et al. 2005), which produced three lines (Figure 2). The upper line indicates the maximum possible number of species present in that time interval, calculated by adding the total number of present species to the total number of NA species, that is, considering that NA species were present. The central line represents the total number of present species divided by the total number of species recorded as present and absent, excluding NA species. This central line highlights the effect that NA values have on the basic pattern. The lower line indicates the minimum number of species present expected for each time interval, assuming that all species with NA values were absent. Because the proportion of present species against time showed little variation between the upper and the central line, it is more likely that species with no available data (NA) were in fact present in earlier years, but with no records taken or kept. Thus, we utilized the maximum number of species (present + NA) for each time interval to conduct the comparison between different periods.

Data was extrapolated between data points to provide the continuous graphical representation (Tait et al. 2005). It means that the same species recorded as present in 1901 and 2018, but with no data sets for intervening years, was presumed as present in all years between these two time points. If a species was recorded as present in 1901, but no records were found for the remaining years, we assumed that the species was locally extinct and categorized it as absent for the next years. We used the software R (R Development Core Team 2020) to conduct the analyses and to produce the graphics.

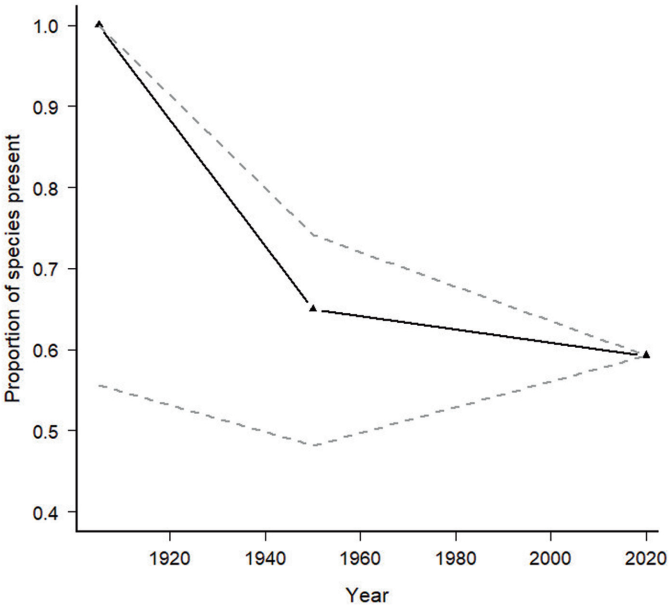

Figure 2. Proportion of species present in three periods. The upper and lower dotted lines (in gray) show the proportion of species present compared to the total number of species (present, absent, and NA). The central line (in black) shows the proportion of species present compared to the total number of species with known status (present and absent), excluding "NA" records.

Results

1. *Fieldwork sampling*

We recorded six species of anurans (Table 1; Figure 3a) during fieldwork in 2016. The most abundant, *Rhinella ornata*, comprises 30% of the total of individuals sampled. Species rarefaction curves from sampling effort stabilized for amphibians (JACK1 = 5.98 ± 0), corresponding fairly to the number of observed species. Within the sampled amphibians, five species (83%) are forest specialists and one (*Physalaemus cuvieri*) is generalist, occurring in both forest and open areas. Three species (*R. ornata, R. icterica* and *P. cuvieri*) are terrestrial while the other three (*Boana faber, Scinax hiemalis* and *Trachycephalus mesophaeus*) are arboreal. All species feed on invertebrates.

We recorded 13 species of reptiles (Table 1; Figure 3b) during fieldwork in 2016. The most abundant, the snake *Xenodon neuwiedii*, comprises 30% of the total of individuals captured. Species rarefaction curves from sampling effort did not stabilize for reptiles (JACK1 = 17.25 ± 0), indicating that at least four species were missed from our effort.

Considering the lizard species, only one (*Ophiodes fragilis*) is common in forested areas. *Notomabuya frenata* and *Tropidurus* gr. *torquatus* are common in open areas, while *Hemidactylus mabouia* and *Salvator merianae* can be found in both open and forest areas. Four species (80%) are terrestrial and only the exotic *H. mabouia* is arboreal. All species feed on invertebrates or are generalists. Amphisbaenids are common in open areas, except for *Leposternon microcephalum*. All amphisbaenids are fossorial and feed on invertebrates or elongate-shape vertebrates.

Within the sampled snakes, four species (*Liotyphlops beui, Tomodon dorsatus*, *Xenodon neuwiedii,* and *Bothrops jararaca*) are common in

Table 1. Sampled species of herpetofauna in the urban green area of the Instituto Butantan (IBu) in three periods: from 1901 to 1950, 1951 to 1989, and 1990 to current days (1 = presence; 0 = absence; NA = non-available data). The table include the methods (Fw = fieldwork; Mc = museum collection), and the following ecological attributes: habitat use (Fo = forest area; Op = open area; Ge = generalist); substrate use (Fs = fossorial; Ar = arboreal; Te = terrestrial; Aq = aquatic); and feeding habits (In = invertebrates; An = anurans; Sn = snakes; Lz = lizards; Ma = mammals; Mo = mollusks; Ew = earthworm; Ge = generalist). Note: Consider NA as presence (see Figure 2 and text).

Order/family	**Species**	**Periods**			**Methods**	**Habitat**	**Substract**	**Diet**
		1901–1950	**1951–1989**	**1990–2020**				
Anura								
Bufonidae	*Rhinella icterica*	0	0	1	Fw	Fo	Te	In
	Rhinella ornata	0	0	1	Fw	Fo	Te	In
Hylidae	*Boana faber*	0	0	1	Fw	Fo	Ar	In
	Scinax hiemalis	0	0	1	Fw	Fo	Ar	In
	Trachycephalus mesophaeus	0	0	1	Fw	Fo	Ar	In
Leptodactylidae	*Physalaemus cuvieri*	0	0	1	Fw	Ge	Te	In
Squamata								
Anguidae	*Ophiodes fragilis*	NA	NA	1	Fw	Fo	Te	In
	Ophiodes striatus	1	0	0	Mc	Ge	Te	In
Gekkonidae	*Hemidactylus mabouia*	1	1	1	Fw/Mc	Ge	Ar	In
Leiosauridae	*Anisolepis grilli*	1	0	0	Mc	Fo	Ar	In
	Enyalius perditus	NA	1	0	Mc	Fo	Ar	In
Mabuyidae	*Notomabuya frenata*	1	1	1	Mc	Op	Te	In
Teiidae	*Salvator merianae*	1	1	1	Fw/Mc	Ge	Te	Ge
Tropiduridae	*Tropidurus* gr. *torquatus*	NA	NA	1	Fw	Op	Te	In
Amphisbaenidae	*Amphisbaena alba*	NA	NA	1	Fw	Op	Fs	In, Sn, Lz
	Amphisbaena dubia	1	1	1	Fw/Mc	Op	Fs	In, Sn, Lz
	Amphisbaena mertensii	NA	NA	1	Fw	Op	Fs	In, Sn, Lz
	Leposternon microcephalum	NA	NA	1	Fw	Fo	Fs	Ew, Sn, Lz
Anomalepididae	*Liotyphlops beui*	NA	1	1	Fw/Mc	Fo	Fs	In
Dipsadidae	*Apostolepis assimilis*	NA	1	1	Mc	Op	Fs	Sn, Lz
	Dipsas mikanii	1	1	1	Fw/Mc	Ge	Te	Mo
	Erythrolamprus aesculapii	NA	1	0	Mc	Ge	Te	Sn
	Erythrolamprus poecilogyrus	NA	1	1	Mc	Ge	Te	An
	Helicops modestus	NA	NA	1	Mc	Op	Aq	Ge
	Oxyrhopus guibei	NA	1	1	Fw/Mc	Ge	Te	Ge
	Philodryas aestiva	NA	NA	1	Mc	Op	Ar, Te	Ge
	Philodryas patagoniensis	NA	1	1	Mc	Op	Te	Ge
	Thamnodynastes cf. *nattereri*	1	0	0	Mc	Fo	Ar, Te	Ge
	Tomodon dorsatus	NA	1	1	Fw/Mc	Fo	Te	Mo
	Xenodon neuwiedii	NA	NA	1	Fw/Mc	Fo	Te	An
Elapidae	*Micrurus corallinus*	NA	1	0	Mc	Fo	Fs, Te	Sn, Lz
Viperidae	*Bothrops jararaca*	NA	NA	1	Mc	Fo	Ar, Te	Ge

forested areas. Four species (*Apostolepis assimilis Helicops modestus, Philodryas aestiva,* and *P. patagoniensis)* dwell in open areas, and *Dipsas mikanii*, *Erythrolamprus poecilogyrus*, and *Oxyrhopus guibei* can be found both in open and forest areas. Six species (54%) are terrestrial, two (18%) are fossorial, two species can use both terrestrial and arboreal substrate (18%), and only *Helicops modestus* is semi-aquatic. There were no predominantly arboreal species sampled. Snakes are mostly generalists (54%). *Liotyphlops beui* feeds on invertebrates, and *D. mikanii* and *T. dorsatus* are malacophagous. *Xenodon neuwiedii* and *E. poecilogyrus* feed on anuran, and *O. guibei* and *B. jararaca* feeds on lizards and small mammals.

2. Reptiles in scientific collections and temporal comparison

We recorded 26 species of reptiles from 1901 to present (Table 1), 13 resulting exclusively from historical data, eight from both historical and fieldwork, and five species sampled exclusively in fieldwork: *Ophiodes fragilis*, *Tropidurus* gr. *torquatus*, *Amphisbaena alba*, *Amphisbaena mertensii*, and *Leposternon microcephalum*. Data from museum records

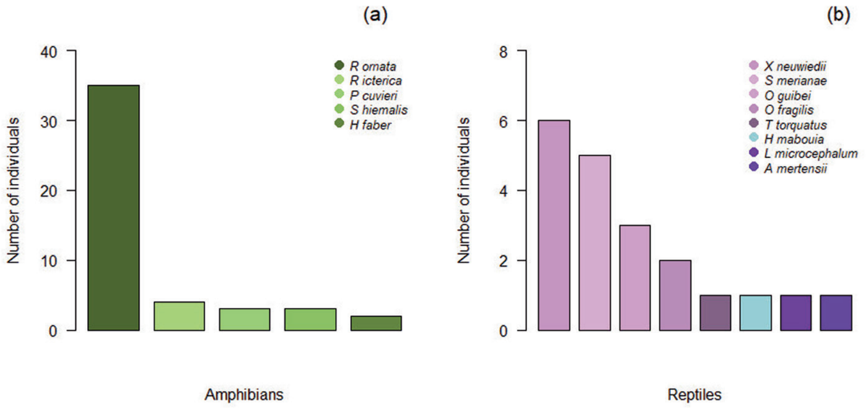

Figure 3. Current herpetofauna community of Instituto Butantan sampled in fieldwork during 2016. (a) Amphibians recorded during fieldwork and number of individuals per species sampled; (b) Reptiles recorded during fieldwork and number of individuals per species samples.

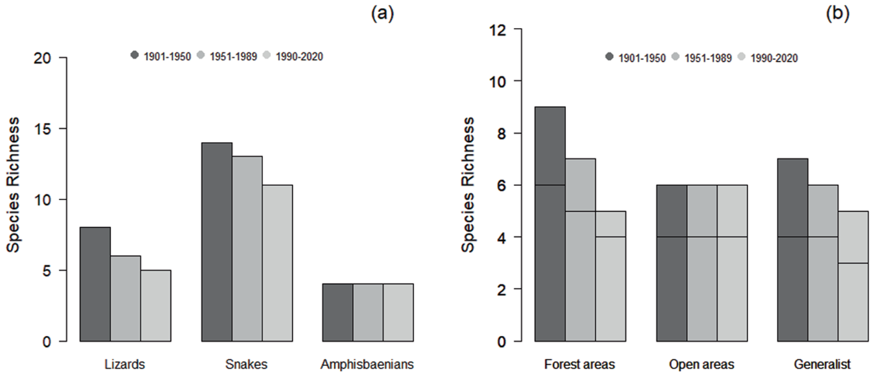

Figure 4. Reptiles species richness in three periods analyzed: 1901-1950 (dark grey), 1951-1989 (grey), 1990-2020 (light gray). (a) Species richness for lizards, snakes and amphisbaenians; (b) Species richness for lizards (upper bar) and snakes (bottom bar) in relation to the ecological attribute of habitat preference (forest areas, open areas, or generalist).

(Table 1) suggests that other seven species of reptiles can be found in current days at IBu, which exceeds the value predicted by the reptile's rarefaction curve (JACK1 = 17.25 ± 0).

The richness of reptile species decreased 23%: 26 species in the first period (1901-1950), to 22 in the second (1951-1990) and 20 in the third period (1991-current). Although the richness of amphisbaenians was constant during all periods (n = 4), species richness decreased 37,5% for lizards, and 21,4% for snakes (Figure 4a). For snakes and lizards, the depletion in species richness occurred regardless the habitat preference of each species, but it kept constant for open-area specialists (Figure 4b).

Discussion

We found six amphibians and 20 reptile species composing the current herpetofauna community at IBu. From 1906 to 2006, IBSP and MZUSP collection gathered a total of 97 species of reptiles from the city of São Paulo, being seven amphisbaenids, 19 lizards and 68 snakes (Marques et al. 2009). From 2003 to 2007, IBu received 38 species of snakes (Barbo 2008). The latest fauna inventory for the city of São Paulo registered 88 species of amphibians and 55 of reptiles sampled in 173 green areas throughout the city, including public and private UGA, water bodies and conservation units (SVMA 2022). However, the IBu area was not assessed in this inventory and our study provides two new records for the city: the lizard *Notomabuya frenata* and the amphisbaenid *Amphisbaenia mertensii*. The species richness and composition we found seem proportionally similar with other urban areas within Atlantic Forest (e.g., Citeli et al. 2016, de Oliveira et al. 2016, França and França 2019, Cavalheri et al. 2021).

The locations in São Paulo city highly sampled for amphibians are the Cantareira and the Serra do Mar State Parks, while other UGA represent sampling gaps (Malagoli 2008). One of these gap areas is the IBu. The amphibians we found are common in urban areas of the city of São Paulo (SVMA 2022), suggesting that these species tolerate environmental change (Santana et al. 2016, Lourenço-de-Moraes et al. 2018). Besides being influenced by climatic variations (Rebouças et al. 2021), declines in amphibians' richness in urban areas are associated with highly anthropic pressure, including habitat loss (Youngquist et al. 2017, Lourenço-de-Moraes et al. 2018; Ganci et al. 2020), low connectivity between fragments (Metzger et al. 2009), and topography of the surrounding matrix (Ribeiro et al. 2018). The low amphibian's richness observed in IBu possibly is related to habitat loss, and to environmental changes in the fragment during urbanization, as already documented in other UGA in the city of São Paulo, for example in the Parque Estadual Fontes do Ipiranga (Lisboa et al. 2021).

Lizards and other snakes sampled in IBu are mostly terrestrial and prey items that seem common in urban environments, which may favor their persistence in UGA. The lizards *O. fragilis, H. mabouia* and *N. frenata* feed on a wide variety of arthropods that occur in undergrowth or herbaceous vegetation (Vrcibradic and Rocha 1998, Bonfiglio et al. 2006, Montechiaro et al. 2011), while *T.* gr. *torquatus* has a diet composed mainly by invertebrates, and occasionally small vertebrates and plants (Teixeira and Giovanelli 1999). *Salvator merianae* is a generalist species with a diversified diet based (Vanzolini et al. 1980, Kiefer and Sazima 2002, Mourthé 2010). *Oxyrhopus guibei* and *B. jararaca* has an ontogenetic shift on diet, and juvenile feeds on lizards while adults feed on small rodents (Andrade and Silvano 1996, Marques et al. 2019). *Dipsas mikanii* and *T. dorsatus* are malacophagous (Bizerra et al. 2005, Marques et al. 2019). *Xenodon neuwiedi* is an anuran-eater (Sazima and Haddad 1992; Marques et al. 2019) and feed mainly on bufonid genus *Rhinella*, including *R. ornata* (Vaz and Chinchilla 2019), the most abundant anuran in IBu.

Fossorial or cryptozoic species (amphisbaenians and snakes) represented 30% of the total reptiles sampled. Considering the historical records, it seems that only one species (*Micrurus corallinus*) was locally extinct or missed from our sampling effort. The snake *A. assimilis* feeds on elongated-shape vertebrates (Ferrarezzi et al. 2005, Marques et al. 2019). Amphisbaenians and the snake *L. beui* are mostly dependent on invertebrates, including earthworm, larvae and pupae of ants (Parpinelli and Marques 2008, Marques et al. 2019). Such prey items are common throughout urban environments (e.g., Pacheco and Vasconcelos 2007, Peng et al. 2020). Although fossorial species may be underestimated (Barbo and Sawaya 2008), their lack of depletion over time suggests that this fauna can remain in small fragments. Thus, subterranean reptiles seem resistant to environmental changes. Additional studies on underground reptiles are still needed to assess the real trend of the impact on this type of fauna due to the loss of habitat and urbanization (cf. Böhm et al. 2013).

Six reptile species, being three lizards and three snakes, were either locally extinct or missed from our sampling effort. *Ophiodes striatus, Anisolepis grilli* and *Thamnodynastes* cf. *nattereri* were recorded only in the first period (from 1901 to 1950), and *Enyalius perditus, Erythrolamprus aesculapii* and *Micrurus corallinus* were recorded only in the second period (from 1951 to 1990). The snakes *T.* cf. *nattereri* and *M. corallinus* are usually found in well-preserved and continuous forests (e.g. Forlani et al. 2010, Giraudo et al. 2012), not the current condition of the IBu. Records available for *A. grilli* and *E. aesculappi* in the city of São Paulo show that these species occur only in continuous forests surrounding the city (SVMA 2022). Therefore, these four species could be locally extinct. However, *O. striatus* and *E. perditus* can be found in urban areas and disturbed habitats, including other UGA in the city of São Paulo (Lisboa et al. 2021, SVMA 2022), indicating that these species might have been missed despite our effort rather than be locally extinct.

Amphibians and reptiles are well-sampled groups in the Brazilian Atlantic Forest, and the urban herpetofauna is becoming a target research subject over the years (e.g., Citeli et al. 2016, Lourenço-de-Moraes et al. 2018, Almeida-Correa et al. 2020, Ganci et al. 2021). Urban environments are typically characterized by lower biodiversity when compared to natural environments (Marzluff 2001; Chace and Walsh 2006). Because the major threat to reptiles and amphibians is habitat loss (Gibbons et al. 2000, Böhm et al. 2013, Vilaseñor et al. 2017), a low diversity of these groups is expected in UGA. For reptiles of different remnants of the Atlantic Forest, the fragment area may be the main predictor for species richness, but matrix quality as well as fragment shape may also be important (Lion et al. 2016). Besides, the likelihood of colonization of new species or exchange with other green areas is restricted due the low connectivity among forest fragments (Laurance et al. 2011), and the intrinsic low dispersion of these animals. Our results show that there was a decline in the species richness of surface reptiles during a temporal gradient, and we did not observe a different pattern of species loss when analyzing ecological attributes separately. Therefore, we believe that the species currently found in IBu were likely present in the past and persisted over changes in land-use.

In this work we evaluate the current herpetofauna community of an urban green area, comparing the current reptile's assemblage with historical data in a temporal scale. Taxa observed are mainly terrestrial, and feed on prey commonly found in UGA, and these traits can favor their persistence within urban environments. In the temporal comparison, there was a general decline in reptiles' richness regardless of ecological attributes, possibly resulting of loss of habitable area in Instituto Butantan. Fossorial reptiles are the group that most resisted habitat loss, with only one species locally extinct. We suggest that future studies keep monitoring this community, investigating other natural history and morphology traits for sampled species, aiming to record possible local adaptations in comparison with populations living in natural areas.

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Authors' Contribution

Eletra de Souza: conceptualization, resources, methodology, writing – original draft, writing – review & editing.

Jade Lima-Santos: conceptualization, resources, methodology, writing – review & editing.

Otavio Augusto Vuolo Marques: resources, writing – review & editing.

Erika Hingst-Zaher: conceptualization, resources, writing – review & editing.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

The datasets generated during and/or analyzed during the current study are available at: https://datadryad.org/stash/share/6qnNreavbbAehOPpBD4-FTY3iWGucvdDgZU2iKIrS0o

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Floristic data to support conservation in the Amazonian *canga*

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SILVA, L.G., LOVO, J., FONSECA-DA-SILVA, T.L., RIUL, P., SILVA-LUZ, C.L., ZAPPI, D.C. **Floristic data to support conservation in the Amazonian *canga***. Biota Neotropica 23(4):e20231517. https://doi.org/10.1590/1676-0611-BN-2023-1517

Abstract: *Canga* is an environment of great natural and economic value because it harbours a considerable number of endemic species on a substrate that is rich in iron ore. In the Amazon, this open vegetation type grows on top of isolated outcrops in a dense forest matrix found in the Carajás region, in southeastern Pará. Of these outcrops, the Parque Nacional dos Campos Ferruginosos (PNCF) is the only area of Amazonian *canga* with a strict protection status. Therefore, industrial activity in the region needs to implement mitigation actions to ensure species and habitat conservation. The objective of this study is to complement and review the floristic list of this recently created protected area, enabling us to compare the floristic similarity between it and other 14 Amazonian *canga* outcrops found outside the conservation units of full protection in the region. This data provides a basis to understand the floristic and phylogenetic complementarity of those patches to support conservation action. For this, six field trips were carried out in the Serra da Bocaina and two in the Serra do Tarzan, respectively, in order to increase the sampling efforts in PNCF and to obtain a more comprehensive plant list. Floristic composition was investigated using multivariate analyses (*non-metric multidimensional scaling and unweighted pair group method with arithmetic mean*) and phylogenetic structure across studied areas. We added 159 species to the floristic list of the PNCF and the results of the analyses showed that all 16 areas (n.b. PNCF comprises two of these sites) have an overall floristic similarity of 42%, with the least similar areas at 35% and the most similar at 50%. The different micro-habitats found in each study site highlight the high beta diversity of the Amazonian *canga* sites, making each area unique. Therefore, even if the Parque Nacional dos Campos Ferruginosos does not harbour all the species found in the other Amazonian *canga* sites, it is strategic for the conservation of the vegetation on ferruginous outcrops in the Amazon, protecting its biodiversity, different habitats, and associated ecosystem services.
Keywords: *edaphic endemismo; floristic list; multivariate analyses; Parque Nacional dos Campos Ferruginosos; phylogenetic structure.*

Dados florísticos para apoiar a conservação nas cangas amazônicas

Resumo: Canga é um ambiente de grande valor natural e econômico por abrigar um número considerável de espécies endêmicas sobre substrato rico em minério de ferro. Na Amazônia, esse tipo de vegetação aberta cresce sobre afloramentos isolados em uma matriz de floresta densa encontrada na região de Carajás, no sudeste do Pará. Dentre esses afloramentos, o Parque Nacional dos Campos Ferruginosos (PNCF) é a única área de canga Amazônica que apresenta *o status* de proteção integral permanente. Dessa forma, a atividade industrial presente na região necessita implementar ações de mitigação para assegurar a conservação de espécies e habitats relacionados às cangas. O objetivo deste estudo é complementar e revisar a lista florística dessa área protegida, recentemente criada, permitindo comparar a sua similaridade florística com outros 14 afloramentos de cangas Amazônicas localizados fora de unidades de conservação de proteção integral encontradas na região. Tais dados fornecem subsídio para entender a complementaridade florística e filogenética desses fragmentos para apoiar ações de conservação.

Para isso, foram realizadas seis viagens de coleta à Serra da Bocaina e à Serra do Tarzan, respectivamente, para aumentar o esforço amostral no PNCF e obter uma lista de plantas mais abrangente. A composição florística foi investigada por meio de análises multivariadas (*non-metric multidimensional scaling and unweighted pair group method with arithmetic mean*) e estrutura filogenética nas áreas estudadas. Nós adicionamos 159 espécies na lista florística do PNCF e os resultados das análises demonstraram que todas as 16 áreas (n.b. o PNCF compreende duas dessas áreas) têm uma similaridade florística total de 42%, com áreas menos similares de 35% e as mais similares de 50%. Os micro-habitats encontrados em cada área de estudo evidenciam a alta diversidade beta das áreas de cangas Amazônicas, o que as tornam únicas. Portanto, ainda que o Parque Nacional dos Campos Ferruginosos não abrigue todas as espécies encontradas em outras áreas de cangas Amazônicas, ele é estratégico para a conservação dos afloramentos ferruginosos na Amazônia, protegendo a sua biodiversidade, os diferentes habitats e os serviços ecossistêmicos associados.

Palavras-chave: *análises multivariadas; composição florística; endemismo edáfico; estrutura filogenética; Parque Nacional dos Campos Ferruginosos.*

Introduction

The creation of protected areas is a global strategy to reduce biodiversity loss and to maintain ecosystem services (Soares et al. 2010, Yang et al. 2021), being one of the 2011-2020 Aichi Targets for halting biodiversity loss (CBD 2013). Recent years have seen an increase of the number of protected areas, and currently, 15.4% of the Earth's surface is protected (UNEP-WCMC 2020). In addition to increasing the number of protected areas, it is also fundamental to invest in their management so that they can contribute significantly to curbing biodiversity loss (Laurance et al. 2012, Geldmann et al. 2015, 2019, Yang et al. 2021). Despite a number of programmes and initiatives towards conserving nature having been created in Brazil (Coelho 2018), detailed knowledge of the organisms protected by parks and reserves is still scant (Moreira et al. 2019, 2020, Oliveira et al. 2017), hindering the advance of more targeted prioritization of areas based in species rarity or genetic distinctiveness (Tucker et al. 2012).

The IUCN provides guidelines on how to implement and develop protected areas by presenting them as six categories: I. Strict protection (Ia. Strict Nature Reserve, Ib. Wilderness Area), II. Ecosystem Conservation and Protection (National Parks), III. Conservation of Natural Features (Natural Monument), IV. Conservation through active Management (Habitat/species management area), V. Landscape/Seascape Conservation and Recreation (Protected Landscape/Seascape), VI. Sustainable use of natural resources (Managed resource protected area) (Dudley 2008). In Brazil, protected areas are known as *Unidades de Conservação* (UC) and have specific legislation under the SNUC law (Brasil 2000), being divided into full protection, such as National Parks (IUCN category II) and sustainable use, as National Forests (VI) (Dudley 2008, ICMBIO 2016a). In addition to the Brazil's UC system, there are other categories of protected areas such as the Indigenous and Quilombola (Brazilian afro-descendent community) lands (Brasil 2006) and Legal Reserves, represented by a fraction of land located inside a rural property that must legally maintain the original native vegetation (Brasil 2012). Currently, 26.6% of the Brazilian Amazon is protected by federal UCs (Fundo Amazônia 2023). It is important to note that this percentage is underestimated as it does not consider state and private conservation units, for example the *Reservas Particulares do Patrimônio Natural* (RPPN).

The protected biodiversity within the Amazonian UCs is still poorly known due to the scarcity of faunistic and floristic inventories in the region (Cardoso et al. 2017, Oliveira et al. 2017). The lack of information regarding the Amazon is related in part to the large extension of this biome, mostly covered by rainforest, but that also includes open vegetation, which comprises approximately 5% of the Brazilian Amazon territory (Devecchi et al. 2020).

In contrast to its relatively small range, recent studies carried out in some of these habitats (Mota et al. 2018, Zappi et al. 2019, Andrino et al. 2020, Devecchi et al. 2020, Fonseca-da-Silva et al. 2020) have been pointing that the open vegetation types occupying these small areas in the Amazon are clearly relevant in terms of biodiversity distinctness, forming a scenarium similar to island habitats (Prance 1996). One example of such insular vegetation is the Amazonian *canga*, which resembles a savanna (despite being different, see Devecchi et al. 2020) but consists of open vegetation growing on iron substrate (Giulietti et al. 2019, Andrino et al. 2020). These steppingstone-like outcrops of *canga* are scattered over an area that is 250 km long east to west, isolated by a matrix of rain forest, and comprises less than 150 km^2, representing c. 0.003% of the Brazilian Amazon. This biodiverse vegetation harbours edaphic endemic plants, including three endemic genera and 38 endemic species (Giulietti et al. 2019), and is fully protected only by the Parque Nacional dos Campos Ferruginosos (PNCF) (IUCN category II). This area has been offered as environmental compensation, a mechanism of Brazilian Legislation (Brasil 2000) aiming to recover the loss of *canga* areas caused by mining within the Floresta Nacional (FLONA) de Carajás (IUCN category VI). The PNCF encompasses an area of 28 km^2 of *canga* in two different blocks, the Serra da Bocaina and the Serra do Tarzan (Figure 1). The FLONA de Carajás is a sustainable use unit (IUCN category VI) and was created with the purpose of managing research, mining, processing, transport and distribution of mineral resources, with Companhia Vale do Rio Doce (VALE) as the holder of technical information on the area, implementer and operator of existing economic activities and beneficiary of relevant environmental licenses (Brasil 1998). This area includes a large mining operation surrounded by pristine rainforest, where the original *canga* outcrops once covered c. 105 km^2, however, satellite images obtained in 2016 highlighted that 28.3 km^2 of the *canga* was suppressed after three decades of exploitation (Souza-Filho et al. 2019). Placed within a mosaic of especially relevant UCs for the conservation of biodiversity, the PNCF is strategically located at the edge of a region known as the "deforestation arc" (Fearnside & Graça 2009, Domingues & Bermann 2012), with the highest rates of deforestation in the Amazon, as a result of vegetation suppression and heavy impact in adjacent areas

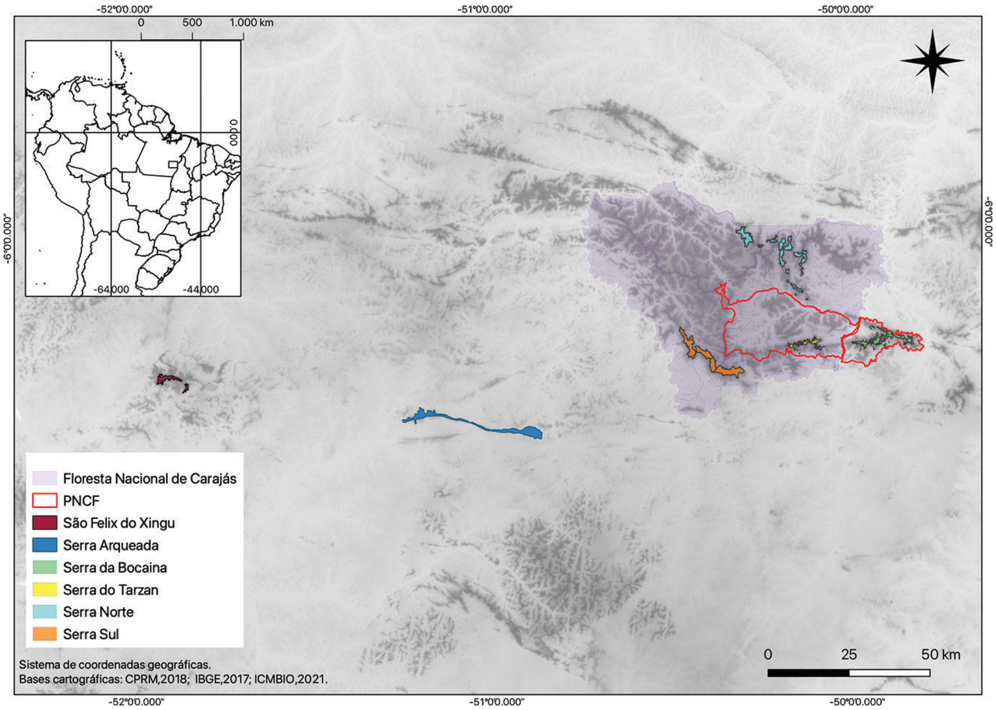

Figure 1. Geographic location of the Parque Nacional dos Campos Ferruginosos (PNCF) and other study sites (Map created by the authors with QGIS 3.18.1-Zürich © 2002-2019 QGIS Development Team available at https://www.qgis.org).

caused by agricultural development and urbanization (Mota et al. 2015, Souza-Filho et al. 2016).

Canga substrate is characterized by a high concentration of heavy metals, such as iron and manganese (Skirycz et al. 2014), high ground temperatures, and low pH, in addition to a high incidence of sunlight and seasonally low water availability that may last several months, enforcing limitations for the establishment of the plant community (Oliveira et al. 2015, Carmo & Jacobi 2016, Vasconcelos et al. 2016).

The high diversity and endemism (Viana et al. 2016, Mota et al. 2018, Giulietti et al. 2019, Zappi et al. 2019) indicate that species inhabiting *canga* have particular mechanisms, possibly adaptations, that allow their survival in such hostile environment (see Porto & Silva 1989, Jacobi et al. 2007, Zappi 2017). The vegetation on the *canga* of Carajás was studied by the Project "*Flora das Cangas da Serra dos Carajás*" (FCC), (Viana et al. 2016) that recorded and described 1131 plant species, including 89 bryophytes (Oliveira-da-Silva & Ilkiu-Borges 2018), 175 ferns, 11 lycophytes (Salino et al. 2018) and 856 seed plants (Mota et al. 2018).

Besides the PNCF and the FLONA de Carajás, there are also *canga* outcrops in the region that are not under any form of legal protection, such as the Serra Arqueada, located in Ourilândia do Norte and the Serra de Campos, near the town of São Félix do Xingu, both with published floristic lists (Andrino et al. 2020, Fonseca-da-Silva et al. 2020). These recent studies show that the distinct *canga* outcrops of Carajás have markedly different floristic composition. Therefore, the suppression of individual *canga* areas, which potentially leads to the imminent loss of part of the species richness (Zappi et al. 2019) found therein, must respect Brazilian legislation (Brasil 2000) that pursues No net loss (NNL) of species. In the core of the concept of NNL there is the idea of seeking for minimal loss of biodiversity while coping with social and economic prosperity (Ermgassen et al. 2019). Although debates concerning their potentials and limitations (Maron et al. 2020, Sonter et al. 2014, 2020, BBOP 2012), governments and industries accross the planet make use of different strategies, mostly offsets aiming to achieve NNL (Ermgassen et al. 2019).

Our major aim is to complement and review the floristic list of this recently created protected area, enabling us to compare the floristic similarity between it and other 14 Amazonian *canga* outcrops found outside the conservation units of full protection in the region. This data provides a basis to understand the floristic and phylogenetic complementarity of those patches to support conservation action. It is a major concern that PNCF reflects the overall plant diversity of Amazonian *canga*, as it is the only strictly protected area for this type of vegetation in the Amazon. We increased the sampling efforts in PNCF to obtain a more comprehensive plant list that would guide conservation practitioners.

Material and Methods

1. Study site

The PNCF located in southeastern Pará includes two *canga* areas on hilltops: Serra da Bocaina and Serra do Tarzan. Serra da Bocaina encompasses nearly 19.98 km^2 of *canga* outcrop at 770 meters a.s.l. surrounded by lowland forests and pastures. The *canga* outcrop can be accessed by the road PA 160 that links the city of Parauapebas to Canaã dos Carajás, entering Vila Sedere I, turning towards the main entrance of the PNCF that is located at coordinates 6°16'59.7''S, 49°58'16.2''W. Serra do Tarzan spreads over nearly 8.3 Km^2 of *canga* at 750 meters a.s.l., surrounded by well-preserved lowland forest formations. Access to the *canga* is gained by the PA 160 road from the town of Canaã dos Carajás towards the road that leads to SS11D (an iron ore mine). Serra do Tarzan's entrance is located at coordinate 6°23'12''S, 50°6'38''W. According to the classification of Köeppen, the climate in the region is tropical (Aw) (Alvares et al. 2013). The rainy season occurs between November and April with monthly mean precipitation of 229 mm, while the dry season occurs between June and September with monthly mean precipitation of 34 mm (ICMBIO 2016a).

Two main vegetation types can be recognized: the rock-dwelling, or *rupestre* ferruginous vegetation and the hydromorphic formations (Mota et al. 2015), see Table 1. The *rupestre* ferruginous vegetation includes shrubby vegetation on or amongst rocks, *campo rupestre* on *canga couraçada*, *campo rupestre* on nodular *canga* and low forest. The hydromorphic vegetation consists of swampy forests, temporary and perennial lagoons, intermittent watercourses, and *buriti* palm (*Mauritia flexuosa* L.) grooves. In addition to these, there are lowland forests associated to the ferruginous mountain ranges. The small heterogeneous environments resulting from the interactions of different substrate types, nutrients, relief, water availability, and vegetation may be recognized as micro-habitats (Jacobi et al. 2007, Alvares et al. 2013, Mota et al. 2018), see Table 1. All collections were conducted under current Brazilian legislation (permission nº 6332401, ICMBIO), processed using standard herbarium techniques (Mori et al. 2011), and deposited at Emilio Goeldi Museum (MG herbarium) in Belém, Pará, Brazil. Voucher material information can be found in the Table S1, Supplementary Material.

Before the creation of the PNCF, the Serra da Bocaina was surrounded by farmland (ICMBIO 2016b) while the Serra do Tarzan, on the other hand, was less exposed to anthropogenic disturbance as its boundary was set within the FLONA de Carajás, created 24 years ago, when the region was pristine (ICMBIO 2016b). Currently, with the expropriation of land around the Serra da Bocaina to integrate the PNCF, the surroundings of this outcrop are undergoing forest regeneration (ICMBIO 2017). Although the flora of the PNCF has been studied in the FCC, this area was undersampled for historic reasons. While sampling efforts were directed towards areas that were licensed or being licensed for mining (Mota et al. 2018), the accesses to the Serra da Bocaina and Serra do Tarzan were very difficult due to roads blocked by fallen trees.

Table 1. Two main types of vegetation are recognized in Amazonian *canga* including their sub-types (following Mota et al. 2015 and Andrino et al. 2020).

Types of vegetation	Sub-types
Rupestre ferruginous vegetation	shrubby vegetation on/or amongst rocks
	campo rupestre on *canga couraçada* or open *canga* slabs
	campo rupestre on nodular *canga* or nodular *canga*
	low forest or *capão de mata*
Hydromorphic vegetation	swamp forests
	temporary lagoons
	perennial lagoons
	intermittent water courses
	Buriti grooves (or Palm swamps)

2. *Plant collections and identification*

Additionally to the pre-existing collections made by the Flora de Carajás project (Viana et al. 2016), six field trips were carried out from September 2018 to February 2020 to the Serra da Bocaina, while the Serra do Tarzan was visited in July 2019 and August 2019. The field trips were performed both during the dry and rainy season in the two areas in order to collect fertile specimens of spermatophyte. We followed the methodology of Filgueiras et al. (1994), making non-systematics walks and collecting spermatophyte specimens in the different vegetation types listed by Mota et al. 2015 (Table 1) associated with the ferruginous *canga* of the study area. This methodology has been used successfully in floristic inventories (Andrino et al. 2020) in *canga* and elsewhere as it allows covering large areas while focusing on fertile material. Given the rarity of some of the species found in the *canga* (Giulietti et al 2019), this method aims to locate small populations occurring in habitats that are hard to sample (rock faces, forest understorey, water courses).

The material was identified by the authors using specific bibliography and comparison with MG herbarium specimens. Moreover, for specific families, plant specialists (referred to in the Acknowledgments session) have been contacted to help with identification (e.g. Myrtaceae, Cyperaceae, Poaceae, etc.).

To prepare our dataset we added the new collections to the final database of the FCC project, which had already listed 230 species for Serra da Bocaina and 228 species for Serra do Tarzan, totalling 351 species for PNCF. We also updated species lists from other areas of *canga* of the FLONA de Carajás including Serra Norte and Serra Sul (Mota et al. 2018), the floristic lists of Serra Arqueada (Fonseca-da-Silva et al. 2020) and Serra de Campos de São Félix do Xingu (Andrino et al. 2020). All floristic lists were added to the Plotsamples module of our Brahms (BRAHMS7 2018) database.

3. *Estimates of floristic sampling on Amazonian canga*

We estimated the completeness of our Amazonian *canga* inventory by performing a rarefaction to assess sampling coverage (Chao & Jost 2012), similarly to those performed in related work (Zappi et al. 2019) using iNEXT package (Hsieh et al. 2016) in R (R Core Team 2022). We considered each of the 16 mountaintops as a sampling site (Figure S2, Supplementary Material).

4. *Floristic and phylogenetic analyses*

The floristic lists of the studied canga were organized according to the respective localities for biogeographical comparisons: FLONA de Carajás Serras Norte (CRJ-SN) and Sul (CRJ-SS). Parque Nacional dos Campos Ferruginosos (Serra da Bocaina and Serra do Tarzan: PNCF-SB and PNCF-ST, respectively), Serra de Campos de São Félix do Xingu (SFX) and Serra Arqueada (ARQ-CAN). Moreover, each Serra of FLONA Carajás was subdivided as follows: Serra Norte: CRJ-SN1, CRJ-SN2, CRJ-SN3, CRJ-SN4, CRJ-SN5, CRJ-SN6, CRJ-SN7, CRJ-SN8; Serra Sul: CRJ-SS11A, CRJ-SS11B, CRJ-SS11C, CRJ-SS11D. The 16 studied sites and their corresponding area code, as well as the number of species at each site, are presented in Table 2.

A total of five species classified as aliens or invasives according to Giulietti et al. (2018) collected at SB were removed from the analysis: *Melinis minutiflora* P.Beauv., *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs, *Cenchrus polystachios* (L.) Morrone,

Table 2. Amazonian *canga* study sites. Numbers in parenthesis correspond to edaphic endemic species.

Code	Area	Area Km^2	Nº of species	Major areas	Species total
ARQ-CAN	Serra Arqueada	1.27Km^2	146	Serra Arqueada	146
CRJ-SS11A	Serra dos Carajás – Serra Sul 11 A	15.27Km^2	184	Serra Sul	539 (3)
CRJ-SS11B	Serra dos Carajás – Serra Sul 11 B	8.44Km^2	208		
CRJ-SS11C	Serra dos Carajás – Serra Sul 11 C	6.26Km^2	185		
CRJ-SS11D	Serra dos Carajás – Serra Sul 11 D	16.41Km^2	434		
CRJ-SN1	Serra dos Carajás – Serra Norte 1	11.81Km^2	388	Serra Norte	647 (3)
CRJ-SN2	Serra dos Carajás – Serra Norte 2	0.86Km^2	130		
CRJ-SN3	Serra dos Carajás – Serra Norte 3	2.1Km^2	222		
CRJ-SN4	Serra dos Carajás – Serra Norte 4	14.83Km^2	312		
CRJ-SN5	Serra dos Carajás – Serra Norte 5	8.26Km^2	294		
CRJ-SN6	Serra dos Carajás – Serra Norte 6	0.97Km^2	100		
CRJ-SN7	Serra dos Carajás – Serra Norte 7	0.34Km^2	114		
CRJ-SN8	Serra dos Carajás – Serra Norte 8	2.69Km^2	104		
PNCF-SB	Parque Nacional dos Campos Ferruginosos – Serra da Bocaina	19.98Km^2	408	Parque Nacional dos Campos Ferruginosos	560 (4)
PNCF-ST	Parque Nacional dos Campos Ferruginosos – Serra do Tarzan	8.3Km^2	333		
SFX	Serra de Campos – São Félix do Xingu	9.04Km^2	246	Serra de Campos	246 (1)

Leonotis nepetifolia (L.) R.Br., and *Urena lobata* L. (S1, Supplementary Material). Taxa not identified at species level were not included in the analyses as well, while new species, currently under publication were kept in both analyses and the spreadsheets, e.g. *Diastema* sp. (Chautems et al. 2018) and *Croton* sp. (Costa et al. 2018).

The species names were standardized following internal and automatic dictionaries of Brahms and also the online tool Plantminer (Carvalho et al. 2010). Once we had the correct scientific names we transformed the final list into a presence and absence matrix (Table S4, Supplementary Material), showing which species occurred at each site.

The data matrix was analyzed in the Past 4.04 software (Hammer et al. 2001) to carry out multivariate analyses using ordination — Non-metric multidimensional scaling (NMDS), and clustering methods — Unweighted Pair Group Method mean (UPGMA), both analyses using Sørensen (Bray-curtis) distance. Moreover, comparative analyses were performed with the online tool jvenn (Bardou et al. 2014), making Venn diagrams to verify the floristic overlap between the studied areas.

We reconstructed a phylogenetic tree with all the species collected across all the areas with the purpose to compare the phylogenetic structure of *canga* species in PNCF and other areas in Carajás. The species list was extracted from the matrix used for biogeographic analysis (S1, Supplementary Material). Names of subspecies and varieties were transformed as follow: Mimosa_acutistipula_var_ferrea changed to Mimosa_acutistipula.ferrea. The list was formatted for uploading into Phylocom 4.2 (Webb et al. 2008) using Plantminer (Carvalho et al. 2010). To reconstruct our phylogenetic tree we used the megatree R20160415.new (Gastauer & Meira Neto 2017) and calibrated with ages estimate proposed by Magallón et al. (2015). The regional tree obtained from our species list and their distribution across the different areas was visualized using iTol (Letunic & Bork 2016). We highlighted selected families with the greater number of species and a few others for discussion.

Results

1. Flora of the PNCF

We collected 410 additional specimens representing 225 species, 178 genera, and 81 families in the *canga* of the PNCF. Our floristic survey added 158 species to the list published in 2018 (Mota et al. 2018). There were specifically 119 new species records for Serra da Bocaina and 39 new records for Serra do Tarzan. Serra da Bocaina currently has 408 listed species while Serra do Tarzan has 333 listed species. Thus, PNCF currently has 559 angiosperms species listed and one gymnosperm species — *Gnetum nodiflorum* Brongn. — totalling 560 species (Table 2; Table S1, Supplementary Material). Some species that represent new records for the PNCF are illustrated in Figure 2.

The 10 families with the greater number of species correspond to 45% of the total sampling for the PNCF. They are Poaceae (53 spp.), Fabaceae (52 spp.), Cyperaceae (36 spp.), Rubiaceae (35 spp.), Asteraceae (20 spp.), Melastomataceae (19 spp.), Convolvulaceae and Solanaceae (13 spp.), Lamiaceae and Malvaceae (12 spp.) (S2, Supplementary Material). We also highlighted a noticeable increase in the sampling of three families, now with double or more species than in the previous list (Mota et al. 2018): Euphorbiaceae (16 spp.), Myrtaceae (16 spp.) and Orchidaceae (12 spp.).

We recorded a further edaphic endemic species (i.e. endemism associated to the type of substrate) to the Serra do Tarzan — *Erythroxylum carajasense* (Plowman) Costa-Lima [Erythroxylaceae] — this area now has 23 listed edaphic endemic species (Giulietti et al. 2019). In the Serra da Bocaina we recorded three extra edaphic endemic species: *Anemopaegma carajasense* A.H.Gentry ex Firetti-Leggieri [Bignoniaceae]*, Syngonanthus discretifolius* (Moldenke) M.T.C.Watan.z , [Eriocaulaceae] and *Peperomia albopilosa* D.Monteiro [Piperaceae], increasing to 26 the number of edaphic endemic species listed in this area (S1, Supplementary Material). Our survey also recorded four new occurrences for Pará state and Amazonian Brazil: *Croton gracilipes* Baill. [Euphorbiaceae], *Gurania eriantha* (Poepp. & Endl.) Cogn. [Curcubitaceae], *Sabicea grisea* Cham. & Schltdl [Rubiaceae], and *Triphora uniflora* A.C.Ferreira, Baptista & Pansarin [Orchidaceae].

2. Sampling cover for Amazonian canga

The specimen number studied for PNCF (650 for the Serra da Bocaina and 500 for the Serra do Tarzan) represent comparable sampling to the other 14 outcrops (12 sites inside the FLONA de Carajás and two outside), which vary between 1699 and 176 specimens per sampled site, depending in the collecting effort and size of the outcrop, with a mean of 565 specimens collected per site. The rarefaction curve (Figure S3, Supplementary Material) indicates that we have a high sample coverage (nearly 90%) for Amazonian *canga*, considering all outcrops.

3. Floristic and phylogenetic similarity among Amazonian canga sites

The matrix used for floristic comparisons included 1021 species (a total of 3807 records) belonging to 16 areas (Table 2; Table S4, Supplementary Material). Our results revealed that 140 species are exclusive from PNCF compared with the other studied *canga* sites, corresponding to 14% of the total sampling in the matrix (S1, Supplementary Material), and 25% of the PNCF flora itself (Figure 3).

We found 61 species shared with Serra Norte, 42 species with Serra Sul, four species with ARQ-CAN, and six species with SFX (Figure 3). When the four *canga* areas are compared separately with the PNCF, our results showed 65% of SFX flora is also present in PNCF, representing the greater overlap. Following that, Serra Sul shares 58% of its flora with PNCF, Serra Arqueada 56%, and Serra Norte 54% (S3, Supplementary Material). Analysing specifically the list from PNCF, SB, and ST have 181 overlapping species, corresponding to respectively 44.47% and 54.35% of the flora of each area (Figure S5, Supplementary Material).

The UPGMA analysis resulted in assemblages with a cophenetic correlation of 0.9548 (Figure 4a). Serra Arqueada appears as the most dissimilar area, outside two major clusters formed by the other sites. A bigger group formed at a mean similarity level of ca. 0.35 includes PNCF, Serra Sul, SFX, and part of Serra Norte, while a smaller cluster is formed by the remaining Serra Norte sites. In the NMDS analysis with a stress of 0.11549, (Figure 4b) we found that areas CRJ-SN1 and CRJ-SN4 had the smallest relative distance from each other. Serra do Tarzan had a smaller relative distance with areas CRJ-SN1, CRJ-SN3, CRJ-SN4, CRJ-SN5, and CRJ-SS11D than with Serra da Bocaina. ARQ-CAN showed again the greatest relative distance from the other sites, followed by SFX, being both not located within any officially protected area.

While in the NMDS (Figure 4b) ST appears more or less equally distant from SB and part of Serra Norte, (CRJ-SN1 – see Table 2 for abbreviations), the UPGMA analysis (Figure 4a) indicates that the two first sites have a greater similarity of c. 50%, and part of Serra Norte is less similar with both ST and SB (c. 42%). In fact, the other blocks of Serra Norte (CRJ-SN2, 6-8, Figure 4a) are even more dissimilar, with c. 35% of similarity in relation to other *canga* areas (except Serra Arqueada).

Our Amazonian *canga* megatree allowed us to visualise a spread of lineages across different areas (Figure 5). As a general pattern, a coinciding representation of lineages was seen in the PNCF and the FLONA de Carajás (Serra Norte and Serra Sul). The same major clades also appear in SFX and ARQ-CAN, however with less diversity. Nonetheless, some frequent lineages occurring in other areas are not well represented in SFX and ARQ-CAN, such as Cyperaceae, Poaceae, and Asteraceae. Some other clades did not present a strict correlation of lineages across all areas. For example, magnoliids and Alismatales appear more consistently represented in Serra Norte and Serra Sul (FLONA de Carajás) than in PNCF, ARQ-CAN, and SFX. Orchidaceae and Poaceae are also better represented in FLONA de Carajás, with several small different lineages absent in the Parque (PNCF). Commelinales is under-represented in the PNCF when compared with FLONA de Carajás. Zingiberales are better represented in the PNCF than elsewhere. Regarding the eudicots, Santalales and Polygonaceae are slightly better represented outside PNCF. The asterid clade (Asteraceae, Convolvulaceae, Solanaceae, Apocynaceae) is roughly equally represented in FLONA and PNCF while within rosids there are a few missing clades of Fabaceae, Melastomataceae, and Sapindaceae in the PNCF.

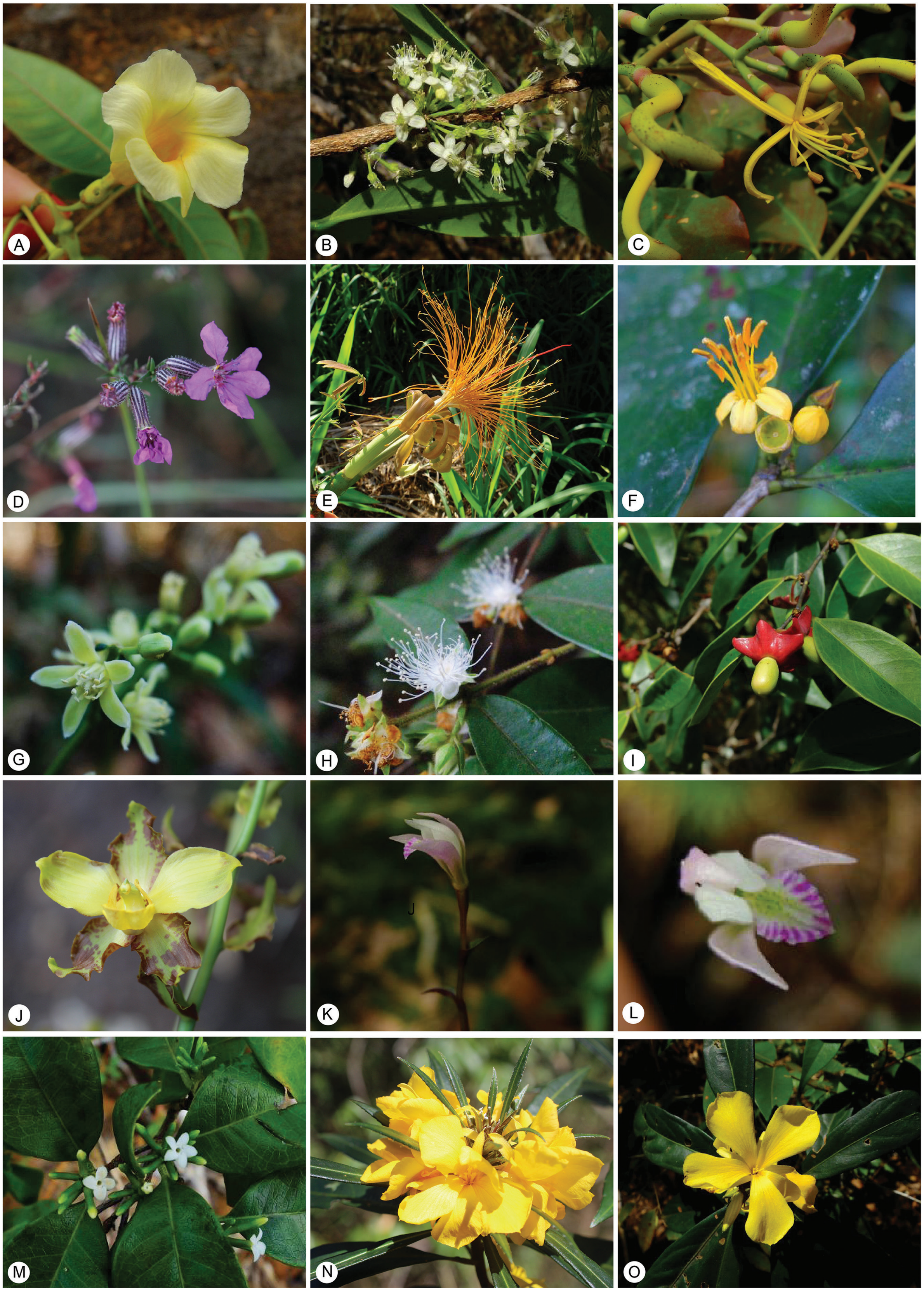

Figure 2. Species found in the Parque Nacional dos Campos Ferruginosos (PNCF), a. *Odontadenia* nitida (Vahl) Müll.Arg., *B,T, b. *Erythroxylum carajasense* (Plowman) Costa-Lima, c. *Psittacanthus eucalyptifolius* (Kunth) G.Don, *B, d. Cuphea *carajasensis* Lourteig, e. *Pachira paraensis* (Ducke) W.S.Alverson, *B, f. *Mouriri cearensis* Huber, *T, g. *Trichilia* micrantha Benth., h. *Myrcia bracteata* (Rich.) DC., *B, i. *Heisteria ovata* Benth, *T, j. *Cyrtopodium andersonii* (Lamb. ex Andrews) R.Br., k-l. *Triphora uniflora* A.C.Ferreira, Baptista & Pansarin, *B, m. *Cordiera myrciifolia* (K.Schum.) C.H.Perss. & Delprete, *B, n-o. *Turnera glaziovii* Urb., *B. *B,T new records for both areas within PNCF. *B new records for the Serra da Bocaina. *T new records for the Serra do Tarzan. s a-i, k-m, o – DCZ; j, n – TLFS.

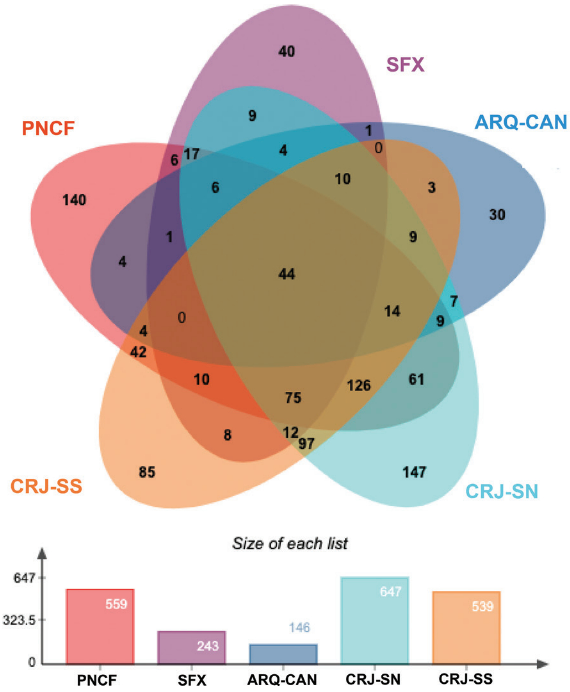

Figure 3. Venn diagram comparing the number of seed plant species exclusively found at the Parque Nacional dos Campos Ferruginosos (PNCF) and shared with other *canga* areas at the Floresta Nacional de Carajás (Serra Norte e Serra Sul), Serra Arqueada (ARQ-CAN), and Serra de Campos de São Félix do Xingu (SFX).

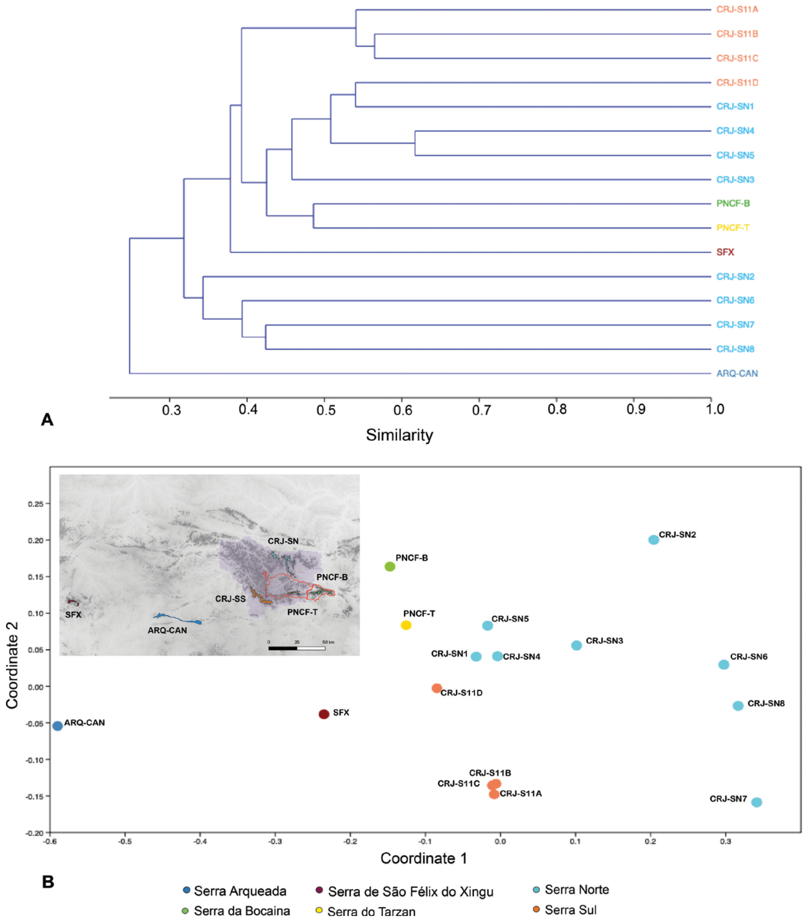

Figure 4. Multivariate analyses of floristic similarity between studied areas. A. Median association (UPGMA), cophenetic correlation coefficient: 0.9548. B. Map showing *canga* outcrop location and ordination analysis using multidimensional, non metric scaling (NMDS), stress value: 0.1549. (Map created by the authors with QGIS 3.18.1-Zürich © 2002-2019 QGIS Development Team available at https://www.qgis.org).

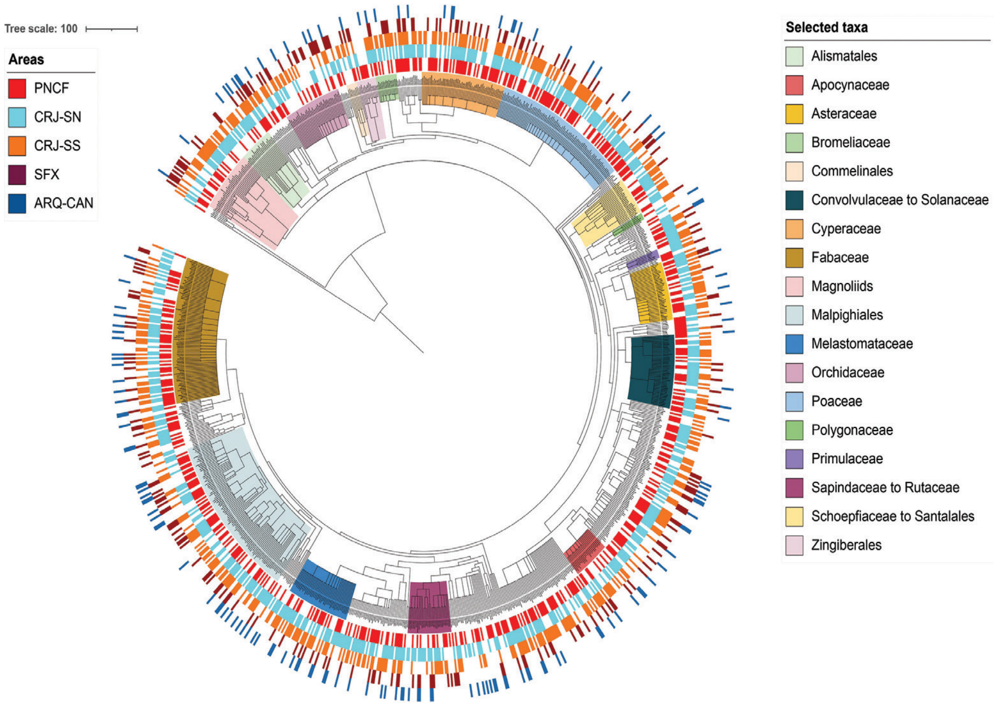

Figure 5. Amazonian *canga* megatree. The innermost ring (red) represents lineages present in the Parque Nacional dos Campos Ferruginosos. Lineages present in the FLONA de Carajás (CRJ-SN (light blue) and CRJ-SS (orange)) and other Amazonian *canga* appear in the following two rings. The two outermost rings, purple and dark blue represent São Félix do Xingu and Serra Arqueada respectively.

Discussion

Following the collecting effort carried out for this specific research, the flora of the Amazonian *canga* now comprises a total of 1022 species of Spermatophyta (1021 angiosperms). This study specifically focused on the PNCF, the only strictly protected area of Amazonian *canga* adding 158 species to the pre-existing list (Mota et al. 2018), resulting in a total of 559 species of angiosperm and one species of gymnosperm (see also item 4, Sample limitations). Regarding the total list of PNCF, we verified that 140 species are found, until the present moment, only in this site, being absent from other Amazonian *canga* sites (Figure 3), highlighting the relevance of PNCF for conservation.

1. *Restricted species and endemism*

The PNCF includes four species restricted to the *canga* of Carajás: *Ichthyothere* sp. 1 (Asteraceae, under description), *Rhynchospora unguinux* C.S.Nunes & A.Gil (Cyperaceae, see Schneider et al. 2019), *Cyperus* sp. 2 (Cyperaceae, under description), and *Spermacoce* sp. 1. (Rubiaceae – under description). The presence of these four new exclusive edaphic endemic species in this area will increase the list of endemics (Giulietti et al. 2019) to 42 species and demonstrates the importance of having a complete management plan for the PNCF. From the other 136 species exclusive to PNCF, 20% are restricted to the Amazon while the other 80% are more broadly distributed. PNCF is a conservation unit dedicated to the protection of *canga* vegetation, however, it also contributes to safeguarding widely distributed species both in the Amazon and in other Brazilian biomes.

PNCF hosts 30 out of the 42 *canga* edaphic endemics from Carajás (Giulietti et al. 2019), representing c. 71% of the Amazonian *canga* species that are unique for the area of Carajás, being crucial for their conservation. The new collections represent an important improvement in the knowledge of the flora of Carajás. The number of species from Serra Norte and Serra Sul (CRJ-SN1-8 and CRJ-SS11A-D) that were not recorded thus far at the PNCF equals 296 and 225, respectively (S4, Supplementary Material). This information is essential for conservation purposes as we can now assure which species are under protection within the PNCF. However, it would be very important to understand the size and dynamics of their populations to ascertain whether those are sufficient for the survival of these species within the study area. Six edaphic endemic species are so far only found in the *canga* vegetation of the FLONA de Carajás (and absent in the PNCF), Serra Norte: *Ipomoea cavalcantei* D.F.Austin (Convolvulaceae), *Paspalum carajasense* S.Denham (Poaceae), and *Daphnopsis filipedunculata* Nevling & Barringer (Thymelaeaceae); and in the Serra Sul: *Parapiqueria cavalcantei* R.M.King & H.Rob. (Asteraceae), *Carajasia cangae* R.M.Salas, E.L.Cabral & Dessein (Rubiaceae), and *Isoetes cangae* J.B.S.Pereira, Salino & Stützel (Isoetaceae). Therefore the concept of NNL of species is not met by the creation of the PNCF, and their survival is being pursued by VALE through other projects involving these species within the area of the FLONA de Carajás (Babiychuk et al. 2017, Watanabe et al. 2018, Zandonadi et al. 2019, Guimarães et al. 2023). Examples are the *ex-situ* cultivation of *Isoetes cangae* and *I. serracarajensis* (Zandonadi et al. 2019) and investigations to support *ex-situ* cultivation of other endemic species (e.g. *Ipomoea cavalcantei*) (Santos et al. 2023). It is very important to highlight that the distribution area of such species will undergo further habitat loss and degradation, with the almost complete disappearance of Serra Norte blocks SN4, SN5 and Serra Sul S11D, which should be considered when planning for the future use of the *canga* outcrops in the FLONA de Carajás. Our study reinforces the importance of evaluate different aspects of sites selected for offseting purposes and integrating an array of different offsets mechanisms in order to guarantee biodiversity conservation (Maron et al 2010, Sonter et al. 2018).

2. *Biogeography of Amazonian canga*

According to Mota et al. (2018), the richest plant families in the PNCF were Poaceae (40 spp.), Rubiaceae and Fabaceae (30 spp. both), Cyperaceae (26 spp.), Asteraceae (17 spp.), Convolvulaceae, Solanaceae, Melastomataceae and Lamiaceae (11 spp. each one), and Malvaceae (nine spp.). These families added up 196 species representing 53% of the total amount recorded in the floristic list published in 2018. The extensive collection effort carried out during our study has also increased the number of species recorded for these families (Figure S2a, Supplementary Material), which keep the status of the top 10 richest but currently represent 45% of the species of the PNCF. As pointed out by previous work, almost half of the *canga* flora is represented by a few or single species which might be related to the existence of several micro-habitats often present in this environment (Mota et al. 2018, Andrino et al. 2020, Fonseca-da-Silva et al. 2020). These micro-habitats are a reflection of the strong topographic variation and different distribution of soil nutrients across the substrate (Borges et al. 2017). The maintenance of the same better represented families also indicates that the sampling resulting from our efforts was evenly distributed, however, for three large plant families (Orchidaceae, Euphorbiaceae and Myrtaceae) the present work brings many newly recorded species for the PNCF. It is possible that the knowledge regarding these plant groups may be still under development, and that the relevant flora chapters (Costa et al. 2018, Kock et al. 2018, Trindade et al. 2018) prepared for them underestimated their diversity.

Our multivariate analysis highlighted that, in general, the Amazonian *canga* sites that compose the Carajás complex have moderate to low floristic similarity (35 to 42% of similarity), pointing to a high beta diversity among the *canga* sites, although some components are shared across all study sites. This can also be seen in our megatree, which obviates a strong correlation of lineages, with certain lineages being better represented in some sites than in others (Figure 5). On the other hand, when taking into account the region´s phytogeography, these *canga* outcrops form a cohesive floristic group distinct from other Amazonian open vegetation (Devecchi et al. 2020), for example the Amazonian savannas.

Comparing all Amazonian *canga* localities studied, the lowest species richness was found in the two totally unprotected sites of Serra Arqueada and São Félix do Xingu (see also item 4, Sample limitations), however, these have rather distinct species composition, with unique endemic species found in the latter site (*Mimosa dasilvae* A.S.Silva & R.Secco and a new species of Lauraceae, currently being described). The contribution of these two sites towards the floristic dissimilarity (Figure 4a) is also clearly visible in our megatree (Figure 5), where both sites contribute with less lineages. Both sites are isolated and geographically distant from the PNCF (Figure 1). Those two sites are also smaller (Table 2), and it has been found that the size of the area occupied by Amazonian *canga* appears to have a positive correlation with the site diversity independently of the geographic proximity between sites (Andrino et al. 2020), because a larger area would foster more variety of species specific to micro-habitats (Andrino et al. 2020). Therefore, the species sharing observed between PNCF and FLONA de Carajás (Figure 3; S5, Supplementary Material) and the higher floristic similarity between these areas (Figures 4a, 5) may be linked to their larger size and higher number of vegetation types found within them (Mota et al. 2015). Nonetheless, São Félix do Xingu presents the greater relative overlap of species with PNCF when compared to the other areas (Figure S5, Supplementary Material) with c. 65% of species shared.

The mosaic of vegetation types found in the *canga* (Table 1) may explain the different floristic composition found between the sites. The absence of deep, perennial lagoons and swamp forest among the hydromorphic vegetation types listed by Mota et al. (2015) for instance, probably meant that aquatic species such as *Apalanthe granatensis* (Humb. & Bonpl.) Planch.*,* *Ottelia brasiliensis* (Planch.) Walp. (Hydrocharitaceae, see Hall & Gil 2016) and *Nymphaea rudgeana* G.Mey (Nymphaeaceae, see Lima 2018) were not found in the PNCF because of the absence of this type of micro-habitat. In this sense, it is not unexpected that magnoliids and Alismatales, lineages often associated with water, appear underrepresented in the PNCF when compared to FLONA de Carajás sites (Figure 5). The less represented Commelinales in the PNCF when compared to the FLONA de Carajás may be a reflection of the absence of some of the hydromorphic vegetation subtypes (perennial lagoons, see Table 1), as many species of this clade are associated with aquatic habitats. The hydromorphic vegetation is significant for the Carajás flora, insofar as it contributes with the majority of *canga* exclusive species (Mota et al. 2018). Therefore, the lack of certain types within this category contributes to the difference between the areas. On the other hand, the more striking presence of Zingiberales in comparison with all other areas may be an effect of the topography of Serra da Bocaina and Serra do Tarzan, where the steep sides are densely forested, an environment preferred by species of this lineage. Therefore, the micro-habitats provided by the vegetation types and sub-types (Table 1) are fundamental to harbour divergent lineages of angiosperms and, despite the difficulty of accurately mapping such environments because of their reduced scale and even temporary absence (i.e. during the dry season some of these may not be noticeable), our megatree serves as a proxy to identify the differences between the study areas.

Our results indicate the proportion of shared overall species between PNCF and the other sites is only moderate to low (S5, Supplementary Material). Furthermore, some species are exclusive from the PNCF even considering that some specific *canga* vegetation types are not found there, making its flora distinct from all other studied sites. These new data reinforce the urgency of having a strong and detailed management plan for the PNCF.

3. *Further conservation needs in the Amazonian canga*

With the exception of the PNCF, the areas contemplated by the present research are under pressure either by mining (FLONA de Carajás) or surrounding deforestation and illegal mining (SFX and ARQ-CAN). Even if legal mining activities are highly regulated by the government at Federal and State level, the present data highlight that the conservation of endemic *canga* species is threatened by large-scale mining, as already recorded by Martins et al. (2018) and also seen in the *canga* of Minas Gerais (Kamino et al. 2020). According to Souza-Filho et al. (2019), the FLONA de Carajás management plan does not specify a minimum area of *canga* that must be preserved. Through our efforts we highlight the importance of the PNCF but also make obvious that more canga areas must be preserved within the FLONA de

Carajás in forthcoming updates of its management plan. Ilegal mining activities also occur in the region and represent an important factor for biodiversity loss (Pivello et al. 2021, Antonelli 2022). Intensification of land conversion into pasture and agribusiness is also responsible for raising greenhouse gas emissions in places such as São Félix do Xingu, the Brazilian municipality that was top of the emission list in 2018 (Albuquerque et al. 2021), as well as being among the 10 municipalities with accumulated fire foci during the last five years (INPE 2021). Serra Arqueada was recently a victim of possibly criminal fires (G1 PA 2021), showing how the *canga* found outside protected areas is under threat due to multiple anthropogenic pressures. These two sites have a distinctive flora (6% of São Félix do Xingu and 20% of Serra Arqueada). The Serra Arqueada (S2, Supplementary Material) is home to 64 species (c. 48% of the flora) that are not protected in the PNCF, such as the orchid *Galeandra cristata* Lindl. and the threatened grass *Axonopus carajasensis* Bastos (Martinelli & Moraes 2013). The latter species is a *canga* edaphic endemic (Giulietti et al. 2019) and is only known from CRJ-SN1 and CRJ-SS11D, requiring special attention. Around 35% of the species recorded at SFX (86 spp. – Figure S3, Supplementary Material) were not recorded for the PNCF, among them the edaphic endemic legume *Mimosa dasilvae* A.S.Silva & R.Secco, only known from this site. Therefore, it is paramount to indicate these two sites as priorities for conservation.

The risk faced by *canga* species, especially the endemics, has been addressed through different research lines. For example, new sampling areas were defined and surveyed, in order to certify the distribution of *canga* specie (Giulietti et al. 2019). The impact of climate change was also anticipated, by defining potentially vulnerable species and priority protection areas under future climate (Giannini et al. 2021). Detailed molecular studies have been carried out (Vasconcelos et al. 2021) especially involving endemic species (Babiychuk et al. 2017, Lanes et al. 2018, Carvalho et al. 2019, Silva et al. 2020). Specifically, endemic hydromorphic species have also been analysed in detail (Nunes et al. 2018, Caldeira et al. 2019, Dalapicolla et al. 2021, Pereira et al. 2021). Loss of biodiversity can also reduce vegetation types, micro-habitats, abiotic values, and ecosystem services (Mace et al. 2012), and needs to be properly addressed through conservation planning. Floristic similarity between the two outcrops found within PNCF, SB and ST was 48% (Figure 4a), close to figures found before (51% in Zappi et al. 2019; 45% in Fonseca-da-Silva et al. 2020 and Andrino et al. 2020). This discrete increment was brought about by the increase in sampling and it does not change the fact that the floristic similarity between these two blocks continues to be moderate signalling to the existence of distinct *canga* floras within the park area. Both UPGMA and NMDS analyses showed that, despite forming a single conservation unit (PNCF), the Serra da Bocaina and the Serra do Tarzan have distinct floras, denoted by their floristic similarity below 50% or by the ordination analysis results.

These two outcrops have a history of different environmental impacts. Originally included within Fazenda São Luís, Serra da Bocaina is surrounded by pasture and the outcrops can be reached from several directions, while Serra do Tarzan, surrounded by relatively untouched dense forest, can be accessed only by an entrance road that is frequently closed due to fallen trees. This difference in surrounding vegetation and land use history justifies the records of exotic species so far only in SB: *Melinis minutiflora* P.Beauv., *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs, *Cenchrus polystachios* (L.) Morrone, *Leonotis nepetifolia* (L.) R.Br., and *Urena lobata* L. In addition to these species, the fern *Pteridium esculentum* (G. Forst.) Cockayne (Dennstaedtiaceae) is widely distributed both on the edges of the trails and near the *canga*, occupying the space opened up by frequent, sometimes criminal fires. This fern is referred for some Brazilian regions and in other countries as a problem plant because, besides producing vast quantities of biomass, it rapidly expands its rhizome making the re-establishment of native vegetation extremely difficult (Guerin & Durigan 2015). Effective control measures for these invasive and native problem species in Serra da Bocaina need to be included in management plans for PNCF.

The presence of alien species in Serra da Bocaina shows that this site is under more pressure from habitat disturbance and native vegetation loss than Serra do Tarzan, which appears to be better preserved. Bellard et al. (2016) demonstrated that the main threats to biodiversity are the loss of plant communities through farming, the use of biological resources, urbanization, and the establishment of exotic species. Thus, although Serra da Bocaina is now included in a strictly protected area (i.e. IUCN category II), carefully applied management measures are needed to recover the vegetation that surrounds the *canga* and prevent uncontrolled access both to the PNCF and its *canga* areas.

4. *Sample limitations*

We acknowledge some limitation in comparing the different areas may exist due to differences in the sampling effort dedicated to the Serra do Tarzan. Considering the great effort carried out by present and previous work, and also by the result of the rarefaction curve (S3, Supplementary Material) we assume that the *canga* is well sampled as a whole for the purpose of this study. The species from Serra do Tarzan are evaluated mostly considering its role as a legal protected area together with Serra da Bocaina. The data here represents an important step towards our overall knowledge on Amazonian *canga* flora. Furthermore, the analysis presented here does not intend to draw a final conclusion, but instead, it aims to investigate whether the protected areas are an appropriate representation of the Amazonian *canga* biodiversity and to point to new directions for research and conservation planning attempting to address any remaining questions. Some of the relationships seen in the present work confirm recent findings (Zappi et al. 2019, Andrino et al. 2020, Fonseca-da-Silva et al. 2020). However, the new collections enabled by this research have increased the sampling of the PNCF, corroborating with more robust floristic comparative data between the *canga* sites of Carajás. Finally, the new data contribute to highlight the high beta diversity of the study area.

Conclusion

The PNCF has a significant role as a strictly protected area, contributing to protect both widespread and unique plant species of the Amazonian *canga*. Detailed study of the flora of PNCF is fundamental as a basis for the authorities and conservation practitioners to develop conservation and management strategies for *canga* vegetation. The extreme importance of this vegetation and the urgency of its conservation are evident, and it is paramount to protect them from all potential threats. The presence of species from *canga* in the PNCF goes towards ensuring their future existence in the *canga* of Carajás. However, in isolation, the PNCF does not adequately cover the entire flora of the Amazonian *canga*. Considering the uniqueness of the Serra Arqueada and São Félix do Xingu, still unprotected *canga* areas of the Carajás complex, we highlight them as priority areas to be included in conservation plans.

Supplementary Material

The following online material is available for this article:

Table S1 – PNCF species list with voucher material information.

Figure S2 – A. Comparison of species number per sampled family in Mota et al. (2018) and current work. B. Families with increased sampled richness in the current study.

Figure S3 – Rarefaction graphic for Amazonian *cangas* showing sample coverage.

Table S4 – Species list showing which species occurred at each site and data matrix used in PNCF analysis.

Figure S5 – Venn diagrams showing exclusive and overlapping species between studied sites**. A-D.** Comparison between PNCF and different areas separately. **E.** Comparison between Serra da Bocaina and Serra do Tarzan.

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Author Contributions

Livia Gadelha Silva: contributed to data collection; contributed to data analysis and interpretation; contributed to data analysis and interpretation; Contributed to manuscript preparation.

Juliana Lovo: substantial contribution in the concept and design of the study; contributed to data collection; contributed to data analysis and interpretation; contributed to manuscript preparation.

Taiana Laura da Fonseca-da-Silva: Contributed to data collection.

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Conflicts of Interest

All authors declare they have no conflict of interest.

Data Availability

All datasets produced during this work is available as Supplementary Material in the Figshare repository: https://figshare.com/s/b7f873442d81f4f6977a

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Article

Threats to health and conservation of free-living sloths (*Bradypus* and *Choloepus*) under anthropic influence in the city of Manaus, Amazonas state, Brazil

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Abstract: Anthropogenic disturbances affecting forest areas can increase disease prevalence and susceptibility in several species of arboreal mammals, such as sloths. Thus, this study aimed to evaluate the most common conditions in body systems of free-ranging sloths admitted at the Wildlife Triage and Rehabilitation Center of Amazonas of the Institute of Environment and Renewable Natural Resources. A total of 227 individuals (139 retrospective cases from 2015 to 2019, and 88 prospective cases from July 2020 to July 2021) were evaluated over a six-year period. Cases from the genus *Bradypus* showed involvement of the following body systems: 44% respiratory, 29% integumentary, 15% musculoskeletal, 5% digestive, 3% visual, 2% auditory, 1% circulatory and 1% genitourinary, while that distribution in the genus *Choloepus* was: 39% musculoskeletal, 27% integumentary, 19% respiratory, 9% digestive, 3% circulatory, 1% visual, 1% auditory and 1% genitourinary. The results reveal significant differences between the condition detected and the genus (*Bradypus* and *Choloepus*), age and case outcome. These results can provide data for future investigations of sloth diseases, confirming lesions, as well as motivating and suggesting adequate management methods.

Keywords: *Wild animals; Xenarthra; Pilosa; clinic; diseases.*

Ameaças à saúde e conservação de preguiças (*Bradypus* e *Choloepus*) de vida livre sob influência antrópica em Manaus, Estado do Amazonas, Brasil

Resumo: Distúrbios antrópicos que afetam áreas florestais podem aumentar a prevalência de afecções e a suscetibilidade às doenças em diversas espécies de mamíferos arborícolas, como as preguiças. Desta forma, este estudo teve como objetivo avaliar as condições mais comuns nos sistemas orgânicos de preguiças de vida livre admitidas no Centro de Triagem e Reabilitação de Animais Silvestres do Amazonas. Um total de 227 indivíduos (139 casos retrospectivos de 2015 a 2019 e 88 casos prospectivos de julho de 2020 a julho de 2021) foram avaliados durante um período de seis anos. Para o gênero *Bradypus* foram diagnosticadas 44% de afecções do sistema respiratório, 29% tegumentar, 15% musculoesquelético, 5% digestório, 3% visual, 2% auditivo, 1% circulatório e 1% genitourinário. Por sua vez, para o gênero *Choloepus* foram detectadas 39% de afecções do sistema musculoesquelético, 27% tegumentar, 19% do sistema respiratório, 19% do sistema respiratório, 9% digestório, 3% circulatório, 1% visual, 1% auditivo e 1% genitourinário. Os resultados mostraram diferenças significativas na prevalência da afecção e gênero das preguiças (*Bradypus* e *Choloepus*), a faixa etária e desfecho do caso. Esses resultados podem fornecer subsídios para investigar as doenças de preguiças, confirmando afecções, motivo e sugerir métodos adequados de manejo.

Palavras-chave: *Animais selvagens; Xenarthra; Pilosa; clínica; doenças.*

Introduction

Urban expansion, habitat loss, fragmentation, and climate change represent complex scenarios that can alter health in the human-animal-plant-environment interface, increasing the risk of zoonotic diseases in the One Health perspective (Jones et al. 2008, Thompson et al. 2016, OHHLEP et al. 2022). The monitoring of diseases in wildlife plays a fundamental role in the zoonotic disease dynamics in nature and may contribute to the evaluation of the prevalence, surveillance, and dissemination of important diseases for animal and environmental health (Jones et al. 2008, Rahman et al. 2020).

The Amazon, the largest biome in South America, is located in a humid tropical area with constant temperatures and climatic oscillations that vary between dry and wet rainy seasons (Veblen et al. 2007). However, more and more Amazon areas are threatened by anthropic factors, such as constant fires and deforestation, which pose threats of extinction to endemic species (Silva et al. 2020). These anthropic factors increase the risks to diversity, even in species with high adaptation to environmental changes, since they become not only vulnerable to habitat fragmentation and degradation but also susceptible to diseases, which can occur in ecotones among wildlife, domestic animals and humans (Santos et al. 2017).

The superorder Xenarthra is divided into two orders, Cingulata (armadillos) and Pilosa (anteaters and sloths), comprising about 31 living species distributed throughout the Neotropical region (Rose & Gaudin 2010, Martins et al. 2015). The order Pilosa, suborder Folivora, includes two distinct families of sloths, Bradypodidae (three-toed sloths), and Megalonychidae (two-toed sloths) (Gardner 2007, Gibb et al. 2016). The family Bradypodidae contains the species *Bradypus pygmaeus*, *B. tridactylus*, *B. variegatus*, *B. torquatus* (Gardner 2007), and more recently *B. crinitus* according to a new taxonomic rearrangement (Miranda et al. 2023). Members of the genus *Bradypus* are generally solitary and arboreal, exhibit strictly folivorous habits, are excellent swimmers and use treetops exposed to the sun for thermoregulation (Medri et al. 2006, Miranda 2014). The family Megalonychidae with two species of the genus *Choloepus* (*Choloepus didactylus* and *Choloepus hoffmanni*) is characterized by omnivorous sloths that consume a greater variety of food items, including fruits, flower buds and small vertebrates (Esbérard 2001, Miranda 2014). In addition, this family is primarily nocturnal, using trees with lianas, vines, and tree-creepers for rest and protection against possible predators (Wetzel 1982, Medri et al. 2006). Sloths depend on trees to perform various daily activities, so deforestation and urban expansion imply habitat loss (Medri et al. 2006, Dünner & Pastor 2017). Besides mortality from anthropic factors, human-wildlife interaction can trigger the increase in diseases that these sloths can acquire, in addition to other conditions.

The Brazilian Amazon harbors four sloth species: two of the genus *Choloepus* (*Choloepus didactylus, Choloepus hoffmanni*) and two of the genus *Bradypus* (*Bradypus tridactylus* and *Bradypus variegatus*) (Wetzel 1985, Medri et al. 2006, Chiarello et al. 2022, Moraes-Barros et al. 2022, Plese et al. 2022, Pool et al. 2022). Three of these species are found in forest areas and neighborhoods in the city of Manaus, Amazonas. Studies in a forest fragment in the Manaus region found that *Bradypus tridactylus* and *Choloepus didactylus* were equally abundant in primary and secondary forests (Carmo 2003, Mata 2009). Despite the threats to which they are exposed, the IUCN (International Union for Conservation of Nature) classifies them as threat status in the Least Concern (LC) category (Chiarello et al. 2022, Moraes-Barros et al. 2022, Plese et al. 2022, Pool et al. 2022). However, urban expansion compromises conservation areas for the species (Andrade et al. 2020), making it one of the leading causes of admissions to the Wildlife Sorting Center that receives animals rescued, trafficked, or delivered by the population.

In order to contribute to the comprehension and conservation of wildlife species, this study aimed to evaluate the most common conditions in body systems of free-ranging sloths admitted at the Wildlife Triage and Rehabilitation Center of Amazonas of the Institute of Environment and Renewable Natural Resources (CETAS/IBAMA-AM) and to discuss the possible implications of the urban expansion for the sloths' health. To the best of the authors' knowledge, data have not yet been documented for sloths in the Manaus region, Amazonas state, Brazil.

Material and Methods

This study was approved by Ethics Committee for Animal Care and Use from the School of Veterinary Medicine and Animal Science, UNESP Campus Botucatu, SP-Brazil (no. 0031/2020–CEUA). Data from sloths (*Choloepus didactylus, Bradypus tridactylus, Bradypus variegatus*) admitted at the CETAS/IBAMA-AM were collected, analyzed and categorized over a period of six years (2015 to 2021). Data were collected from two different information sources: (a) retrospective consultation of the files and database from the Supervisory nucleus (NUFIS) of the CETAS/IBAMA-AM from 2015 to 2019; (b) prospective analysis, with follow-up for one year (July 2020 to July 2021) of all sloths received at CETAS/IBAMA-AM with an individualized clinical record. In both data collections, the variables checked included locality, admission history, sex, age group (adult, juvenile, young), body weight, clinical diagnosis and case outcome (survival or death).

To categorize the data, a nominal qualitative variable analysis was performed, identifying the main conditions according to the following systems: musculoskeletal, integumentary, visual, auditory, digestive, respiratory, circulatory and genitourinary. Since the cases of the musculoskeletal system presented a high percentage, the occurrence registration was based on the degree of case concentration in the neighborhoods. A map of Manaus city, Amazonas state, enabled estimation of the locations where the highest numbers of animals were found. By calculating Cramér's V coefficient (Cramér 1999), implemented in the R package "rcompanion" (R Core Team, 2021; Mangiafico 2022), we evaluated the existence of a prevalence between age and condition type, as well as animal mortality and/or outcome predictors (death or survival). The correlation was defined as strong, moderate, or weak (range, 0.63–0.81) (Cohen 1988). The calculation was repeated for 10,000 bootstraps to obtain a 95% confidence interval.

Results

Data were analyzed from 227 sloths, including 139 retrospective (2015 to 2019) and 88 prospective cases (July 2020 to July 2021). A total of 60% of the animals were males, 30% were females, and 10% had no information as to sex. Sloths were 48% adult animals, 31% young and 21% juveniles. Body weight was not provided in the retrospective data.

Body weight information was only obtained in prospective cases (n = 88), ranging from 203 g to 6.3 kg for the genus *Bradypus* and from 307 g to 8.8 kg for the genus *Choloepus*. The reasons for admission included: rescue (73%) carried out by a competent authority associated with electrocution, dog attacks, or having been hit by a car; and admission volunteer agreement to deliver the animal (27%) was usually related to being orphaned/abandoned or dispersed in backyards. There was no information in 8% of the cases. The conditions according to the systems are shown in Table 1.

Table 1. Conditions on body systems in 227 free-ranging sloths of the genera *Bradypus* and *Choloepus* admitted at the Wildlife Triage and Rehabilitation Center of Amazonas of the Institute of Environment and Renewable Natural Resources (CETAS/IBAMA-AM).

Body systems	Conditions	*Bradypus* (n = 125)	*Choloepus* (n = 102)
Musculoskeletal	Fractures, Luxations	15%	39%
Integumentary	Skin laceration, Burns Ectoparasites	29%	27%
Visual	Corneal ulcer	3%	1%
Auditory	Fungal otitis	2%	1%
Digestive	Bloat, Parasitism, Malabsorption syndrome, Fecaloma	5%	9%
Respiratory	Bronchopneumonia, Pneumonia	44%	19%
Circulatory	Endocarditis, Cardiomyopathy	1%	3%
Genitourinary	Obstruction, Prolapse	1%	1%

The highest percentage of cases occurred in *Bradypus* related to respiratory (44%) and integumentary (29%) systems. In the respiratory system were observed mainly bronchopneumonia and pneumonia, but there were cases with no specific cause. A correlation coefficient of 0.72 was detected between these respiratory diseases and age, which was most frequent in young *Bradypus* sloths (20%). In relation to the integumentary system, the most significant lesions were skin lacerations caused by dog bites and burns due to electrocution, besides mites (*Sarcoptes scabiei*) and ticks (*Amblyomma* spp.), which had a high prevalence of infestation.

In the genus *Choloepus*, the prevalence of lesions were associated with the integumentary (39%) and musculoskeletal (27%) systems. Cases related to the integumentary system involved skin lacerations of the head caused by an attack by a domestic animal (dog); palmar and facial lacerations due to conflict with conspecifics; and burns of different degrees due to electrocution. The lesions in the musculoskeletal system consisted of fractures and luxations. In general, the fractures involved the femur, radius/ulna, or multiple ones involving more than one bone, caused by being hit by a car or by electrocution. There was also one case of osteosclerosis of the interphalangeal joints attributable to deformity of the claws/nails.

Figure 1 shows primary conditions according to the following systems: musculoskeletal, integumentary, visual, auditory, digestive, respiratory, circulatory, and genitourinary. The west zone of Manaus presented the greatest concentration of musculoskeletal and integumentary system conditions (example fractures, skin laceration and burns), and reported the second-highest rate of population growth of the decade in the region and ecological consequences of forest fragmentation in the Amazon rainforests (Figure 2).

Regarding the other systems, the percentage was very similar for both genera, as shown in Table 1. Eye injuries and traumatic corneal

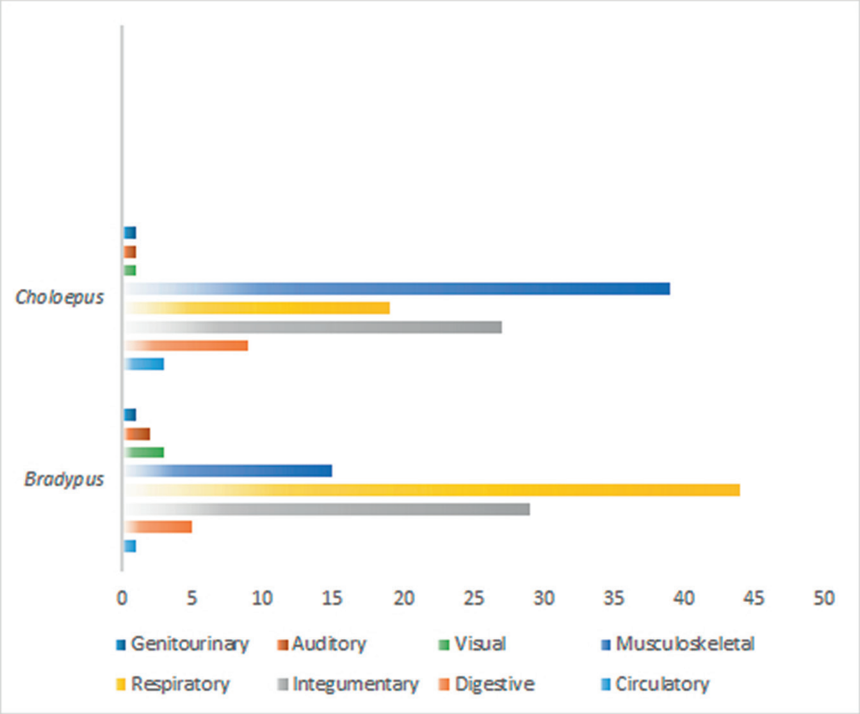

Figure 1. Prevalence between affected system and sloth genus (*Bradypus* and *Choloepus*).

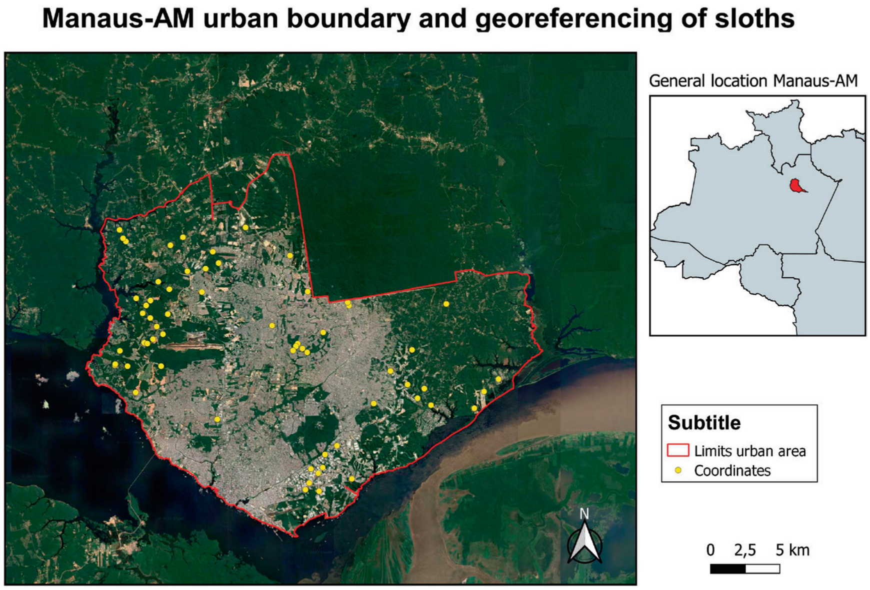

Figure 2. Records of the occurrence of musculoskeletal and integumentary lesions in sloths verified in the city of Manaus-AM.

ulcers were detected in the visual system. In the auditory system, only one sloth of the genus *Bradypus* presented fungal otitis (*Malassezia* spp.) with no defined history. In the digestive system, the following were observed: bloat associated with multiple traumas and gastric impaction, parasite infections (coccidiosis/giardiasis), malabsorption syndrome and fecaloma because of inadequate diet, and rectal prolapse due to constipation. Age was associated with the affected system, and a correlation coefficient of 0.81 was detected between these digestive diseases and age, which was most frequent in young *Choloepus* (22%). As to the circulatory system, animals were identified with endocarditis and cardiomyopathy, whereas the genitourinary system presented cases of urinary obstruction.

The outcome of the cases (death or survival) did not differ statistically in relation to age ($p > 0.05$), but there was a significant difference when comparing outcome and affected system ($p < 0.001$). The musculoskeletal, respiratory and integumentary systems were the main outcome predictors of death, comprising 70% of the cases, while the survival outcome corresponded to 30%.

Discussion

The present study showed data on the principal conditions in body systems of sloths that live in the region of Manaus, Amazonas state, Brazil. Studies on sloths are generally focused on taxonomy and ecology (Xenarthra, Pilosa) (Carmo 2003, Aguiar 2004, Réus & Sousa 2007, Moraes-Barros 2011, Vidal 2018). Although such studies contribute to conservation, they have not address the health problems resulting from anthropization, which may threaten the conservation of these mammals (Lopes & Ferrari 2000).

In the genus *Choloepus*, the highest percentage of lesions corresponded to the integumentary and musculoskeletal systems confirming that free-living sloths are subject to traumas such as being hit by a car, falling trees, electrocution, and fights with domestic animals. A sloth rehabilitation center in Colombia also verified that *C. hoffmanni* has been subjected to several injuries, including being hit by cars, stoned by children, or electrical shocks, even with the greater adaptability of these sloths to habitat alteration (Moreno & Plese 2006).

The integumentary system presents a similar morphology for both genus (Dünner & Pastor 2017), which probably influenced this system to have a high proportion of cases in both genera in the current study. Two ectoparasites were detected in the sloths, *Sarcoptes scabiei* and *Amblyomma* spp. The infestation by *Sarcoptes scabiei* has also be described in *C. hoffmanni* and *B. variegatus* in captivity from Costa Rica (Sibaja-Morales et al. 2009). The zoonotic potential of this mite must be considered due to the risk of transmission to the persons who rescued or are responsible for the care of the infested animal. Sarcoptic mange has a global distribution and is considered an emerging disease in some wildlife species (Escobar et al. 2022), as observed in the current study. Among the ticks, the *Amblyomma varium* presents high host specificity and is frequently verified in the *Choloepus* and *Bradypus* genera (Marques et al. 2002). Ticks, lice, and algae are observed in sloths under natural conditions but must be treated in captivity animals if a host-parasite imbalance occurs.

The musculoskeletal system of sloths presents disparities in functional adaptations, and variation may occur eventually in individuals within the same species (Mendel 1985, Miranda 2014, Dünner & Pastor 2017). The activity pattern and movement rates also differ between sloths, since *Cholloepus* ones are nocturnal with a cyclic activity pattern, and *Bradypus* decrease activity before and after sunrise (Sunquist & Montgomery 1973). Therefore, injuries may have different impacts between species and individuals, as verified in the current study, since the musculoskeletal system had the second-highest percentage of lesions in the sloths of the genus *Choloepus* and the third in the genus *Bradypus*.

Sloth genera differ in ecological behavior, which may have influenced the differences in lesion percentages, especially in the integumentary, musculoskeletal, and respiratory systems. While sloths of the *Bradypus* genus move less often, staying for a prolonged period in a tree and with both daytime and nighttime activity in less frequency, sloths of the *Choloepus* genus present strictly nocturnal activities spending little time at a single location (Sunquist & Montgomery 1973, Chiarello 2008).

In addition, a study showed that activity patterns in *Bradypus variegatus* are influenced by environmental temperature, besides nutritional requirements and protection against predators (Chiarello 1998, Castro-Sá et al. 2021, Lopes et al. 2023). These differences influenced the high percentage of electrocution and being hit by a car for *Choloepus*, resulting in fractures, injuries, lacerations, and burns. Therefore, the activity pattern of these species, associated with the high rates of destruction and fragmentation of the habitat, may be considered the main consequence of the large number of traumatic conditions distributed throughout the neighborhoods, as verified in the spatial analysis. There were significantly more abundant rescue points in forested areas, where anthropization levels are considered high (De Andrade et al. 2020).

Electrocution, for example, is a common problem for several arboreal mammals due to urban expansion, causing injuries of different degrees and complexity (Dünner & Pastor 2017, Carmo et al. 2019, Almeida et al. 2022). In this context, the modified ecological matrix (roads, constructions, invasions in neighborhoods, industries, electrical wiring) can account for the high number of traumatic conditions related to the behavior of species in search of new areas to establish territory and use for foraging. The evaluation area of the present study has been subjected to different types of anthropic pressure due to exacerbated urban growth of the city and population growth with a percentage increase of 25.5% in a decade. Manaus, the state capital of Amazonas, has experienced significant population growth in recent years (IBGE, 2023), a fact that has resulted in areas of destroyed or fragmented forests. Faced with such challenges, public bodies must seek solutions through public policies that guarantee the sustainable development of the city, including by actions that conserve the environment and species of the Amazon region. The houses present lands adjacent to forest fragments that generate direct and indirect human - domestic animal - wildlife interactions (Gontijo 2008, Santos et al. 2017). Consequently, the populations of free-living sloths (*Choloepus didactylus, Bradypus tridactylus, B. variegatus*) that inhabit green areas, anthropic forest fragments, and permanently protected areas in Manaus presented lacerations or injuries from attacks/bites by dogs when descending to the soil or when trying to connect to another forest fragment through the resident backyards. A study around a cacao farm in Costa Rica detected that most mortality of the adult sloths of the genera *Bradypus* and *Choloepus* was associated with predation by coyotes and domestic dogs and suggested that *B. variegatus* require immigration to maintain a stable population (Peery & Pauli 2014).

Since the present study did not consider specific variables for spatial epidemiological analysis, further studies must be conducted to correlate clinical lesions with negative interactions that threaten sloth survival and to promote conservation strategies. The urban matrix directly influences threats to arboreal mammals, such as sloths and primates (Gordo et al. 2013, Katsis et al. 2018, Brandão et al. 2019, Carmo et al. 2019), vulnerable to urbanization because of the high dependence on trees for displacement.

The lesions verified in the respiratory and digestive systems probably were influenced by the time in captivity. Stressed animals can develop enteric and respiratory disturbances (Messias-Costa & Esbérard 2001). Bronchopneumonia and pneumonia were the most frequent respiratory diseases in the present study. Severe climate changes were associated with developing pneumonia among sloths living in captivity at one São Paulo zoo (Diniz & Oliveira 1999). Bronchopneumonia and acute edema of the lungs was also the major causes of death in sloths received at a zoo in Belém, Amazonia (Messias-Costa 2001). The sloths have poor thermoregulatory ability and are sensitive to temperature changes (Gilmore et al. 2000), which must be considered in captivity animals.

Parasitism, bloat, and malabsorption syndrome were some of the digestive alterations detected in the present study. Coccidia and Giardia were the intestinal protozoan parasites identified. Free-living sloths may have coccidian infection without disease manifestation (Messias-Costa 2001). *Giardia duodenalis* has already been diagnosed in baby sloths rescued in Manaus and must be considered part of the zoonotic scenario in the region (Reis et al. 2023). Traumas and gastric impaction occurred in cases of bloat in the present study, evidencing that veterinary complications and the maintenance of a standardized and adequate nutritional protocol for the pup in captivity are crucial for its maintenance. (Gage 2002, Larrazábal 2004, Dünner & Pastor 2017). Tympanism and constipation are common in sloths and related to stress factors (Messias-Costa 2001). A study of young *Choloepus didactylus* in captivity reported tympanism as the most frequent alteration, which was attributed to the food and position feeding position (Larrazábal 2004). In addition, tympanism has been observed in politraumatized free-ranging sloths (Dünner & Pastor 2017), as verified in animals with bloat in the present study.

It should be *mentioned that* the respiratory and digestive conditions in the current study were mainly associated with young sloths of the genus *Bradypus* removed from their natural environment, often resulting in death. Common problems reported in young and newly arrived captive animals include milk maladaptation (diarrhea), bloat, and fecaloma, as well as malabsorption syndrome that causes poor body condition and peeling of the skin and nails (Messias-Costa 2001, Dünner & Pastor 2017). Furthermore, the lesions related to the visual, auditory, circulatory and genitourinary systems were considered isolated and punctual cases.

In conclusion, the most common conditions in body systems of free-ranging sloths from Manaus included the musculoskeletal, integumentary, and respiratory systems; however, there were differences between genera. These results can provide data for future investigations about clinical diseases in sloths and their respective causes, to support adequate management measures that contribute to the health and conservation of these mammals.

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Author Contributions

Laynara Silva dos Santos: substantial contribution in the concept and design of the study, contribution to data collection, contribution to manuscript preparation.

Carlos Roberto Teixeira: substantial contribution in the concept and design of the study, contribution to data collection.

Sheila Canevese Rahal: contribution to manuscript preparation and substantial contribution in the concept and design of the study.

Alessandra Ferreira Dales Nava: contributed to data collection and manuscript preparation.

Laerzio Chiesorin Neto: contribution to data collect and interpretation.

Ricardo Shoiti Ichikawa: contribution adding intellectual content and analyzes.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives. This study was approved by the Ethics Committee for Animal Care and Use of the School of Veterinary Medicine and Animal Science, Unesp Campus Botucatu (no. 0031/2020– CEUA), and it was authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) through the System of Authorization and Information on Biodiversity (SISBIO) (no. 74815/1).

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on a reasonable request.

All data supporting the results in papers published in the journal must be archived in an appropriate public archive offering open access and guaranteed preservation (http://hdl.handle.net/11449/236499).

The datasets generated during and/or analyzed during the current study are available at: https://doi.org/10.48331/scielodata.7YO9P7.

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Inventory

Amazonian forest termites: a species checklist from the State of Acre, Brazil

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Abstract: The Brazilian state of Acre is located in the southwestern Amazon and it is characterized by a humid tropical forest vegetation that covers plains and mountains. Up to this point, the composition of termite species in the state is not known. The aim of this study was to provide a checklist of termite species or recognizable taxonomic units for the state of Acre. Sampling was conducted through field expeditions at the Serra do Divisor National Park, Chandless State Park, Humaitá Forest Reserve, and Chico Mendes Environmental Park using a standardized rapid termite inventory protocol in the first two areas and active searching collections in the others, without a specific protocol. This study also included occurrence records published in the scientific literature. A total of 128 species and morphospecies of termites were found in Acre, distributed across 59 genera and four families. The most frequently occurring species in Acre was *Heterotermes tenuis* (Hagen, 1858). The study also identified six new species records for Brazil. The predominant feeding groups were soil-feeders and wood-feeders, as expected from data obtained from surveys in humid tropical forests. Despite the significant number of new records for Acre (112), it is concluded that a larger sampling effort is still required, as many areas of the state have not yet been studied for termites.
Keywords: *insect fauna; biodiversity; feeding groups; Isoptera; Neotropics.*

Térmitas da Floresta Amazônica: uma lista de espécies do Estado do Acre, Brasil

Resumo: O estado brasileiro do Acre está localizado no sudoeste da Amazônia e é caracterizado por uma vegetação de floresta tropical úmida que cobre planícies e montanhas. Até então, a composição de espécies de térmitas no estado não é conhecida. O objetivo desse estudo foi construir um checklist de espécies ou unidades taxonômicas reconhecíveis de térmitas para o estado do Acre. A amostragem foi conduzida através de expedições de campo no Parque Nacional da Serra do Divisor, no Parque Estadual Chandless, na Reserva Florestal Humaitá, e no Parque Ambiental Chico Mendes utilizando o protocolo rápido de diversidade de térmitas nas duas primeiras áreas e coletas avulsas nas demais, sem um protocolo específico. Este estudo também incluiu registros de ocorrência publicados na literature científica. Um total de 128 espécies e morfoespécies de térmitas foram encontradas no Acre, distribuídas em 59 gêneros e quatro famílias. A espécie de ocorrência mais frequente no Acre foi *Heterotermes tenuis* (Hagen, 1858). O estudo também identificou seis novos registros de espécies para o Brasil. Os grupos alimentares predominantes foram os humívoros e xilófagos, como esperado a partir de dados obtidos de pesquisas em florestas tropicais úmidas. Apesar do número significativo de novos registros para o Acre (112), conclui-se que ainda é necessário um esforço amostral maior, uma vez que muitas áreas do estado ainda não foram estudadas para térmitas.
Palavras-chave: *fauna de insetos; biodiversidade; grupos alimentares; Isoptera; Neotropical.*

Introduction

Termites are abundant eusocial insects that act as decomposers and ecosystem engineers in tropical and subtropical regions of the world (Bignell et al. 2011). Their presence alters the environment as a result of their foraging behavior and nest-building, making nutrients trapped in plant organic matter more readily available to the soil, and creating new microhabitats for a wide variety of organisms of different trophic categories (Bignell & Eggleton 2000, Holt & Lepage 2000). Low-latitude regions tend to have higher temperatures and higher precipitation, which contribute to elevated levels of species diversity (Lomolino 2001). This is true for Brazil, one of the countries with the greatest biodiversity, which has a recorded 348 termite species, a value clearly underestimated (Cancello et al. 2014, Constantino 2022). Currently, 2,970 extant termite species are known worldwide, 615 of which species are recorded in the Neotropical region, surpassed only by the Oriental and Ethiopian regions (Constantino 2022).

Termite studies in Brazil have increased our understanding about the diversity of this group and its distribution across different biomes (Constantino 2005, Vasconcellos & Moura 2014, Feitosa et al. 2021, Vasconcellos et al. 2023). Some species lists have already been published for the Amazon (Emerson 1925, Bandeira 1979, Constantino 1992, Constantino & Cancello 1992, Issa 2000, Bahder et al. 2009, Palin et al. 2011, Dambros et al. 2012, Castro et al. 2021a) but to date there are no published checklists for any of the federal states of northern Brazil. The lack of a species inventory is especially detrimental because of the importance of these data for establishing ecological and biogeographical baselines to better inform conservation strategies (Silveira et al. 2010, Koroiva et al. 2020).

The Amazon biome is characterized by dense tropical forests. About 60% of its extent is in Brazil, but it also extends into Peru, Bolivia, Ecuador, Colombia, Venezuela, French Guiana, Guyana and Suriname. Far beyond its recognized biodiversity, the biome is of great importance to global ecology as it regulates regional hydrological cycles and climate patterns (Hutyra et al. 2007, Silva & Rezende 2022), and stores up to 100 billion tons of carbon (Copertino et al. 2019). However, the Amazon Basin continues to experience rapid deforestation and forest degradation (Vergopolan & Fisher 2016, INPE 2021), which threaten the balance of the entire ecosystem.

The state of Acre, located in the westernmost part of the Brazilian Amazon, has particular environmental characteristics that favor high biodiversity due to the influence of the Andean Cordillera (Sombroek 2000). Despite several protected areas, Acre has experienced particularly high rates of deforestation in recent decades (INPE 2021), highlighting the need to rapidly improve our knowledge of the region. One example is the Serra do Divisor National Park, which harbors some of the greatest biodiversity on earth, and is threatened by proposed bills that seek to reduce its protected status, likely leading to increased degradation within the park boundaries (Koga et al. 2022). For many termite species, there are only a few records from Acre, which suggests an underestimation of termite gamma diversity and highlights the need for further studies to better understand their distribution in the area. Improving the current knowledge of this taxon will be useful in developing conservation strategies and contributing to protected area management plans. Our aim here is therefore to provide a comprehensive checklist of the species or recognizable taxonomic units for termites occurring within four important protected areas within the Brazilian state of Acre.

Material and Methods

1. *Study areas*

The state of Acre (Figure 1) has an area of 164,173.431 km², is located entirely in the Amazon biome and belongs to the northern region of Brazil.

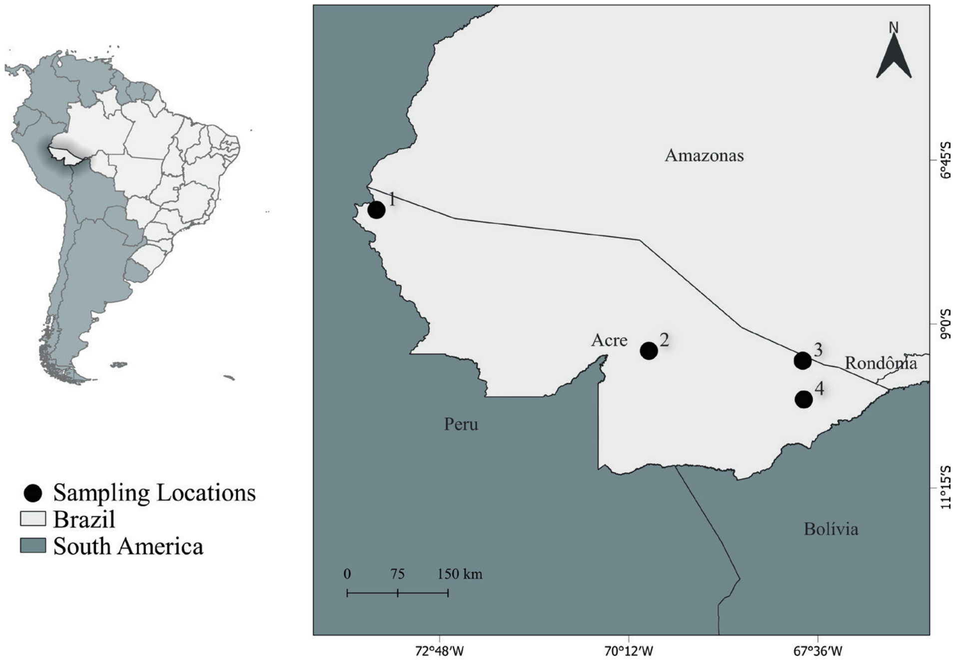

Figure 1. Locality map of the areas where termite sampling was conducted in the state of Acre, Brazil. 1, Serra do Divisor National Park; 2, Chandless State Park; 3, Humaitá Forest Reserve; 4, Chico Mendes Environmental Park.

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It borders the Amazon River to the north, Rondônia to the east, and the international borders with Peru to the west and Bolivia to the south. The relief of Acre is mainly composed of depressions, except for a small plateau in the north, and lowlands closely related to the hydrographic basin of the state (Cavalcante 2006). The hydrography of Acre consists of extensive rivers that are part of the Amazon River system, flowing from the southwest to the northeast and forming two important hydrographic basins for the state: the Acre-Purus Basin and the Juruá Basin.

According to Koppen's climate classification, Acre has a tropical savanna, equatorial and monsoon climate, with an average temperature of 25.1 °C, an annual precipitation of 2,169 mm. Although the vegetation of Acre is predominantly humid tropical forest, it has variations that include 11 forest typologies (IBGE 2005). Acre is the second-largest state in the legal Amazon region in terms of vegetation coverage, with 86% of its territory covered by the Amazon rainforest, second only to the state of Amazonas, which boasts 91% coverage (INPE 2021).

For this study, sampling was conducted in four protected areas across the state of Acre: Serra do Divisor National Park (SDNP—8°02'39.0" S 73°33'55.0" W), Chandless State Park (CSP—9°55'15" S 70°09'45" W), Humaitá Forest Reserve (HFR—9°45'49" S 67°38'40" W) and Chico Mendes Environmental Park (CMEP—10°02'14" S 67°47'38" W). The SDNP and CSP protected areas are under special protection regimes that aim to preserve biodiversity, natural ecosystems, and unique environmental characteristics. These areas are recognized as important for the preservation of biological diversity and have a territorial extension of 843,012.28 ha and 695,303.00 ha, respectively. In contrast, HMF (2,000 ha) is located in a forest fragment near the state capital of Rio Branco, and CMEP (57 ha) is an urban park located in the Rio Branco suburbs. These two sites have lower protected status and clearly present different environmental pressures than those observed at SDNP and CSP.

2. *Species sampling*

Five sample expeditions were carried out in May and December 2019. In May, collections were carried out in SDNP, CSP, HFR, and CMEP, and in December, an additional collection was conducted in SDNP. The rapid inventory protocol adapted by Cancello et al. (2014) was applied twice in SDNP and once in CSP. The application of this protocol is important not only because it captures a high number of species and feeding groups with a quick field effort, but also because it is a standardized method that allows for more reliable comparisons between different locations, enabling the development of ecological studies at larger scales. This protocol consists of six transects spaced at least 100 m apart, and divided into five plots of 5 × 2 m spaced 10 m apart. Sampling effort was 1 hour/plot/person, searching in all possible environments within the plot, such as on the ground, in fallen logs, in the leaf litter, under rocks, and on trees up to about 2 m high. Collected material was placed in flasks containing 80 °GL ethyl alcohol for sorting in the laboratory. In HFR and CMEP, collections were conducted using active searching for termites along the path traversed in those areas, without a specific protocol. The reduced number of days in the HFR and CMEP expeditions restricted the implementation of the rapid inventory protocol at these sites.

Termite sampling and transport were authorized by the Brazilian Biodiversity Information and Authorization System (SISBIO), Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ministry of Environment (MMA) (SISBIO authorization number 68077-1, 50565-2 and 72337-1). All specimens sampled in this project were deposited in the Termite Collection of the Department of Systematics and Ecology at the Federal University of Paraíba (UFPB).

Identification of species and morphospecies was based on material available in the scientific literature (Constantino 1998, 2002, Bourguignon et al. 2016a, Carrijo et al. 2023), and comparison of specimens with previously identified material deposited in the UFPB termite collection. Given the limited identifiability of specimens of the soldierless subfamily Apicotermitinae, it was necessary to dissect their enteric valves (see Supplementary Material, Figures S1, S2 and S3), which contain essential taxonomic characters for this subfamily, in addition to their gut topology (Bourguignon et al. 2016b). This necessitates the loss of specimens subjected to this procedure because part of their intestine is destroyed. Therefore, nine termite samples deposited in the UFPB termite collection were not included in this checklist because they contained five or fewer specimens, and as a result, could not be identified.

To complete the list, data were added from the Taxonomic Catalog of Isoptera from Brazil (Constantino 2022) and from the Web of Science (Clarivate Analytics 2023) and Google Scholar (Google Scholar 2023) databases using the keywords "Termite" and "Acre state". Of the termites found in the scientific literature for the state of Acre, only those represented at the species or genus morphotype level that were not part of the sampling conducted in this work are considered, since it is impossible to know whether the morphotypes designated in third party studies are different from those analyzed in this work.

3. *Feeding groups*

Termites were classified into feeding groups according to the proposals of Plaza (2019) and Donovan et al. (2001). The first classification of feeding groups (FG1) is part of one of the criteria analyzed for the characterization of Amazonian termite guilds (Plaza 2019). The characterization of these feeding groups follows criteria adapted from earlier studies (De Souza & Brown 1994, Eggleton et al. 1995, Vasconcellos et al. 2010, Cancello et al. 2014). Although it is gray literature, the use of this classification was appropriate due to the regional proximity of the studied material, i.e., termites from the Amazon. Five categories are proposed, considering the feeding habits of termites: (1) W = wood-feeders that feed on wood and litter, regardless of the stage of decomposition; (2) L = leaf-feeders that cut living plant material during foraging to feed; (3) S = soil feeders that feed on decaying organic material and soil; (4) W/S = wood/soil interface feeders that use wood in an advanced stage of decomposition, already in the phase of incorporation into the soil; and (5) I = obligate inquiline termites that feed on organic compounds of the nest-building species of which they are inquilines. The second food group classification (FG2) establishes a humification gradient and divides termites into four groups based on differences in stomach contents, morphology, and internal anatomy (Donovan et al. 2001).

Results

For the state of Acre, a total of 128 species and morphospecies of termites were recorded, including those from this study and from other previously published studies. Of these, 89 species (69%) could be identified at the species level, while for 39, it was only possible to identify morphospecies (for 17 it was possible to identify the genus level, for 15 it was possible to identify only the subfamily level, and for the remaining seven species, there was some uncertainty, and we attributed a qualifying word, like aff. and cf., to each to indicate the degree of uncertainty).

In this study, 59 genera were recorded distributed across four families: Kalotermitidae (four genera, six species), Rhinotermitidae (four genera, six species), Serritermitidae (one genus, one species), and Termitidae (50 genera, 115 species and morphospecies). The high diversity within the Termitidae can be examined at the subfamily level, with 15 genera and 40 species and/or morphospecies for Apicotermitinae, 12 genera and 31 species and/or morphospecies for Nasutitermitinae, 12 genera and 23 species and/or morphospecies for Syntermitinae, and 11 genera and 21 species and/or morphospecies for Termitinae. The resulting checklist, across all families, includes 112 new records of termite species for Acre and six new records of termite species for Brazil (Figures 2–6).

The SDNP was the site with the greatest species richness (89), followed by CSP (62), HFR (19) and CMEP (4). Literature data registered only 16 species for the whole of Acre. Of the 128 records for Acre, 52 were collected only in the SDNP, 22 only in the CSP, two species in the HFR, two in the CMEP and four only found in the literature. Only nine species occurred in three or more localities. Seventy-four species (58%) were exclusively sampled through the rapid inventory protocol, while 27 species (21%) were exclusively sampled through active searching. Overall, 23 species were recorded building conspicuous nests, both in this study and in the literature (Mathews 1977, Bandeira & Fontes 1979, Constantino 1991, 1992, 1995, Laffont et al. 2004, Fougeyrollas et al. 2017).

The soil-feeding termites (category S from FG1) were predominant across Acre, representing 42% of the species found in samples from this study, followed by wood-feeders, represented by 34% of the species. According to the classification based on worker morphology by Donovan et al. (2001) (FG2), feeding groups II and III were most represented, with 28% and 46% of the species found, respectively (Table 1).

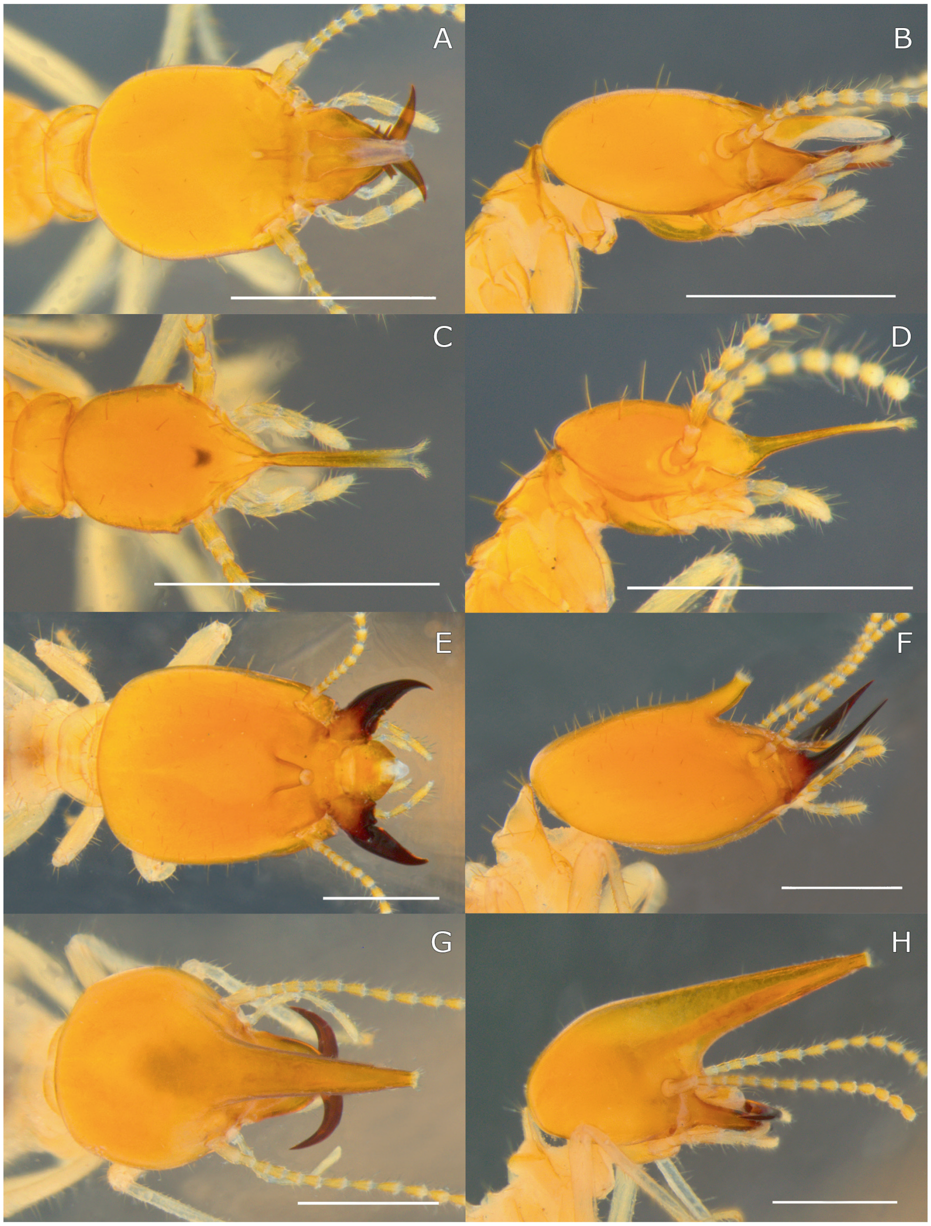

Figure 2. Soldiers of termite species with a new occurrence record for Brazil. *Dolichorhinotermes tenebrosus* major soldier (A, B); *Dolichorhinotermes tenebrosus* minor soldier (C, D); *Cornitermes bolivianus* (E, F); *Uncitermes almeriae* (G, H). Scale bar: 1 mm.

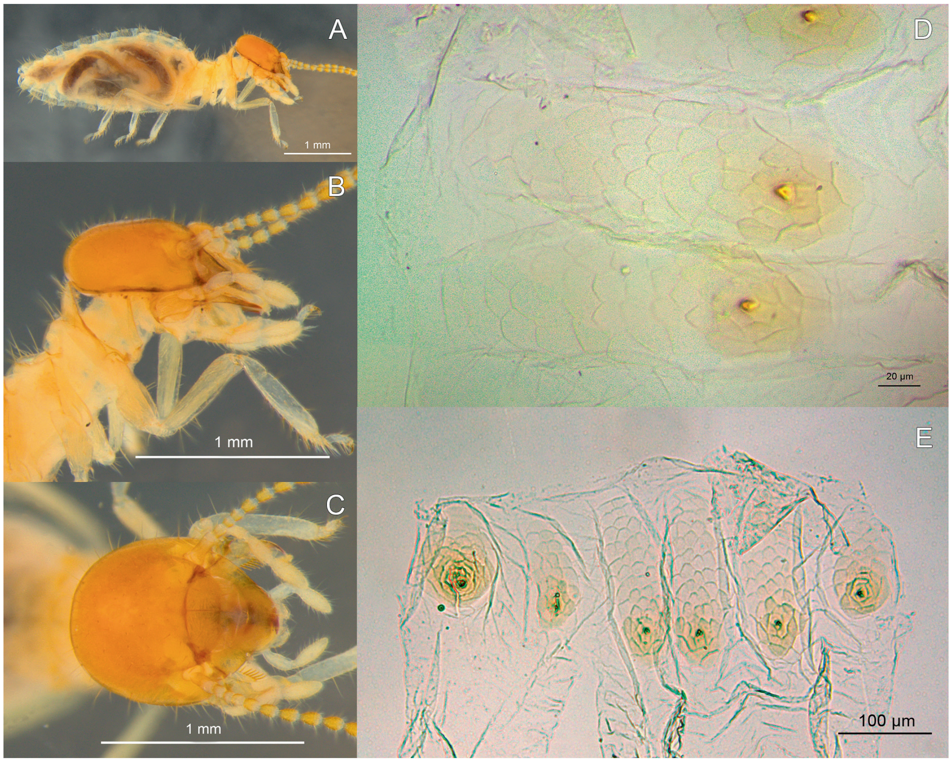

Figure 3. *Aparatermes thornatus* worker, new occurrence for Brazil. Lateral view of habitus (A); profile view, with lateral view of head and thorax (B); dorsal view of head (C); enteric valve in detail (D); complete view of enteric valve cushions (E).

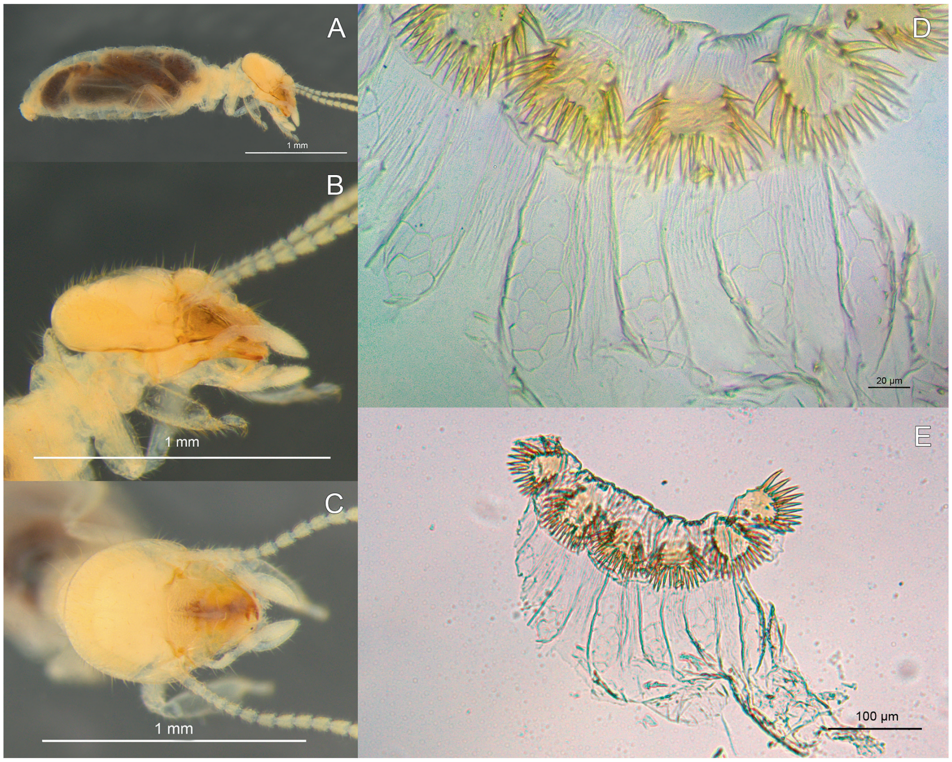

Figure 4. *Humutermes krishnai* worker, new occurrence for Brazil. Lateral view of habitus (A); profile view, with lateral view of head and thorax (B); dorsal view of head (C); enteric valve in detail (D); complete view of enteric valve cushions (E).

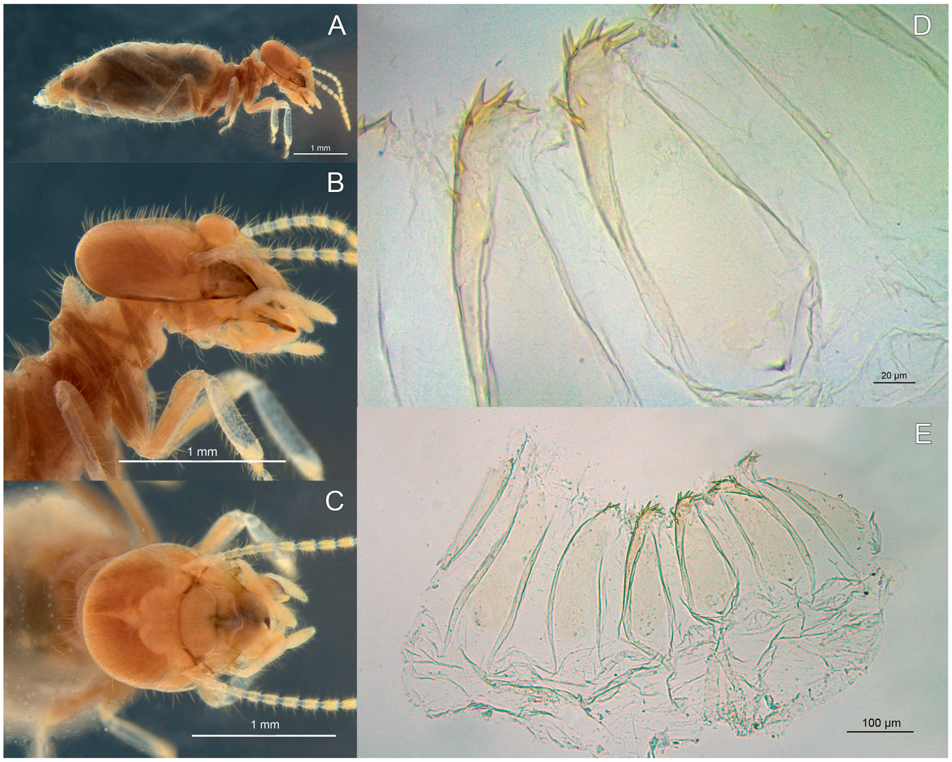

Figure 5. *Rubeotermes jheringi* worker, new occurrence for Brazil. Lateral view of habitus (A); profile view, with lateral view of head and thorax (B); dorsal view of head (C); enteric valve in detail (D); complete view of enteric valve cushions (E).

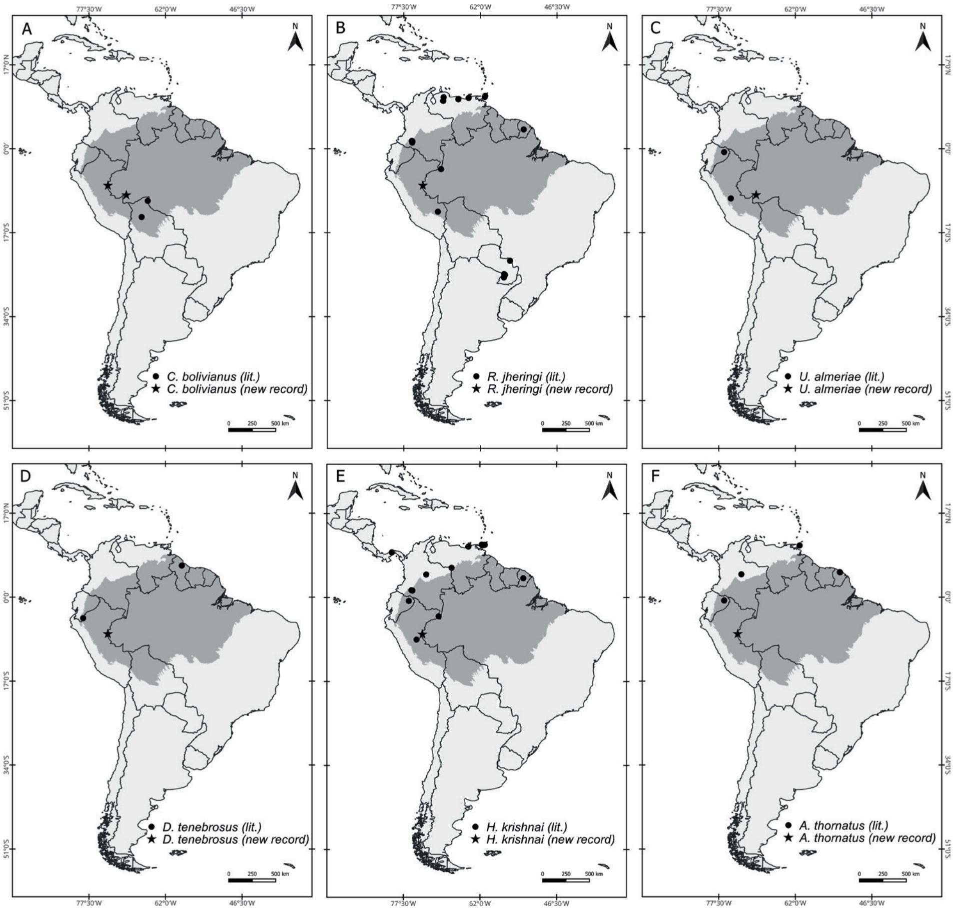

Figure 6. Location map of new records for Brazil of the species *Cornitermes bolivianus* (A), *Rubeotermes jheringi* (B), *Uncitermes almeriae* (C), *Dolichorhinotermes tenebrosus* (D), *Humutermes krishnai* (E), *Aparatermes thornatus* (F). Dark grey: Amazonia.

Table 1. List of termite species from the state of Acre, Brazil, and classification of feeding groups. Sampling locality: SDNP, Serra do Divisor National Park; CSP, Chandless State Park; HFR, Humaitá Forest Reserve; CMEP, Chico Mendes Environmental Park. Previously published records (Literature): 1, (Santos et al. 2022); 2, (Constantino 2022); 3, (Thomazini & Thomazini 2002); 4, (Santos 2022). FG, Feeding-Groups: FG1 (Plaza 2019): L, leaf-feeders; S, soil-feeders; I, inquiline; W/S, wood/soil interface feeders; W, wood-feeders. FG2: (Donovan et al. 2001). [1]Rapid termite inventory protocol (Cancello et al. 2014). ★New incident record for Brazil. *Species that build conspicuous nests according to literature records. **Species that were collected in conspicuous nests in this study.

Family/subfamily/species	Sampling locality				Literature	Feeding groups	
	SDNP	CSP	HFR	CMEP		FG1	FG2
KALOTERMITIDAE							
Calcaritermes rioensis Krishna, 1962		X[1]				W	I
Calcaritermes aff. *temnocephalus* (Silvestri, 1901)		X	X			W	I
Eucryptotermes hagenii (Müller, 1873)	X[1]					W	I
Glyptotermes sp.	X					W	I
Rugitermes sp.1		X[1]				W	I
Rugitermes sp.2		X[1]				W	I
RHINOTERMITIDAE							
Coptotermes testaceus (Linnaeus, 1758)	X[1]	X[1]	X		1;2	W	I
Dolichorhinotermes longilabius (Emerson, 1924)	X[1]	X[1]	X		2	W	I
Dolichorhinotermes tenebrosus (Emerson, 1925)★	X[1]					W	I
Heterotermes tenuis (Hagen, 1858)	X[1]	X[1]	X		2;3;4	W	I
Rhinotermes hispidus Emerson, 1925	X				2	W	I
Rhinotermes marginalis (Linnaeus, 1758)	X[1]	X[1]			2	W	I
SERRITERMITIDAE							
Glossotermes sulcatus Cancello & DeSouza, 2005	X[1]					W	I
TERMITIDAE							
Apicotermitinae							
Anoplotermes banksi Emerson, 1925**	X[1]					S	III
Anoplotermes meridianus Emerson, 1925	X[1]	X[1]				S	III
Anoplotermes sp.1	X[1]	X[1]	X			S	III
Anoplotermes sp.2	X[1]					S	III
Anoplotermes aff. *pacificus* Müller, 1873	X[1]					S	III
Anoplotermes susanae Scheffrahn, Carrijo & Castro, 2023	X[1]					S	III
Aparatermes silvestrii (Emerson, 1925)		X		X		W/S	III
Aparatermes thornatus Pinzon & Scheffrahn, 2019★	X[1]					W/S	III
Apicotermitinae sp.1	X[1]					S	III
Apicotermitinae sp.2	X[1]	X[1]				S	III
Apicotermitinae sp.3	X[1]	X[1]				S	III
Apicotermitinae sp.4	X[1]	X[1]	X			S	III
Apicotermitinae sp.5		X[1]				S	III
Apicotermitinae sp.6	X[1]					S	III
Apicotermitinae sp.7	X[1]					S	III
Apicotermitinae sp.8	X[1]					S	III
Apicotermitinae sp.9	X[1]					S	III
Apicotermitinae sp.10		X[1]				S	III
Apicotermitinae sp.11	X[1]					S	III
Apicotermitinae sp.12		X				S	III
Apicotermitinae sp.13	X[1]	X[1]	X			S	III

Continue...

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Family/subfamily/species	**Sampling locality**				**Literature**	**Feeding groups**	
	SDNP	**CSP**	**HFR**	**CMEP**		**FG1**	**FG2**
Apicotermitinae sp.14		X[1]				S	III
Apicotermitinae sp.15		X[1]	X			S	III
Compositermes vindai Scheffrahn, 2013	X[1]				2	S	IV
Disjunctitermes aff. *insularis* Scheffrahn, 2017	X[1]					S	IV
Disjunctitermes insularis Scheffrahn, 2017	X					S	III
Grigiotermes hageni (Snyder & Emerson, 1949)					2	S	IV
Hirsutitermes kanzakii Scheffrahn, Carrijo & Castro, 2023	X					S	III
Humutermes krishnai Bourguignon & Roisin, 2016★	X[1]					S	IV
Hydrecotermes kawaii Bourguignon & Roisin, 2016	X[1]					S	III
Krecekitermes daironi Scheffrahn, Carrijo & Castro, 2023	X[1]					S	III
Longustitermes manni (Snyder, 1922)	X[1]					S	IV
Mangolditermes curveileum Scheffrahn, Carrijo & Castro, 2023	X[1]	X[1]				S	III
Patawatermes nigripunctatus (Emerson, 1925)	X	X				S	IV
Patawatermes turricola (Silvestri, 1901)	X[1]					S	IV
Rubeotermes jheringi (Holmgren, 1906)★	X[1]					S	IV
Ruptitermes franciscoi (Snyder, 1959)	X[1]					L	III
Ruptitermes piliceps Acioli & Constantino, 2015	X[1]	X[1]				L	III
Ruptitermes sp.	X[1]					L	III
Rustitermes boteroi Constantini, Castro & Scheffrahn, 2020	X[1]		X			W/S	III
Nasutitermitinae							
Agnathotermes aff. *crassinasus* Constantino, 1990				X		S	IV
Agnathotermes glaber (Snyder, 1926)	X[1]					S	IV
Atlantitermes oculatissimus (Emerson, 1925)	X[1]					W/S	IV
Atlantitermes osborni (Emerson, 1925)		X[1]				W/S	IV
Atlantitermes sp.	X[1]					W/S	IV
Coatitermes clevelandi (Snyder, 1926)	X[1]	X				W/S	III
Coatitermes kartaboensis (Emerson, 1925)	X	X[1]				W/S	III
Constrictotermes cavifrons (Holmgren, 1910)[**]	X					W	II
Convexitermes convexifrons (Holmgren, 1906)	X	X[1]				W/S	III
Diversitermes diversimiles (Silvestri, 1901)		X				W	II
Hyleotermes brevipilus (Emerson, 1925)	X[1]					W	II
Nasutitermes acangussu Bandeira & Fontes, 1979[*]	X	X[1]			2	W	II
Nasutitermes banksi Emerson, 1925	X[1]					W	II
Nasutitermes callimorphus Mathews, 1977		X[1]	X			W	II
Nasutitermes cf. *bivalens* (Holmgren, 1910)	X	X[1]	X			W	II
Nasutitermes cf. *bolivari* (Snyder, 1959)		X[1]				W	II
Nasutitermes chaquimayensis (Holmgren, 1906)	X[1]					W	II
Nasutitermes corniger (Motschulsky, 1855)[*]		X[1]	X		1;2	W	II
Nasutitermes ephratae (Holmgren, 1910)[**]		X[1]				W	II

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Family/subfamily/species	**Sampling locality**				**Literature**	**Feeding groups**	
	SDNP	**CSP**	**HFR**	**CMEP**		**FG1**	**FG2**
Nasutitermes guayanae (Holmgren, 1910)**	X[1]	X[1]				W	II
Nasutitermes longirostratus (Holmgren, 1906)		X[1]	X			W	II
Nasutitermes rotundatus (Holmgren, 1906)		X[1]	X			W	II
Nasutitermes sp.	X[1]					W	II
Nasutitermes surinamensis (Holmgren, 1910)*	X[1]	X[1]				W	II
Nasutitermes wheeleri Emerson, 1925*		X				W	II
Rotunditermes bragantinus (Roonwal & Rathore, 1976)*	X					W	II
Subulitermes sp.			X			S	III
Triangularitermes triangulariceps Mathews, 1977	X[1]					S	III
Velocitermes sp.1	X[1]	X[1]				W	II
Velocitermes sp.2	X[1]	X[1]				W	II
Velocitermes sp.3			X			W	II
Syntermitinae							
Armitermes sp.					1	S	III
Cornitermes bequaerti Emerson, 1952**	X				2	L	II
Cornitermes pugnax Emerson, 1925*	X	X[1]	X			L	II
Cornitermes bolivianus Snyder, 1926★	X[1]	X[1]				L	II
Cornitermes pilosus Holmgren, 1906		X				L	II
Curvitermes odontognathus (Silvestri, 1901)				X		S	III
Cyrilliotermes angulariceps (Mathews, 1977)	X[1]					S	III
Embiratermes neotenicus (Holmgren, 1906)**	X[1]	X				W/S	III
Embiratermes sp.	X[1]					S	III
Labiotermes labralis (Holmgren, 1906)*		X				S	IV
Mapinguaritermes peruanus (Holmgren, 1906)	X[1]					W/S	III
Rhynchotermes amazonensis Constantini & Cancello, 2016	X[1]					L	II
Silvestritermes heyeri (Wasmann, 1915)*	X[1]	X				W/S	III
Silvestritermes minutus (Emerson, 1925)*	X[1]					W/S	III
Syntermes aculeosus Emerson, 1945*	X[1]					L	II
Syntermes brevimalatus Emerson, 1945	X[1]					L	II
Syntermes chaquimayensis (Holmgren, 1906)*					2	L	II
Syntermes molestus (Burmeister, 1839)	X[1]	X[1]				L	II
Syntermes spinosus (Latreille, 1804)*		X				L	II
Syntermes cf. *territus* Emerson, 1924	X[1]					L	II
Uncitermes almeriae Carrijo, 2016★		X				W/S	III
Uncitermes teevani (Emerson, 1925)	X	X				W/S	III
Vaninitermes ignotus (Constantino, 1991)	X[1]					S	III
Termitinae							
Cavitermes tuberosus (Emerson, 1925)		X		X		S	III
Cornicapritermes mucronatus Emerson, 1950	X[1]					S	III
Crepititermes verruculosus (Emerson, 1925)	X[1]					S	III

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Family/subfamily/species	**Sampling locality**				**Literature**	**Feeding groups**	
	SDNP	**CSP**	**HFR**	**CMEP**		**FG1**	**FG2**
Cylindrotermes flangiatus Mathews, 1977	X[1]					W	II
Cylindrotermes parvignathus Emerson, 1949	X[1]					W	II
Inquilinitermes sp.					1	I	III
Microcerotermes arboreus Emerson, 1925*		X[1]				W	II
Microcerotermes strunckii (Sörensen, 1884)*	X[1]	X[1]	X		1	W	II
Neocapritermes guyana Krishna & Araujo, 1968	X[1]					W	III
Neocapritermes pumilis Constantino, 1991	X[1]				2	W	III
Neocapritermes talpa (Holmgren, 1906)		X[1]	X		2	W	III
Neocapritermes talpoides Krishna & Araujo, 1968	X[1]					W	III
Neocapritermes utiariti Krishna & Araujo, 1968		X				W	III
Orthognathotermes sp.1		X				S	IV
Orthognathotermes sp.2		X				S	IV
Planicapritermes planiceps (Emerson, 1925)	X[1]					W	III
Spinitermes trispinosus (Hagen, 1858)	X[1]					S	III
Termes ayri Bandeira & Cancello, 1992	X[1]	X				S	III
Termes fatalis Linnaeus, 1758*		X				S	III
Termes hispaniolae (Banks, 1918)*		X[1]				S	III
Termes medioculatus Emerson, 1949**	X[1]	X[1]				S	III
Species Richness	**89**	**62**	**19**	**4**	**16**		

Discussion

Considering the data presented in this study, together with the species and morphospecies already published (Thomazini & Thomazini 2002, Constantino 2022, Santos 2022, Santos et al. 2022), there are a total of 128 species recorded for the Brazilian state of Acre, representing an increase of 112 new species now known in Acre. Six species are new records for Brazil, revealing that although much is already known about Brazilian biodiversity, improved sampling in even a few sites can contribute new records for Brazil, plus new taxa to science.

The geographic proximity of Acre state to other South American countries, such as Peru and Bolivia, means that species already known to occur in these countries may also occur in Brazilian territory, since the similar climatic conditions and the short distance between these countries can favor their dispersion (Tatem & Hay 2007, Seebens et al. 2013, 2015). An example of this is the species *Uncitermes almeriae* Carrijo, 2016 (Figure 2G, 2H), which was recorded here in Brazil for the first time but which was detected previously in Peru and Ecuador in 2016 (Carrijo et al. 2016). Faunal inventories, such as this one, can decrease gaps in sampling (*Wallacean shortfall*), improving information on the geographical distribution of taxa. They can also provide important taxonomic contributions, especially considering that, in the case of termites, the precise and correct identification of some taxa can be complicated, and specific groups have many species still undescribed (*Linnean shortfall*) (Hortal et al. 2015).

Although not a megadiverse insect group, termite taxonomy is relatively difficult. Considering the new vouchers found here for the state of Acre, 32 morphospecies are identified at the genus (17) and subfamily (15) levels. The subfamily Apicotermitinae has the largest number of morphotyped records, 18, including all morphospecies at the subfamily level. In many published termite species lists, the subfamily Apicotermitinae appears with notable taxonomic inaccuracies (Bandeira & Vasconcellos 2002, Bandeira et al. 2003, Vasconcellos 2010, Araújo et al. 2015, Couto et al. 2015, Silva et al. 2019). This problem is largely due to the absence of the soldier caste in Neotropical species of the subfamily Apicotermitinae, since this caste comprises the main morphological characters important for termite taxonomy (Carrijo et al. 2023). Identification in this subfamily is also difficult due to differences among workers of the same species, which can indicate intrapopulation variations (Bourguignon et al. 2016b). As a result, comparative analyses at the species level are not always possible, making it harder to carry out ecological studies for different assemblages using literature data (Dambros et al. 2017, Cerezer et al. 2020).

The pattern of species richness for sites sampled in this study roughly follows those found in other studies conducted in humid forests (Roisin et al. 2006, Jones & Eggleton 2011), where Termitidae has the highest species richness, with Apicotermitinae being the most species-rich subfamily within this family. Regarding feeding groups, a similar pattern to literature data is also observed, where the majority of termite species feed on soil (42%) and wood (34%) (Davies et al. 2003, Ernesto et al. 2014, Silva et al. 2019, Castro et al. 2021b).

The higher species richness of soil-feeders is possibly related to soil type and use of sampling protocols that use time per area, which favors more soil sampling in search of termite colonies. In general, sandy soils provide little stability for building tunnels and underground nests by termites, disfavoring soil-feeders, which apparently prefer clayier soils (Lee & Wood 1971, Vasconcellos et al. 2005). Several other studies carried out in Amazonia also found a higher proportion of soil-feeder species (Davies 2002, Bourguignon et al. 2011). Termite soil-feeders tend to be more abundant and more evenly distributed in areas with denser forests because environmental conditions favor the development of more humus-rich soils (Davies et al. 2021). In other ecosystems in Brazil, there are records of higher species richness of wood-feeders, such as in the Caatinga and some locations in the Atlantic Forest (Vasconcellos 2010, Souza et al. 2012, Cancello et al. 2014).

Regarding the presence of conspicuous nests, most nest-building species are widely distributed throughout the Amazon Rainforest (Constantino 1992, Palin et al. 2011, Dambros et al. 2012, Castro et al. 2021b). Termite nests, both active and abandoned, are part of the structure of tropical ecosystem and can serve as shelter and sites for predation and reproduction for various species of distinct functional groups, including other insect, arachnids, amphibians, reptiles, and birds (Cunha & Brandão 2001, Vasconcelos et al. 2015). Furthermore, the type (epigeal, arboreal, etc.) and density of nests, in addition to the composition of the building termite community, can provide relevant information about the levels of anthropogenic disturbance at local and regional scales (Vasconcellos et al. 2008).

This study provides an essential contribution to our knowledge of biodiversity in Acre and Brazil, even though only four areas were sampled, and only two of these using the rapid termite inventory protocol. Thus, in the case of the Amazon, which hosts the greatest species diversity on Earth (Flores et al. 2010), the number of species presented here is certainly less than the actual diversity. The Amazon hosts about 70% of all termite species known in Brazil (Constantino 2022), yet many species are still unknown in the Amazon. In addition to Serra do Divisor National Park, there are ten other federal protected areas in Acre, as well as state parks such as Chandless State Park and other smaller protected areas such as Humaitá Forest Reserve and Chico Mendes Environmental Park, which were sampled in this study. Conducting and expanding expeditions beyond these areas is essential for improving our knowledge of termite diversity in Acre and Brazil.

The urgent need for these studies is exemplified by the case of Serra do Divisor National Park, considered one of the most diverse areas in the world (Koga et al. 2022) but now threatened by a highway development to connect the municipalities of Cruzeiro do Sul, in Acre, and Pucallpa, in Peru, that would cross the park, causing irreparable environmental impacts (Koga et al. 2022). Limited species inventories have been conducted here for some groups, such as bats (Nogueira et al. 1999), but the biodiversity for SDNP is still notably undersampled in comparison to other areas of Acre that have been better covered by surveys for taxa including ants (Miranda et al. 2012), snakes (Silva et al. 2010, Bernarde et al. 2011), butterflies (Mielke et al. 2010), mammals (Botelho et al. 2012), scorpions (Lira et al. 2021) and dragonflies (Miranda Filho et al. 2022). New inventories contribute not only to biodiversity knowledge for the area, but also influence conservation policies for protected areas.

The SDNP has a high rate of endemism (Souza et al. 2003, Almeida et al. 2022). One of the key factors that make this area special is the influence exerted by the Andes Mountains. Currently, through paleoclimatic modeling and molecular analyses, it is known that geomorphological and phylogenetic modifications in the Amazon occurred in parallel (Hoorn et al. 2010). The uplift of the Andes created an orographic barrier, altering the influence of moisture from the Pacific Ocean on the Amazon (Poulsen et al. 2010), and changes in the topography affected the direction of river flow within the Amazon basin (Costa et al. 1996, Figueiredo et al. 2009). All of these dynamics also influenced the composition of the soil in the region, with the soil in the western portion being much richer than that in the eastern portion (Quesada et al. 2010).

Among the nine existing state Conservation Units in Acre, CSP presents the highest known biodiversity, with 1,392 species including insects, reptiles, amphibians, birds, mammals, and plants, and this value is obviously underestimated (Lacerda et al. 2022). This study contributes to the knowledge of the park's biodiversity, adding to its preservation as a Conservation Unit, which provides important socio-environmental services such as protection of flora and fauna, an area for scientific research, significant for the development of environmental education activities, ecotourism, protection and maintenance of water sources, and the promotion of health and well-being for those who interact with the natural landscape (Lacerda et al. 2022).

The HFR and CMEP areas, despite being smaller, encompass important diversity of animal and plant groups, having already been the subject of several studies (Pinheiro et al. 2015, Venâncio & Souza 2016, Lima et al. 2021). However, termites had not yet been sampled in these areas, given the importance of this work for the knowledge of biodiversity in these forest fragments. Being smaller and relatively closer to urban centers, these protected areas are excellent spaces for the development of environmental education activities, especially with children, since they are close to schools, making the fragments accessible didactic resources for the production of local environmental knowledge (Cerati & Lazarini 2009). Additionally, urban forest fragments, such as CMEP, promote sociability by providing environments that stimulate physical exercise, reduce stress and attention deficit in adolescents and children, contributing to the improvement of the population's well-being by enhancing the city's environmental quality, regulating temperature and air humidity, protecting water bodies, reducing pollution, among many other benefits (Rosso et al. 2021).

Species inventories are important and have an impact on several areas of knowledge beyond taxonomy, being fundamental to compose ecological analyses (Wijas et al. 2022), for example. In this study, we have an increase of 128 species for one Brazilian state, plus six new records for the country. Because it harbors the greatest biodiversity on the planet (Vieira et al. 2005), it is crucial to know which species exist in the Amazon, so that new and rare species can be identified, helping to conserve entire ecosystems (Ngiam & Cheong 2016). Species inventories can help to understand the degree of threat that species face and as a consequence, guide conservation efforts and allocate resources more effectively to protect biodiversity, as many species may be at risk of extinction but not yet identified as threatened or endangered (Costello et al. 2013).

In addition, knowledge of the diversity of the Amazon can reveal the presence of bioindicator species (Rodríguez et al. 1998), contributing to the monitoring of environmental changes, such as habitat loss, climate change, as well as other factors that affect biodiversity.

Supplementary Material

The following online material is available for this article:

Figure S1 – Enteric valves of Apicotermitinae morphospecies presented in this article. Apicotermitinae sp.1 (A), Apicotermitinae sp.2 (B), Apicotermitinae sp.3 (C), Apicotermitinae sp.4 (D), Apicotermitinae sp.5 (E), Apicotermitinae sp.6 (F), Apicotermitinae sp.7 (G), Apicotermitinae sp.8 (H), Apicotermitinae sp.9 (I), Apicotermitinae sp.10 (J), Apicotermitinae sp.11 (K), Apicotermitinae sp.12 (L), Apicotermitinae sp.13 (M), Apicotermitinae sp.14 (N) and Apicotermitinae sp.15 (O).

Figure S2 – Enteric valves and junction S/P3 (only A) of Apicotermitinae species presented in this article. *Compositermes vindai* (A, B), *Longustitermes manni* (C), *Hirsutitermes kanzakii* (D), *Patawatermes turricola* (E), *Rustitermes boteroi* (F), *Mangolditermes curveileum* (G), *Aparatermes silvestrii* (H), *Anoplotermes banksi* (I), *Krecekitermes daironi* (J), *Anoplotermes susanae* (K), *Anoplotermes* aff. *pacificus* (L), *Anoplotermes meridianus* (M), *Disjunctitermes* aff. *insularis* (N), *Disjunctitermes insularis* (O), *Hydrecotermes kawaii* (P) and *Patawatermes nigripunctatus* (Q).

Figure S3 – Enteric valves of *Anoplotermes* morphospecies presented in this article. *Anoplotermes* sp.1 (A) and *Anoplotermes* sp.2 (B).

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Author Contributions

Renan Rodrigues Ferreira: methodology; specimens' identification; writing – original draft; writing – review & editing.

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Renato Almeida de Azevedo: sampling; writing – original draft; writing – review & editing.

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Conflicts of Interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author(s) used ChatGPT-3.5, an AI language model developed by OpenAI, exclusively for the purpose of enhancing readability and language quality during the writing process. After using this AI tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Data Availability

The datasets generated during and/or analyzed during the current study are available at: https://doi.org/10.48331/scielodata.ENCMUK

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Short Communication

First occurrence in mangroves of South America of the exotic species *Sonneratia apetala* Buch.-Ham. from the Indo-Malayan region

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Abstract: This is the 1st record of the *Sonneratia apetala* Buch.-Ham., in the mangroves of South America – Cubatão-Baixada Santista, State of São Paulo, Brazil. The record was made in an area located 2 km apart from the port of Santos. Its arrival was probably made through the ballast water. S. apetala as well an exotic it is also an aggressive mangrove species. All 85 individuals identified were georeferenced. In May 2023 they were flowering and in August they already had ripe fruits/propagules. The presence of exotic species in mangroves is worrying, they can cause profound ecological and economic impacts and negatively influence the restoration of native ecosystems. The presence of S. apetala in the mangroves of Cubatão region could bring about a radical change in the biological structure of the mangrove ecosystem, in addition to being a new point of dispersal to others areas.

Keywords: *Ballast water; biological invasions; invasive species; mangrove; Sonneratia apetala; South America.*

Primeiro registro nos manguezais da América do Sul da espécie exótica *Sonneratia apetala* Buch.-Ham da região Indo-Malaia

Resumo: Este é o primeiro registro da ocorrência de *Sonneratia apetala* Buch.-Ham., na América do Sul, Cubatão-Baixada Santista, Estado de São Paulo, Brasil. O registro ocorreu em área situada a 2 km do porto de Santos. Sua presença, se fez, provavelmente, por meio da água de lastro dos navios. A S. apetala é uma espécie exótica agressiva nos manguezais dessa região. Todos os 85 exemplares identificados foram georeferenciados. Em maio de 2023 estavam floridas e em agosto já tinha frutos/propágulos maduros. A presença de espécies exóticas nos manguezais é preocupante, pois são capazes de gerar impactos ecológicos e econômicos e influenciar, negativamente, na restauração desse ecossistema no estuário de Cubatão. A presença da S.apetala, no manguezal da região de Cubatão pode povocar uma mudança radical na estrutura biológica do ecossistema manguezal, além de ser um novo ponto de dispersão para outras áreas.

Palavras-chave: *Água de lastro; invasão biológica; espécies invasoras; manguezal; Sonneratia apetala; América do Sul.*

Introduction

The presence of exotic species in natural ecosystems can have profound ecological and economic impacts. Sometimes alien species are accidentally introduced, others due to negligence or lack of norms.

The Port of Santos (23°57'S latitude and 046°19'W longitude), located in the state of São Paulo, Brazil, is one of the busiest and most important ports in Latin America.

Due to its strategic location and extensive infrastructure, the Port of Santos attracts a diverse range of commercial vessels, connecting Brazil to global markets and facilitating trade with countries around the world. Located approximately 2 kilometers northeast of the port, an extensive mangrove area (6,000 ha or 60 km^2) forms part of the larger coastal ecosystem known as the Santos Estuary (approximately 23°54´S latitude and 046°23´W longitude).

The mangroves of Baixada Santista-Brazil, where the Cubatão mangroves are located, has in total more than 12 thousand hectares. Our restauration project area is in the Municipality of Cubatão has 2,725 hectares. The study area, has the referenced central point (23°54´10.62″W and 046°23´48.28″W), is approximately 1,550 hectares, whose mangroves, until the mid-1990s, were practically degraded. Today the recovery stage is quite advanced because of the numerous actions to control the pollution and various impacts were also controlled, in parallel to the fact that, since 1993 we are developing a restauration mangrove project in the Baixada Santista Estuary region.

The Baixada Santista Estuary main environmental and climate characteristics can be described as a coastal plain formed by Holocene origin sandy islands, exposed twice a day to tides with maximum amplitude of 1.8 m, salinity values ranging from oceanic to fresh water related to the combined effects of the tides and the freshwater inputs, regulated by high precipitation rates in the summer months (December, January and February) and mean annual values of 1,922 mm.

The relative closeness of the mangrove area to the Port of Santos highlights the potential for ecological interactions between the two areas, particularly in terms of water quality and the introduction of exotic species through ballast water discharge.

This proximity underscores the importance of monitoring and managing the ecological integrity of the mangroves of Cubatão, especially considering the potential risks posed by exotic species and other well-known disturbances associated with the port's area operations.

S. apetala presence in the mangroves of Cubatão Baixada Santista Estuary is cause of great concern, as it seems to adapt very quickly and some specimens already have reached more than 12 meters in height. Most of the specimens identified so far are located on the mangrove fringe, although 5 (five) specimens have already been identified more than 70 meters into the mangrove basin stand.

Sonneratia apetala Buch.-Ham., a pioneer species in ecological succession, is native of the mangroves of the Indo-Malayan region (Tomlinson, 1986). The trees are known to occur on soil under a range of salinity conditions (Rahman et al., 2020).

It is very likely that the exotic specie's presence in Cubatão mangroves, was due to the release of ballast water from international ships and the proximity to port facilities.

In Brazil the CONABIO Resolution nr 7, May 29, 2018, concerned with the identification of new introduced species, emphasizes that introduction of exotic species can cause changes in biomes and this fact has been a concern that requires profound consideration of adopting recommended measures that when identifying the presence of an exotic species, preventive and corrective management measures are urged to be taken.

Material and Methods

First species were located in the monitoring area (Figure 1), then boat incursions were made in the Perequê river, Cubatão river and the area near to the harbor of Santos to check the extension of presence of *Sonneratia apetala* in the mangroves. GPS positioning were registered by Garmin Etrex 10 and photograph records were done.

After the first identification of a specimens of *S. apetala*, it was decided to use a drone, aiming to facilitate the presence of other specimens so, the mangrove area was scanned by using the TOPODRONE 200 Ultra embedded in FJI M300 RTK drone, the profiler includes a Velodyne Ultra Puck sensor, a high-precision IMU (Honeywell), and a receiver HNSS L1/L2 Survey (U-BÇOX) (Figure 2).

Drone images were analyzed, some specimens were localized, and their presence was confirmed locally.

Results and Discussion

Sonnetratia apetala are easily recognized in the field by the peculiar shape of the canopy (Figure 3), size and number of pneumatophores (Figures 4 and 5), leaf format and color (Figure 6) and the distinctive fruits (Figures 7 and 8) and flowers (Figure 9).

The presence of *S. apetala* was noted in May 2023. To date, more than 80 specimens have been found in the Cubatão-Baixada Santista mangrove (São Paulo-Brazil) (Figure 10). All specimens were georeferenced with the aim of being able to track them individually for later studies but also to investigate their possible dispersal route, from the Santos harbor to more distant locations.

Some observed specimens are up to 12 meters tall; pneumatophores are four times higher than those of native species.

A large quantity of fruits was observed, in one tree more than 1,000 propagules were counted and within each fruit up 60 seeds (Figure 11). Ripe fruits and seeds float, which facilitates their dispersion by the tide. It was also observed that the fallen fruits are already part of the animals' diet (as some specimens were partially eaten).

Its distribution is in a still relatively restricted area but is clear that it is related to the route of ships, hence the assumption that the fruits/propagules (and/or seeds) have been transported through ship ballast

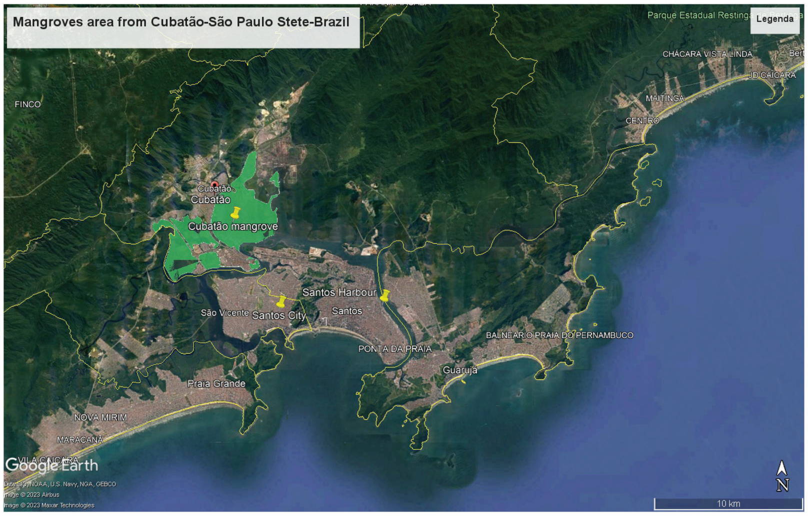

Figure 1. Mangroves of the Cubatão-Baixada Santista estuary where the Port of Santos is located.

Figure 2. TOPODRONE 200 Ultra embedded in FJI M300 RTK drone, the profiler includes a Velodyne Ultra Puck sensor, a high-precision IMU (Honeywell), and a receiver HNSS L1/L2 Survey (U-BÇOX).

Figure 3. One of the specimens of *Sonneratia apetala* (Photo E. Hatamura).

water. A probable route was traced, and the location of the identified specimens corroborate this option.

All these factors support with its ability to rapid dispersal and adaptation (Ren *et al.* 2009). Although this species is widely found in China's restoration projects (Chen *et al*. 2009; Fazlioglu *et al.* 2020).

In addition to this first record of *S. apetala* in South America, it is urgent that we consider the ecological issue of the presence

Figure 4. Roots of *Sonneratia apetala* (extend up to 20 meters) (Photo E. Hatamura).

Figure 5. Characteristics of pneumatophores of *Sonneratia apetala* (up to 80 cm high) (Photo E. Hatamura).

Figure 6. Leaf of *Sonneratia apetala* (Photo E. Hatamura).

Figure 7. Trees of *Sonneratia apetala* with fruit (Photo E. Hatamura).

Figure 8. Fruits of *Sonneratia apetala* (Photo E. Hatamura).

Figure 9. Flower of *Sonneratia apetala* (Photo G.G.J. Eysink).

of this species, aiming to assess the need for its total eradication, before the situation becomes irreversible mainly due to the size of the trees; the large amount of fruits and seeds, without forgetting to mention the species aggressiveness of its roots and structure of the pneumatophores.

All these factors highlight the aggressiveness of the species whether in the occupation of the area, competition with native species and its great potential and success for dispersal. It is worth noting here that the mangroves in the Cubatão region and the Baixada Santista Estuary, previously degraded, are now at an advanced stage of recovery, but there are still places to be restored.

Figure 10. Location of georeferenced *Sonneratia apetala*.

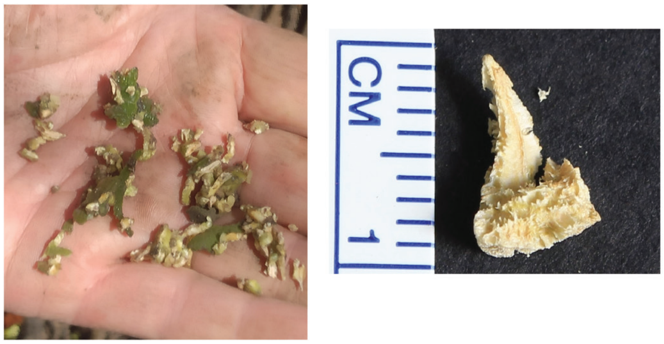

Figure 11. Seeds of *Sonneratia apetala* (Photo E. Hatamura).

The presence of *S. apetala* in South American soil (actually in Brazilian territory) can lead to a radical change in the ecosystem configuration and the biological diversity of local native species.

The presence of this exotic species (especially due to its aggressive characteristics and ability to occupy the still unoccupied niches), could negatively influence the reestablishment of all mangroves with species native to the region and could even cause significant socio-environmental impacts.

Sonneratia apetala presence is a casualty and brings into question enormous impacts that exotic species can cause prejudices where the mangroves have a very strong and important relationship with the presence of artisanal fishermen and also due to the multiple ecosystem's role (including the not silting up the harbors).

It should be considered the issue that mangroves are a nursery for important commercial fishes and other species (birds, crabs, and fish), and there are fish species of great economic value that move more than 2,000 km to come to this estuary to spawn.

An urgent decision to eradicate this invasive species is strongly recommended by the authors, as well as the implementation of an Environmental Management Policy.

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Author Contributions

Geraldo Guilherme José Eysink: substantial contribution to the concept and design of the study; data collection; analysis and interpretation; manuscript preparation and critical revision; adding intellectual content.

Edmar Hatamura: data collection; analysis and interpretation; manuscript preparation and critical revision.

Yara Schaffer-Novelli: substantial contribution to the concept and design of the study; analysis and interpretation; manuscript preparation and critical revision; adding intellectual content.

Conflicts of Interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data availability

Supporting data are available at: https://data.scielo.org/dataset.xhtml?persistentId=doi:10.48331/scielodata.A5MQL.

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Inventory

Insect galls from the Serra da Bandeira (Barreiras, Western Bahia, Brazil)

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MENEZES J.B., LIMA V.P., CALADO D.C. **Insect galls from the Serra da Bandeira (Barreiras, Western Bahia, Brazil)**. Biota Neotropica 23(4): e20231527. https://doi.org/10.1590/1676-0611-BN-2023-1527

Abstract: Understanding the diversity of insect galls is pivotal to the establishment of conservation planning in different Brazilian ecosystems. Here, we (1) characterize the insect galls found on plant host species, (2) identify the gall-inducing insects to the lowest possible taxonomic level, and (3) record the presence of gall-associated fauna. Our study was carried out monthly from August, 2017 to July, 2018 along nine trails in Serra da Bandeira, which is located in an area of Cerrado stricto sensu over a year. We found 48 distinct gall morphotypes, belonging to 12 botanical families. The most morphotypes commonly found were globoid (27.1%), lenticular (27.1%) and cylindrical (25%). The plant organs most affected by galling insects were the leaves (73%), followed by the stem (19%), flower (4%), inflorescence (2%) and fruits (2%). The host botanical families holding the highest number of galls were Fabaceae (26), Malpighiaceae (5) and Anacardiaceae, Combretaceae and Euphorbiaceae (3). We observed that the genus *Copaifera* Lindl. (Fabaceae) had the greatest diversity of gall morphotypes, harboring 19 morphotypes distributed in three species that occur sympatrically - *Copaifera sabulicola* J. Costa (Fabaceae), *Copaifera depilis* Dwyer (Fabaceae) and *Copaifera luetzelburgii* Harms (Fabaceae). For the first time in Brazil, we recorded the occurrence of galls on flowers of *Manihot caerulescens* (Euphorbiaceae), on inflorescences of *Mimosa acutistipula* (Fabaceae) and flower buds of *Anacardium humile* (Anacardiaceae), which were induced by Cecidomyiidae. Inducers of the order Diptera (Family Cecidomyiidae) were the most abundant, found in 14 morphotypes of galls. Regarding the associated fauna, we found insects primarily belonging the order Hymenoptera, and identified them as parasitoids. The information provided can be used highly by decision makers for conservation programs, as well as in other strategies for the conservation of biological diversity in the Brazilian Cerrado.

Keywords: *Cerrado; Cecidomyiidae; Gall inducing insects; Galling insects; Insect-plant interaction.*

Galhas de insetos da Serra da Bandeira (Barreiras, Oeste da Bahia, Brasil)

Resumo: Compreender a diversidade de galhas de insetos é fundamental para o estabelecimento de planos de conservação em diferentes ecossistemas brasileiros. Aqui, nós (1) caracterizamos as galhas de insetos encontradas em espécies de plantas hospedeiras, (2) identificamos os insetos indutores de galhas até o nível taxonômico mais baixo possível e (3) registramos a presença de fauna associada a galhas. Nosso estudo foi realizado mensalmente de agosto de 2017 a julho de 2018 ao longo de nove trilhas na Serra da Bandeira, que está localizada em uma área de Cerrado stricto sensu ao longo de um ano. Encontramos 48 morfotipos distintos de galhas, pertencentes a 12 famílias botânicas. Os morfotipos mais comumente encontrados foram globoide (27,1%), lenticular (27,1%) e cilíndrico (25%). Os órgãos vegetais mais afetados pelos insetos galhadores foram as folhas (73%), seguidas do caule (19%), flor (4%), inflorescência (2%) e frutos (2%). As famílias botânicas hospedeiras com maior número de galhas foram Fabaceae (26), Malpighiaceae (5) e Anacardiaceae, Combretaceae e Euphorbiaceae (3). Observamos que o gênero *Copaifera* Lindl. (Fabaceae) apresentou a maior diversidade de morfotipos de galhas, abrigando 19 morfotipos distribuídos em três espécies que ocorrem simpatriicamente – *Copaifera sabulicola* J. Costa (Fabaceae), *Copaifera depilis* Dwyer (Fabaceae) e *Copaifera luetzelburgii* Harms (Fabaceae). Pela primeira vez no Brasil, registramos a ocorrência de galhas em flores de *Manihot caerulescens* (Euphorbiaceae), em inflorescências de *Mimosa acutistipula* (Fabaceae) e em botões florais de *Anacardium humile* (Anacardiaceae), induzidas por Cecidomyiidae. Indutores da ordem Diptera (Família Cecidomyiidae) foram os mais abundantes, encontrados em 14 morfotipos de galhas. Em relação à fauna associada, encontramos insetos pertencentes principalmente à ordem Hymenoptera, e os identificamos como parasitóides. As informações fornecidas podem ser utilizadas por tomadores de decisão para programas de conservação, bem como em outras estratégias para a conservação da diversidade biológica no Cerrado brasileiro.

Palavras-chave: *Cerrado; Cecidomyiidae; Insetos indutores de galhas; Insetos galhadores; Interação inseto-planta.*

Introduction

Galling insects are specialized herbivores that redirect plant resources in order to develop themselves during their cycle in host plants (Marini-Filho & Fernandes 2012, Gagné & Jaschhof 2017, Lima & Calado 2020). Such development is an adaptive evolutionary strategy of certain groups for obtaining food and protection against predators (Gonçalves-Alvim & Fernandes 2001a). Insect galls are developed from tissue hyperplasia and cell hypertrophy (Martini et al. 2019, Isaias et al. 2013, Mani 1964) due to abnormal differentiation in mechanical and chemical response of inducers, resulting in characteristic growth that harbor gall-inducing insects (Santos-Silva & Araujo 2020). Currently, six orders of gall-inducing insects are known to science: Diptera, Hymenoptera, Thysanoptera, Coleoptera, Lepidoptera and Hemiptera (Maia & Monteiro 1999). Among Diptera, the Cecidomyiidae family is the main family of galling insects not only in the Neotropics, but also in other regions (Maia 2013a). To this date, 280 species and 103 genera have already been described belonging to the Cecidomyiidae family in Brazil (Maia 2023).

Many species of galling insects are economically important, inducing galls on cultivated plants used daily as food, such as *Anacardium humile* A.St.Hil. (Anacardiaceae), and *Caryocar brasiliense* Cambess (Caryocaraceae), as well as other medicinal and ornamental plants (Fernandes & Price 1988). Galling insects are recognized as ecosystem engineers, as they can directly or indirectly modulate the availability of resources for other species, responsible for modifying, creating and maintaining microhabitats (Jones et al. 1994). Furthermore, a large number of associated fauna (parasitoids, tenants, predators and successors) are regularly found in insect galls with great richness and abundance, thus increasing the ecological importance of galling insects (Maia & Monteiro 1999, Santos et al. 2018, Soares et al. 2021, Lima & Calado 2018, Maia 2013b).

Although several inventories of insect galls have been carried out in Cerrado areas, particularly in the states of Minas Gerais and Goiás (Araújo 2018), few studies have covered the Cerrado of western Bahia (Lima & Calado 2018). Besides holding about 5% of the planet's biological diversity, the Cerrado is one of the 25 world biodiversity hotspots, due to the high diversity of endemic species and extensive loss of habitats through anthropogenic actions (MMA 2017). The vegetation of the Cerrado is diverse, presenting characteristics of forest, savannah and grassland formations. Among the various vegetation formations of the Cerrado, there is the Cerrado stricto sensu, which is characterized by trees randomly distributed on the ground in different densities, as well as by the presence of defined tree and shrub-herbaceous strata (Sano et al. 2008). It is estimated that the invertebrate fauna of the Cerrado is approximately 90 thousand species. However, this fauna is still poorly known (Oliveira & Frizzas 2008). Few efforts have sought to study the diversity of insects in Western Bahia, and these have been suffering significant declines mainly because of the destruction of their suitable habitats to meet the demand of agribusiness in the region (Soares et al. 2021, Lima & Calado 2018). Despite this chaotic scenario in the region, recent assessments in the region have characterized the ecology of galling species and described new species in heavily degraded areas (Garcia et al. 2017, Santos et al. 2018, Soares et al. 2021).

Besides their important role as bioindicators of environmental quality (Santana & Isaias 2014), understanding the diversity of galling insects and the associated flora in tropical Brazilian ecosystems is extremely important to comprehend the patterns of distribution and occurrence of these herbivores in the Cerrado of Western Bahia, which effectively contributes as a tool for ecological study and conservation. Here, we (1) characterize the insect galls found on plant host species, (2) identify the gall-inducing insects to the lowest taxonomic level, and (3) record the presence of gall-associated fauna.

Material and Methods

Our study was carried out along trails in the Serra da Bandeira (–12° 05' 29'S and –45° 02' 23'W), located in the municipality of Barreiras (Figure 1). The area has a typical Cerrado vegetation with herbaceous and shrubby vegetation, predominance of subshrubs and shrubs, and great diversity of pioneer species in the secondary phase of succession (Cruz Ruggiero et al. 2002). The region has a seasonal climate, with two well-defined seasons, a rainy one, lasting from October to March, and a dry one, which lasts from April to September. The average annual rainfall in this biome is 1,500mm and temperatures are generally mild throughout the year (Nascimento & Novais 2020).

The study area was monthly sampled by the first author throughout one year, between August 2017 and July 2018, through walks between trails lasting 2 hours, which is a method commonly used in inventories of galling insects in Brazil (Soares et al. 2021, Lima & Calado 2018, Bregonci et al. 2010, Maia & Fernandes 2004). All plant organs were inspected on the host plants from ground level to the top of the canopy for the presence of insect galls. We then photographed the galls found to better characterize the morphological characteristics such as shape, hairiness and distribution on the plant (Isaias et al. 2013). Next, branches containing flowers and fruits were collected from the host plant for further identification of the species. Collected specimens were stored and transported them to the Laboratory of Invertebrate Zoology at the Federal University of Western Bahia (UFOB). The plant samples collected were stored in newspapers and cardboard containing 70% alcohol for subsequent assembly of specimens. These samples were sent to the BRBA Herbarium of UFOB for species identification by botanical taxonomists and incorporated into the collection under numbers from 7657 to 7676.

In the laboratory, part of the collected galls was dissected using tweezers and a stereomicroscope (Leica S8-APO) to look for the inducer and its associated fauna. The remains were kept in plastic containers containing cotton moistened with water and were stored in a BOD (SL-224) oven at a temperature of approximately 25 ^{o}C to obtain the insects in the adult phase. We monitored the containers daily for emergence of adult insects. The emerged adults' insects were used to mount permanent slides following the methodology proposed by Gagné (1994), and later identification using dichotomous keys (Gagné 1994) and other descriptive studies of galling insects. The associated fauna was preserved in 70% alcohol and identified at the lowest possible taxonomic level using identification keys from the Insects from Brazil: Diversity and Taxonomy (Rafael et al. 2012) and Luz & Mendonça Júnior (2019) as reference. Most of the associated fauna was obtained only at an immature stage and therefore we identified them only at the order level. Lastly, we summarized information on cecidomyids species, host plant species and associated fauna in a table.

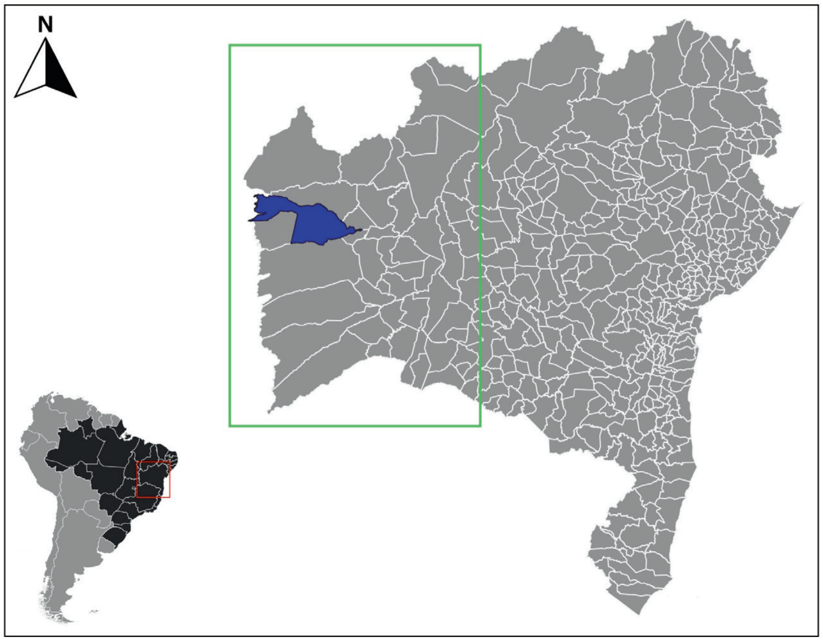

Figure 1. Study area. Location of the municipality of Barreiras, Bahia, Brazil depicted in blue. The west region of Bahia is highlighted in light green.

Results

We found and characterized 48 distinct gall morphotypes, belonging to 12 plant families (Figures 2, 3 and 4, Table 1). Insect galls showed different morphotypes such as globoid, cylindrical, lenticular, conical, fusiform and marginal leaf fold. The most commonly found were globoid (27.1%), lenticular (27.1%) and cylindrical (25%), followed by conical (12.5%) and fusiform (6.2%). The least abundant shape was marginal leaf fold (2.1%) (Figure 5). We observed insect galls developed on leaves, stems, flowers, inflorescences and fruits. The plant organs most affected by galling insects in the Serra da Bandeira were the leaves (73%), followed by the stem (19%), flower (4%), inflorescence (2%) and fruits (2%) (Figure 6). Green was the most frequent color found among the morphotypes (32), followed by brown (25) and red (11) (Table 1).

The host plant families with the highest number of gall morphotypes in Serra da Bandeira were Fabaceae (26), Malpighiaceae (5) and Anacardiaceae, Combretaceae and Euphorbiaceae (3) (Figure 7, Table 1). Furthermore, we found that the genus *Copaifera* Lindl. (Fabaceae) had the greatest diversity of gall morphotypes, harboring 19 morphotypes distributed in three species that occur sympatrically – *Copaifera sabulicola* J. Costa (Fabaceae), *Copaifera depilis* Dwyer (Fabaceae) and *Copaifera luetzelburgii* Harms (Fabaceae) (Table 1). Here, for the first time in Brazil, we recorded the occurrence of galls on flowers of *Manihot caerulescens* (Euphorbiaceae), on inflorescences of *Mimosa acutistipula* (Fabaceae) and flower buds of *Anacardium humile* (Anacardiaceae), which were induced by Cecidomyiidae. Galls on flowers of *Manihot caerulescens* Pohl (Euphorbiaceae) was induced by the species *Iatrophobia brasiliensis* (Rübsaamen, 1908).

Cecidomyiidae were found inducing galls in 14 morphotypes. Out of 48 morphotypes found in our study, inducers were not determined for 32 morphotypes. Additionally, we found Hymenoptera in 24 gall morphotypes, Lepidoptera in 2 gall morphotypes and Acari and Pseudoscorpiones in only one gall morphotype (Table 1).

Discussion

Inventories of gall-inducing insects in Brazil indicate Fabaceae as the most common host plant family for galling insects (Marinho et al. 2023, Maia 2013a, Lima & Calado 2018, Araujo et al. 2019), which confirms the richness hypothesis (Southwood 1960), also recorded in other studies carried out in the Cerrado (Gonçalves-Alvim & Fernandes 2001b, Santana & Isaias 2014). Among the Fabaceae family, *Copaifera* Lindl. (Fabaceae) had a great diversity of gall morphotypes, a result found by Santos et al. (2018), in the same area with three species of the *Copaifera* (*C. sabulicola C. depilis* and *C. luetzelburgii*). Furthermore, Nogueira et al. (2016), in a study in Serra Geral in the city of Caetité, Bahia, characterized ten morphotypes for *Copaifera langsdorffii* Desf. (Fabaceae) in transition areas between Cerrado and Caatinga. Therefore, our study contributes to scientific knowledge on insect galls by reinforcing that *Copaifera* species are super hosts of insect galls.

Here, we describe for the first time the occurrence of insect gall on flowers of *Manihot caerulescens* Pohl (Euphorbiaceae), which had not been observed in other studies carried out in different Brazilian ecosystems (Carneiro, Borges, et al. 2009, Araújo et al. 2011, Maia 2013a, Scareli-Santos et al. 2018, Saito & Urso-Guimarães 2012). Many studies have observed the occurrence of galls exclusively on leaves for the genus *Manihot* and the inducing insect has been identified as belonging to the genus *Iatrophobia* (Rübsaamen, 1915) (Diptera, Cecidomyiidae). The occurrence of the species *Iatrophobia brasiliensis* (Rübsaamen, 1908) in several species of the genus *Manihot* can be explained by the fact that the species is oligophagous. Carneiro et al. (2009) investigated the level of specificity of inducers and identified that about 5.6% of the 196 galling species studied are oligophagous, inducing galls on more than one host plant species of the same genus. Another new record is the occurrence of cylindrical and glabrous galls in the inflorescence of

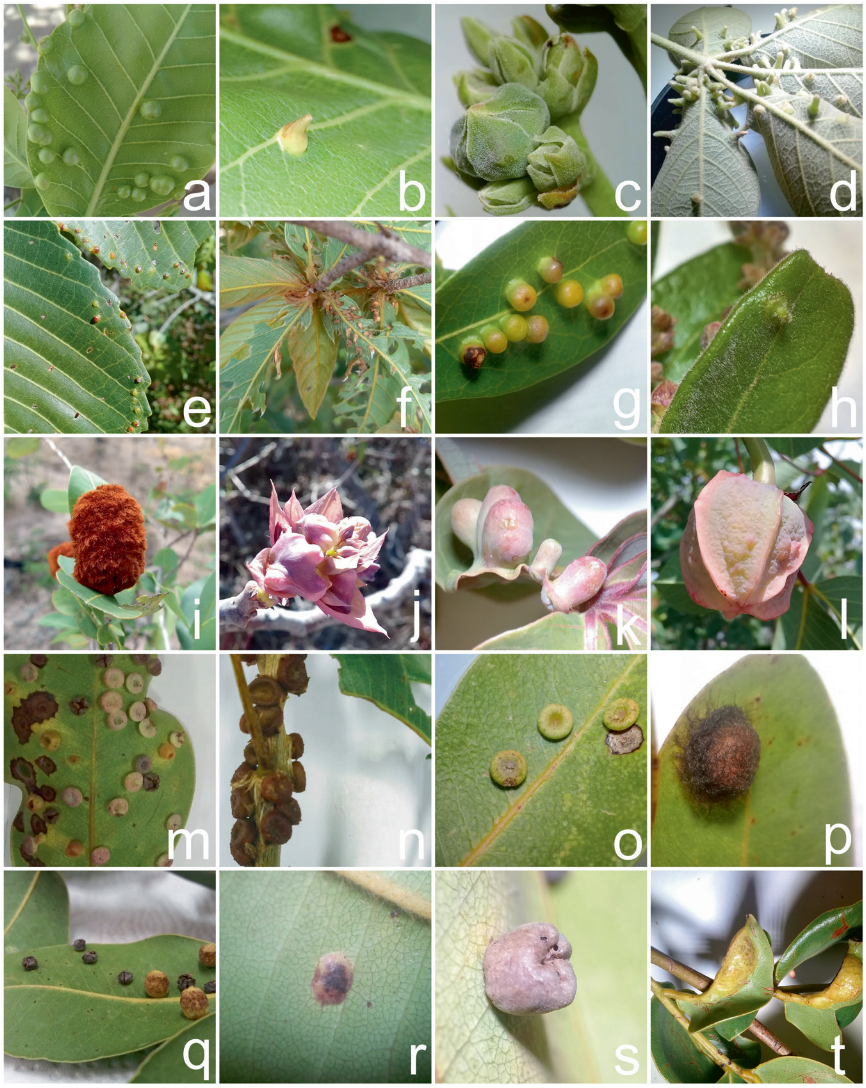

Figure 2. Insect galls found in the Serra da Bandeira (Barreiras, Bahia, Brazil). a. *Anacardium humile* – Globoid, b. *Anacardium humile* – Conical, c. *Anacardium humile* – Globoid, d. Bignoniaceae – Conical, e. *Caryocar brasiliense* – Globoid, f. *Terminalia fagifolia* – Conical, g. *Terminalia* sp. – Cylindrical, h. Combretaceae R. – Conical, i. *Erythroxylum* sp. – Globoid, j. *Manihot caerulescens* – Cylindrical, k. *Manihot caerulescens* – Cylindrical, l. *Manihot caerulescens* – Cylindrical, m. *Copaifera sabulicola* – Lenticular, n. *Copaifera sabulicola* – Lenticular, o. *Copaifera sabulicola* – Lenticular, p. *Copaifera sabulicola* – Globoid, q. *Copaifera sabulicola* – Globoid, r. *Copaifera sabulicola* – Lenticular, s. *Copaifera sabulicola* – Lenticular and t. *Copaifera sabulicola* – Marginal Leaf Fold.

Mimosa acutistipula (Fabaceae), whose inducer is an undetermined species of Cecidomyiidae. Many studies have reported the occurrence of insect galls in species of the genus *Mimosa* on leaves and stems (Nogueira et al. 2018, Vieira et al. 2018, Nogueira et al. 2016, Costa et al. 2014, Coelho et al. 2013), however, none for the species *Mimosa acutistipula*. Thus, this is the first record of the insect gall on this plant species, which is rare in field.

Furthermore, we found an unprecedented record of galls on flower buds, induced by a cecidomyiids on *Anacardium humile* (Anacardiaceae), a species popularly known as cajuí or cajuzinho do Cerrado. The record of galls in the *Anacardium* genus is very common in leaves (Vieira et al. 2018, Araújo et al. 2011). Such occurrence on flowers is relevant due to the economic importance, since the insect galls caused deformities in the flower buds, preventing the opening of the flowers and, consequently, the formation of the accessory fruit and the fruit (chestnut). According to ethnobotanical studies, o cajuzinho do Cerrado creates income, serves as food for local communities and consequently improves people's livelihoods (Vieira et al. 2016). In this context, investigations on the phenology of the plant, aiming at a better understanding of the incidence of the infection, as well as the damage caused in fruits and pseudofruits, are fundamental.

About 65% of the inducer species have not yet been determined, due to the absence of individuals in the adult (male and female), pupa and larva stages. Maia (2013) highlights the need to obtain gallers at each stage of the life cycle, as this is the only way to describe new species. Inducers of the order Diptera (Family Cecidomyiidae) were the most abundant, found in 14 morphotypes of galls. Cecidomyiidae is the main family of gall-inducing insects throughout Brazil and the Neotropics (Gagné & Jaschhof 2017, Fernandes et al. 1988). The distribution of gallers in the different orders of insects and their host plants, observed

Figure 3. Insect galls found in the Serra da Bandeira (Barreiras, Bahia, Brazil). a. *Copaifera sabulicola* – Globoid, b. *Copaifera sabulicola* – Lenticular, c. *Copaifera depilis* – Lenticular, d. *Copaifera depilis* – Globoid, e. *Copaifera depilis* – Cylindrical, f. *Copaifera depilis* – Lenticular, g. *Copaifera depilis* – Globoid, h. *Copaifera luetzelburgii* – Cylindrical, i. *Copaifera luetzelburgii* – Lenticular, j. *Copaifera luetzelburgii* – Lenticular, k. *Mimosa sericantha* – Cylindrical, l. *Mimosa sericantha* – Cylindrical, m. *Caliandra* sp. – Globoid, n. *Caliandra* sp. – Fusiform, o. *Mimosa acutistipula* – Cylindrical, p. *Fabaceae* – Fusiform, q. *Bauhinia* sp1 – Globoid, r. *Bauhinia* sp2 – Fusiform, s. *Diplopterys* sp. – Conical and t. *Diplopterys* sp. – Globoid.

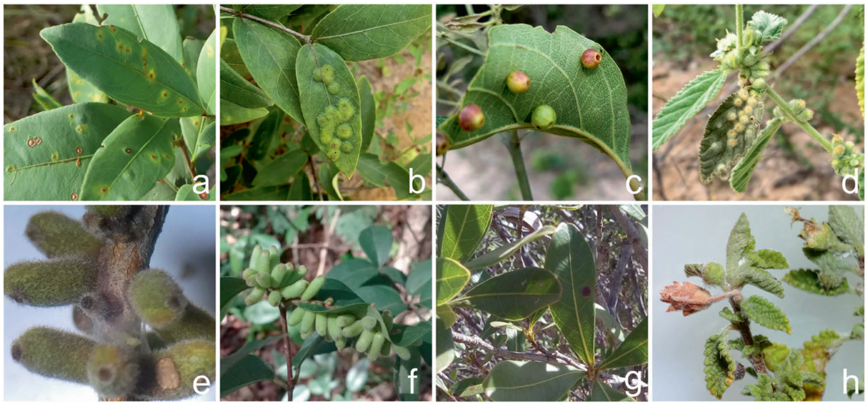

Figure 4. Insect galls found in the Serra da Bandeira (Barreiras, Bahia, Brazil). a. *Byrsonima* sp. – Lenticular, b. Malpighiaceae sp1 – Lenticular, c. Malpighiaceae sp2 – Conical, d. Malvaceae – Cylindrical, e. *Eugenia* sp. – Cylindrical, f. *Eugenia* sp. – Cylindrical, g. *Ouratea* sp. – Lenticular and h. Verbenaceae – Globoid.

Table 1. Characterization of insect galls found in Serra da Bandeira, Barreiras, Bahia, Brazil.

Gall	Host plant	Organ	Shape	Foliar surface	Color	Trichomes	Occurrence	Inducer	Associated fauna	Fig.
ANACARDIACEAE										
1	*Anacardium humile* A.St.Hil	Leaf	Globoid	Abaxial	Green	Absent	Grouped	Cecidomyiidae	Hymenoptera	1a
2	*Anacardium humile* A.St.Hil	Leaf	Conical	Abaxial	Green	Absent	Isolated	Not determined	–	1b
3	*Anacardium humile* A.St.Hil	Flower	Globoid	–	Green	Absent	Isolated	Cecidomyiidae	–	1c
BIGNONIACEAE										
4	Bignoniaceae Juss.	Leaf	Conical	Both	Green	Absent	Grouped	Not determined	Hymenoptera	1d
CARYOCARACEAE										
5	*Caryocar brasiliense* Cambess	Leaf	Globoid	Adaxial	Green/Brown	Absent	Grouped	Not determined	–	1e
COMBRETACEAE										
6	*Terminalia fagifolia* Mart.	Leaf	Conical	Both	Brown	Present	Grouped	Not determined	–	1f
7	*Terminalia* sp. L.	Leaf	Cylindrical	Adaxial	Green	Absent	Grouped	Not determined	–	1g
8	Combretaceae R. Br.	Leaf	Conical	Abaxial	Green/Red	Absent	Grouped	Not determined	Hymenoptera	1h
ERYTHROXYLACEAE										
9	*Erythroxylum* sp Kunth	Leaf	Globoid	Adaxial	Red	Present	Grouped	Cecidomyiidae	Hymenoptera	1i
EUPHORBIACEAE										
10	*Manihot caerulescens* Pohl.	Flower	Cylindrical	–	Green/Red	Absent	Grouped	*Iatrophobia brasiliensis* (Rübsaamen, 1908)	Hymenoptera	1j
11	*Manihot caerulescens* Pohl.	Leaf	Cylindrical	Adaxial	Green/Red	Absent	Grouped	*Iatrophobia brasiliensis* (Rübsaamen, 1908)	Hymenoptera	1k
12	*Manihot caerulescens* Pohl.	Fruit	Cylindrical	–	Green/Red	Absent	Grouped	Cecidomyiidae	–	1l
FABACEAE										
13	*Copaifera sabulicola* J. Costa	Leaf	Lenticular	Extralaminar	Green/Red	Absent	Grouped	Not determined	–	1m
14	*Copaifera sabulicola* J. Costa	Stem	Lenticular	–	Brown	Absent	Grouped	Not determined	–	1n
15	*Copaifera sabulicola* J. Costa	Leaf	Lenticular	Extralaminar	Green	Absent	Grouped	Not determined	–	1o
16	*Copaifera sabulicola* J. Costa	Leaf	Globoid	Abaxial	Brown	Present	Isolated	Not determined	–	1p
17	*Copaifera sabulicola* J. Costa	Leaf	Globoid	Abaxial	Brown	Absent	Grouped	Hymenoptera	Hymenoptera	1q

Continue...

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Gall	Host plant	Organ	Shape	Foliar surface	Color	Trichomes	Occurrence	Inducer	Associated fauna	Fig.
18	*Copaifera sabulicola* J. Costa	Leaf	Lenticular	Intralaminar	Brown	Absent	Isolated	Not determined	Hymenoptera/ Acari	1r
19	*Copaifera sabulicola* J. Costa	Leaf	Globoid	Abaxial	Green/Brown	Absent	Isolated	Not determined	Hymenoptera	1s
20	*Copaifera sabulicola* J. Costa	Leaf	Leaf Fold	Both	Green/Brown	Absent	Isolated	Not determined	Hymenoptera	1t
21	*Copaifera sabulicola* J. Costa	Stem	Globoid	–	Brown	Absent	Grouped	Not determined	Hymenoptera	2a
22	*Copaifera sabulicola* J. Costa	Leaf	Lenticular	Extralaminar	Brown	Present	Isolated	Not determined	–	2b
23	*Copaifera depilis* Dwyer	Stem	Lenticular	–	Brown	Absent	Grouped	Not determined	Hymenoptera	2c
24	*Copaifera depilis* Dwyer	Stem	Globoid	–	Red/Brown	Absent	Isolated	Not determined	Hymenoptera	2d
25	*Copaifera depilis* Dwyer	Leaf	Cylindrical	Both	Red/Brown	Absent	Isolated	Not determined	Hymenoptera	2e
26	*Copaifera depilis* Dwyer	Leaf	Lenticular	Extralaminar	Green/Brown	Absent	Grouped	Not determined	Hymenoptera	2f
27	*Copaifera depilis* Dwyer	Leaf	Globoid	Abaxial	Brown	Absent	Isolated	Not determined	Hymenoptera	2g
28	*Copaifera luetzelburgii* Harms	Leaf	Cylindrical	Abaxial	Red/Brown	Present	Grouped	Not determined	Hymenoptera	2h
29	*Copaifera luetzelburgii* Harms	Leaf	Lenticular	Extralaminar	Brown	Present	Grouped	Not determined	Hymenoptera	2i
30	*Copaifera luetzelburgii* Harms	Leaf	Lenticular	Intralaminar	Green/Brown	Absent	Isolated	Cecidomyiidae	Hymenoptera	2j
31	*Mimosa sericantha* Benth	Stem	Cylindrical	–	Green/Brown	Present	Grouped	Cecidomyiidae	Hymenoptera	2k
32	*Mimosa sericantha* Benth	Leaf	Cylindrical	Adaxial	Green/Brown	Present	Grouped	Cecidomyiidae	Hymenoptera	2l
33	*Caliandra* sp. Benth	Stem	Globoid	–	Green/Red/ Brown	Present	Grouped	Hymenoptera	Lepidoptera	2m
34	*Caliandra* sp. Benth	Leaf	Fusiform	Both	Green	Absent	Grouped	Not determined	Lepidoptera	2n
35	*Mimosa acutistipula* Benth	Inflorescence	Cylindrical	–	Green	Absent	Grouped	Cecidomyiidae	Hymenoptera	2o
36	Fabaceae Lindl.	Leaf	Fusiform	–	Green	Present	Isolated	Not determined	–	2p

Continue...

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Gall	Host plant	Organ	Shape	Foliar surface	Color	Trichomes	Occurrence	Inducer	Associated fauna	Fig.
37	*Bauhinia* sp1 L.	Leaf	Globoid	Adaxial	Brown	Present	Grouped	Not determined	–	2q
38	*Bauhinia* sp2 L.	Stem	Fusiform	–	Brown	Absent	Grouped	Cecidomyiidae	–	2r
MALPIGHIACEAE										
39	*Diplopterys* sp. A. Juss.	Leaf	Conical	Abaxial	Green	Absent	Grouped	Not determined	Hymenoptera/ Pseudoscorpiones	2s
40	*Diplopterys* sp. A. Juss.	Stem	Globoid	–	Green/Red	Absent	Grouped	Not determined	Hymenoptera	2t
41	*Byrsonima* sp. Rich	Leaf	Lenticular	Intralaminar	Green/Brown	Absent	Isolated	Not determined	–	3a
42	Malpighiaceae sp1 Juss	Leaf	Lenticular	Intralaminar	Green	Absent	Grouped	Not determined	–	3b
43	Malpighiaceae sp2 Juss	Leaf	Conical	Adaxial	Green	Present	Isolated	Not determined	–	3c
MALVACEAE										
44	Malvaceae Juss	Leaf	Cylindrical	Adaxial	Green	Present	Grouped	Cecidomyiidae	–	3d
MYRTACEAE										
45	*Eugenia* sp. L.	Stem	Cylindrical	Abaxial	Green	Present	Grouped	Cecidomyiidae	–	3e
46	*Eugenia* sp. L.	Leaf	Cylindrical	–	Green	Present	Grouped	Cecidomyiidae	–	3f
OCHNACEAE										
47	*Ouratea* sp. Aubl.	Leaf	Lenticular	Intralaminar	Green/Brown	Absent	Grouped	Not determined	–	3g
VERBENACEAE										
48	Verbenaceae J. St.–Hill	Leaf	Globoid	Adaxial	Green	Present	Isolated	Not determined	–	3h

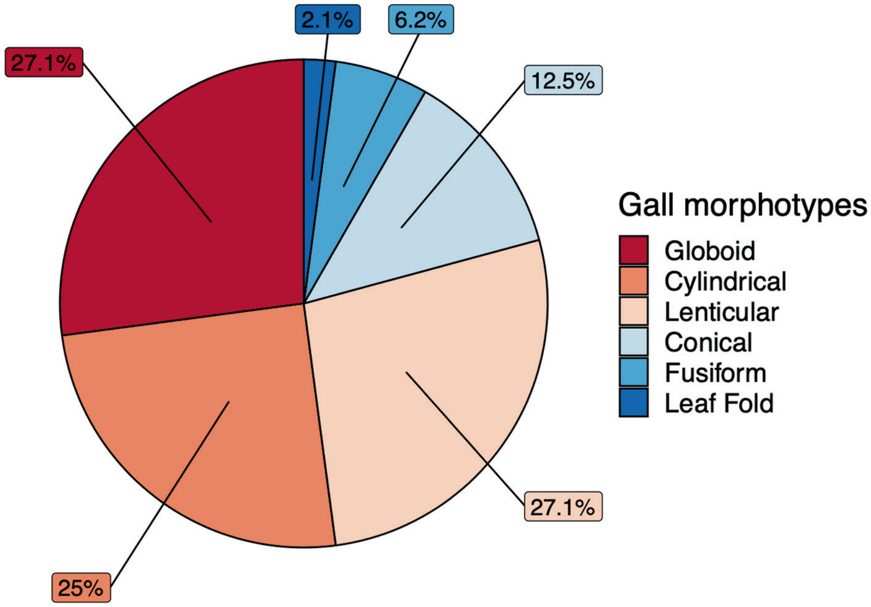

Figure 5. Percentage of gall morphotypes found in the Serra da Bandeira (Barreiras, Bahia, Brazil).

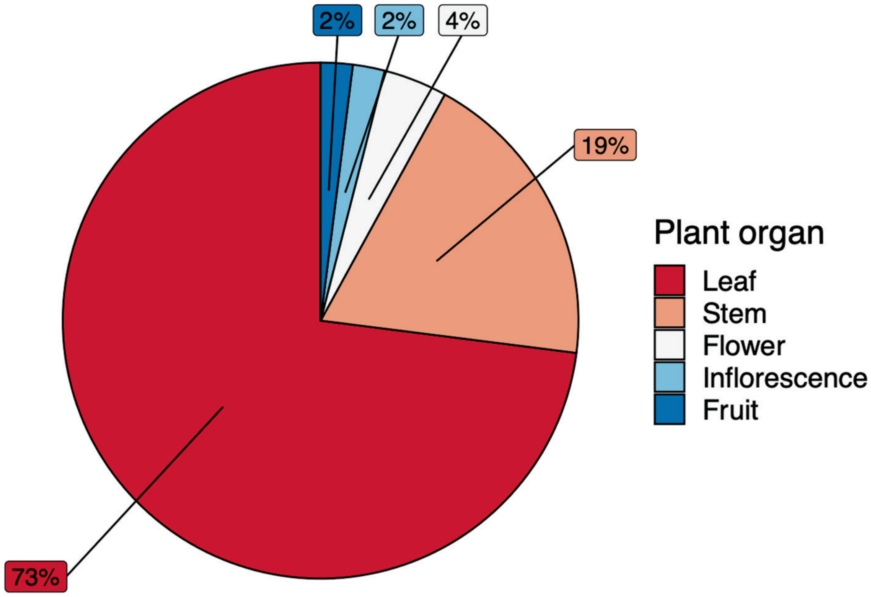

Figure 6. Percentage of plant organs affected by galling insects in the Serra da Bandeira (Barreiras, Bahia, Brazil).

in this study, has also been detected by other authors (Carneiro et al. 2009, Araújo et al. 2011, Maia 2013a, Scareli-Santos et al. 2018, Saito & Urso-Guimarães 2012), confirming that the cecidomyids are the main group of galling insects in the Cerrado.

We showed that the leaf was the organ with the highest number of registered galls as shown in other inventories in the Cerrado (Marinho et al. 2023, Maia 2013a). Such preference has been justified due to the availability of resources in the leaves being constantly abundant (Maia 2001). For this study, the globoid and cylindrical morphotypes were the most expressive, similar to studies conducted by Maia (2014). Bregonci et al. (2010) found that the majority of galls had the same color as the plant organ, with a predominance of green color as in the leaves. Here, we observe my pattern and that the coloration of some morphotypes can vary over time. Regarding the associated fauna, we found insects primarily belonging the order Hymenoptera, and identified them as parasitoids. According to Gagné (1994), hymenoptera are the main natural enemies of the Cecidomyiidae family. Further, the presence of these parasitoids has been considered a problem for obtaining insect galls at different stages of development and subsequent description (Maia 2013). Although we noticed a pseudoscorpion in the conical morphotype on *Diplopterys* sp. A. Juss, the occurrence of pseudoscorpions in Brazilian inventories seems to be scarce. Nogueira et al. 2016 also observed the presence of pseudoscorpions in a single gall morphotype and highlighted this likely scarcity.

In short, we characterized the insect galls found on plant host species, identified the gall-inducing insects to the lowest taxonomic

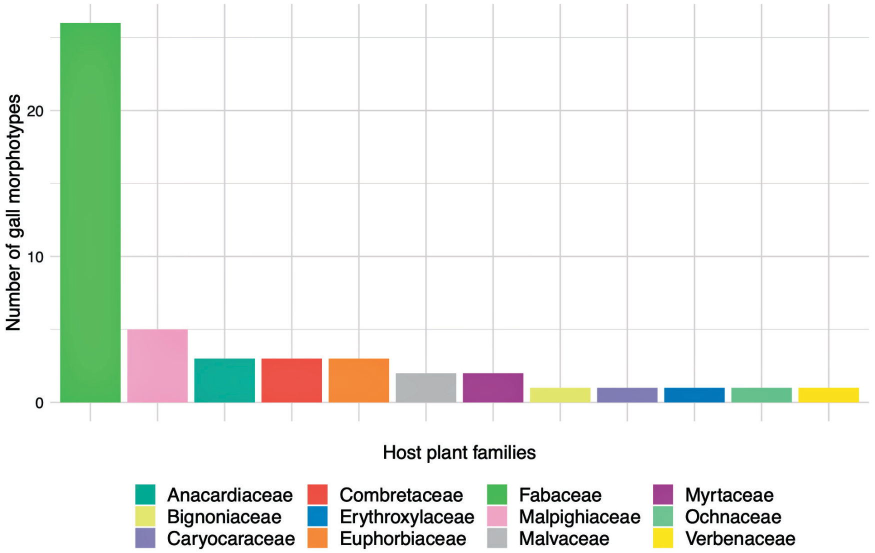

Figure 7. Distribution of insect galls in host plant families found in Serra da Bandeira (Barreiras, Bahia, Brazil).

level, and recorded the presence of gall-associated fauna. The information provided can be used highly by decision makers for conservation programs, as well as in other strategies for the conservation of biological diversity in the Brazilian Cerrado.

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Associate Editor

Gustavo Graciolli

Author Contributions

Jeferson Brito de Menezes: design of the study, data collection, data analysis and interpretation, manuscript preparation, critical revision, adding intellectual content.

Valdeir Pereira Lima: design of the study, data analysis and interpretation, manuscript preparation, critical revision, adding intellectual content.

Daniéla Cristina Calado: design of the study, data analysis and interpretation, manuscript preparation, critical revision, adding intellectual content.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics

This study did not involve human subjects or clinical trials which require authorization by an Institutional Committee.

Data Availability

Codes are available on https://doi.org/10.5281/zenodo.8104868

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Article

Reassessment of the extinction risk status of the ponytail palm *Beaucarnea inermis*

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Abstract: *Beaucarnea inermis* is an endemic species from Northeast Mexico, in the states of San Luis Potosí and Tamaulipas. It is appreciated as an ornamental plant, so its populations are subject to the poaching of individuals for illegal trade. Previous studies determined that their populations have been affected due to the disturbance since the incidence of anthropogenic activities affects the viability of the species. Here we determine the current conservation status of *B. inermis* and identify their main risk factor by performing an extinction risk assessment based on the Annex II "Method for Evaluation of Risk of Extinction of Plants in Mexico". We studied 10 populations of *B. inermis* from protected and non-protected areas in San Luis Potosí and Tamaulipas. We considered the MER criteria: A) geographical distribution characteristics, B) habitat characteristics, C) intrinsic biological vulnerability, and D) impact of human activity. Using field and analyzed data, the MER assessment gives 1.91 points that confirm *B. inermis* is correctly classified as an Endangered species. The natural protected areas where the species occurs represent cores for its protection; however, the surface of these areas may not be sufficient without biological corridors that connect them.
Keywords*: Endangered species; endemism; Mexico; natural protected areas; seasonally dry tropical forests; Sierra Madre Oriental.*

Reevaluación del riesgo de extinción de la pata de elefante *Beaucarnea inermis*

Resumen: *Beaucarnea inermis* es una especie endémica del Noroeste de México distribuida en los estados de San Luis Potosí y Tamaulipas. Es apreciada como planta ornamental, por lo que sus poblaciones están sujetas al saqueo de individuos para su comercialización ilegal. En trabajos anteriores se determinó que sus poblaciones han sido afectadas debido al disturbio por la incidencia de actividades antrópicas que afectan su viabilidad. En la presente contribución se determina el nivel de riesgo de *B. inermis* con base en el Anexo Normativo II "Método de Evaluación de Riesgo de Extinción de Plantas en México". Se estudiaron 10 poblaciones de *B. inermis* en San Luis Potosí y Tamaulipas, tanto en áreas naturales protegidas como en sitios no protegidos. Los criterios MER considerados fueron: A) características de la distribución geográfica, B) características del hábitat, C) vulnerabilidad biológica intrínseca y D) impacto de la actividad humana. El análisis MER arroja un valor de 1.91 que, confirma a *B. inermis* dentro de la categoría de Amenazada. Las áreas naturales protegidas donde se distribuye la especie funcionan como núcleos de protección, sin embargo, su superficie puede no ser suficiente sin la presencia de corredores biológicos que las conecten.
Palabras-clave: *Especie amenazada; endemismo; México; áreas naturales protegidas; bosque tropical estacionalmente seco; Sierra Madre Oriental.*

Introduction

Beaucarnea inermis (S. Watson) Rose (Asparagaceae) is an endemic species to the seasonal tropical forests of northwestern Mexico. It is distributed in the biocultural region of Huasteca, in the northeast of San Luis Potosí and the south of Tamaulipas. It is an arborescent and massive species, dominant within its natural habitat, where it can reach up to 18 m in height and 3 m in base diameter (Castillo-Gómez 2011, Hernández-Sandoval et al. 2012, Rubio-Méndez et al. 2018a, 2018b; Figure 1). Although *B. inermis* has been considered a synonym of *B. recurvata* Lem., some authors separate them by morphological differences in the habit, the bases of the branches, rosettes, and inflorescences (Hernández-Sandoval et al. 2012, Rojas-Piña et al. 2014). Due to its striking morphology, with massive bulbous trunk, the species has a relevant ornamental use at regional, national, and international levels, so illegal poaching of natural populations threatens its long-term conservation (Rubio-Méndez et al. 2018a, 2018b). In addition, their habitat has been subject to changes in land use for agricultural production, so the size of their populations, sex proportions, and establishment of new individuals have been altered (Hernández-Sandoval et al. 2012), which could negatively affect the genetic diversity of the species. For this reason, *B. inermis* is in the category of Threatened (A) within the Mexican norm NOM-059-SEMARNAT-2010 (SEMARNAT 2010) and in Appendix II of CITES (2022). One of its main populations is located within the Sierra del Abra Tanchipa Biosphere Reserve (SATBR), a protected natural area that stands out as an important refuge for the conservation of the species in terms of its demography and population structure (Rubio-Méndez et al. 2018a, 2018b). Other protected populations are located in the El Cielo Biosphere Reserve (ECBR) and the Sierra de Tamaulipas Biosphere Reserve (STBR) (Castillo-Gómez, 2011). They all belong to the national category of Biosphere Reserves in Mexico, which includes ecosystems that have not been significantly altered and are home to endemic or threatened species. The SATBR and STBR are recognized in the category VI "Protected area with sustainable use of natural resources" of the IUCN World Commission on Protected Areas (WCPA), which conserve ecosystems and habitats, together with associated cultural values and traditional natural resource management systems and where conservation and sustainable use can be mutually beneficial (Dudley 2008). However, the populations of *B. inermis* have yet to be properly evaluated just as some other *Beaucarnea* species considered endangered: *B. gracilis* (Fuentes et al. 2020a) *B. guatemalensis* (Coronado et al. 2022), *B hiriartieae* (Fuentes et al. 2020b), *B. olsonii* (Fuentes et al. 2020c), *B. purpusii* (Martínez Salas et al. 2020a), *B. recurvata* (Martínez Salas et al. 2020b), and *B. sanctomariana* (Fuentes et al. 2020d).

Figure 1. Adult specimen of *Beaucarnea inermis* at the RBSAT, San Luis Potosí.

As a way of promoting objectivity and thus being able to offer greater relevance, credibility, and legitimacy in the assignment of categories of risk of extinction of wild species, a Wild Species Extinction Risk Assessment Method, included in the NOM-059-ECOL-1994 updated in 2001, was developed in Mexico, to identify and weigh the factors that affect and threat a species (Tambutti et al. 2001, Sánchez et al. 2007, Castillo-Gómez 2011, *Pérez-Paredes 2013*, *Sánchez-Salas* et al. 2013). In the update of the NOM-059 in 2010, the Normative Annex II "Method of Evaluation of Risk of Extinction of Plants in Mexico" (MER-Plants) is included, which considers intrinsic attributes of the plants such as geographical distribution, specificity of habitat, and demographic issues that require to be evaluated differently from animals (Sánchez-Salas et al. 2013, SEMARNAT 2010). The MER-Plants is based on two indices, the Rarity Index (IR) and the Anthropogenic Impact Index (IIA) both encompassing four independent decision criteria. The IR includes: Criterion A) Characteristics of geographic distribution; Criterion B) Characteristics of the habitat with respect to the natural development of the taxon; Criterion C) Intrinsic biological vulnerability. And the IIA includes Criterion D) Impact of human activity. Considering this, our aim was to reassess the extinction risk status of *Beaucarnea inermis* and identify the main threatening factors by evaluating the criteria of the MER-Plants, based on published and new information about the species.

Materials and Methods

To obtain MER-Plants indexes, we used information regarding distribution, demographics, genetic diversity, and habitat degradation of 10 representative populations of the natural distribution of *Beaucarnea inermis* in the states of San Luis Potosí and Tamaulipas (El Abra, El Sabinito, Estación Micos, Grutas de Quintero, La Chaca, Ocampo, RBSAT, San Dieguito, San Gerónimo and Sótano del Arroyo). The risk category for the species is determined by summing all criterion' values, which weight the same and are normalized between 0 and 1 (SEMARNAT 2010, Ruiz-Jiménez et al. 2018).

1. *Rarity index*

Criterion A) Characteristics of geographical distribution. We included presence-only data of 82 species records of previously studied populations from 10 localities of San Luis Potosí and Tamaulipas, geographical records from herbaria specimens from collections SLPM, MEXU, and QMEX, as well as the databases of the Naturalista portal (CONABIO 2020) and the Global Biodiversity Information Facility (GBIF), all of them validated in the field during the year 2020 (Figure 2). To reduce spatial autocorrelation, we previously eliminated the duplicate records and used the rest to delimit the extension of the occupied area (Solano & Feria 2007). For these records, we used a non-spatial partition by selecting two random k-fold, dividing the presence records for *B. inermis* into two independent data sets. For the delimitation of the calibration area –referred to as the accessibility area *sensu* Soberon and Peterson (2005)– we use the intersection of the biogeographic provinces proposed by Morrone et al. (2017) with the ecoregions proposed by Dinerstein et al. (2017), and physical factors that may have meant a barrier to the dispersal of species (Soberon & Peterson 2005, Barve et al. 2011). Since the resulting surface greatly exceeded the area with a concentration of species records, we considered a 100 km buffer to determine the extreme limits.

According to Hijmans et al. (2005) and Fick & Hijmans (2017) we used 19 climate variables from WorldClim Global Climate Data V.1.4 (http://www.worldclim.org), with a resolution of 30 arcsec (1 km^2), from which we selected only a set of 15 climate variables excluding four layers (i.e., Bio 8, Bio 9, Bio 18, and Bio 19), as these present spatial anomalies in the form of odd discontinuities between neighboring pixels (Escobar et al. 2014, Booth, 2022). Then, in order to minimize the repeated information from layers, we performed a normality test and as 13 of the 15 remanent variables had a non-normal distribution, we conducted a non-parametric Spearman correlation analysis using the variable values corresponding to the species' records and selected only seven variables (i.e. Bio 1, Bio 2, Bio 3, Bio 4, Bio 12, Bio 15, Bio 17) with a correlation coefficient < 0.7.

To obtain the geographic distribution of the species we used MaxEnt v.3.4.1 (Phillips et al. 2006) via Wallace, a shiny application for modeling species distributions (Muscarella et al. 2014, Kass et al. 2022). The Maxent parametrization was performed considering the feature clases: linear (*l*), linear and quadratic (*lq*) and linear, quadratic and hinge (*lqh*), as well as the regularization in a sequence from 0.5 to 3.5 in intervals of 0.5. The background sampling (representing potentially suitable but unsampled locations) was 20,000 points out of a total of 74,000 available in the calibration area extent. From this

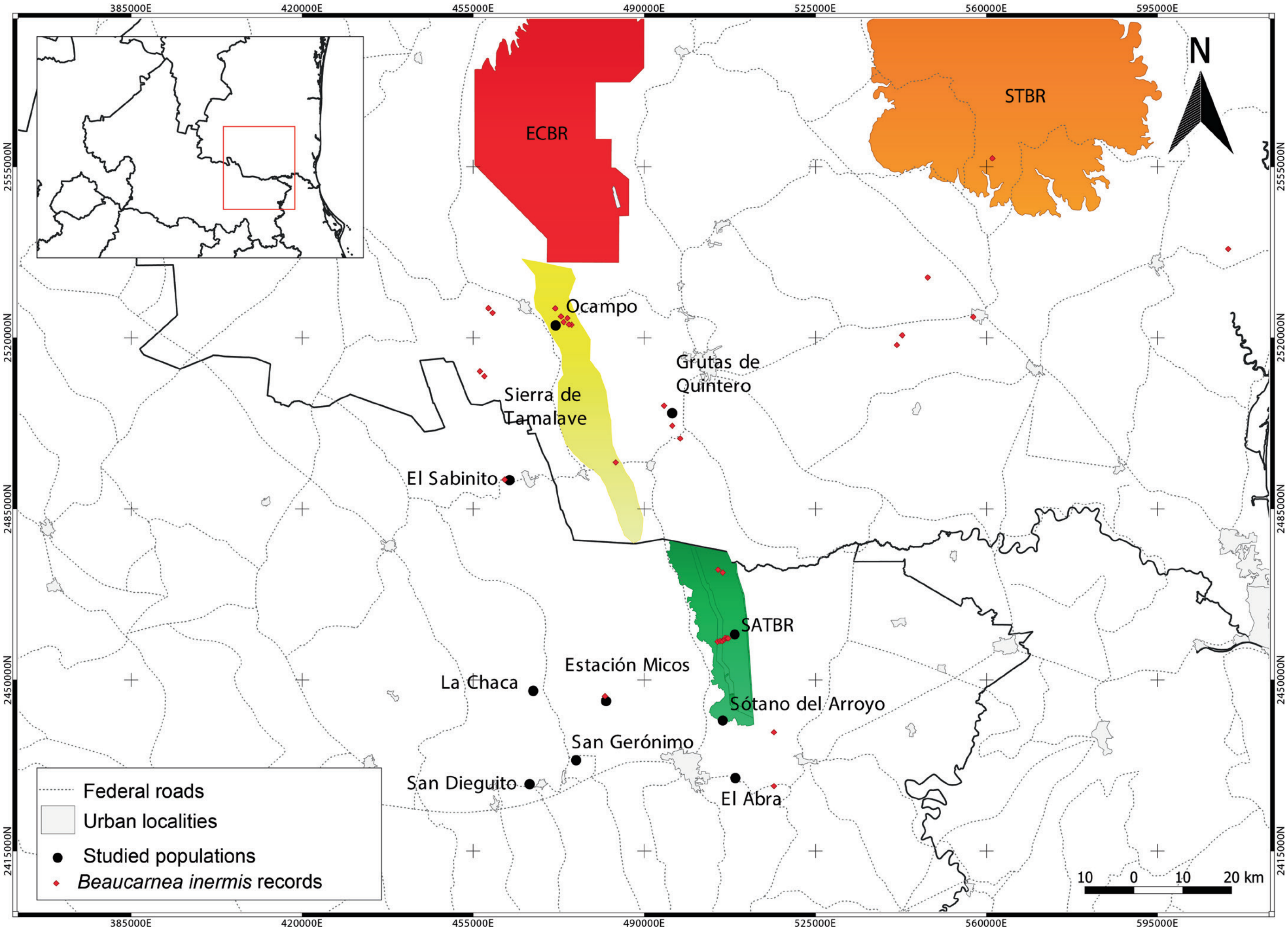

Figure 2. Locations of analyzed records and populations of *Beaucarnea inermis* for this study.

parametrization, we obtained 21 different models and the best model selection was conducted by favoring the omission rates as the first filter, followed by the higher values of the Continuous Boyce Index, and as the last the AIC.

Criterion B) Characteristics of the habitat. The current state of the habitat and the requirements for the establishment of *Beaucarnea inermis* were determined according to the characteristics of the sites, mainly regarding the level of disturbance, types of vegetation, and associations presented, which have been indicated in previous studies (Castillo-Gómez 2011, Hernández-Sandoval et al. 2012, Rubio-Méndez et al. 2018a).

Criterion C) Intrinsic biological vulnerability. Six components were considered. 1) Demography: The demographic information and conservation status of the species have been previously analyzed by Rubio-Méndez et al. (2018a, 2018b). All individuals found within plots of 100 m × 10 m (0.1 ha) at each site were counted and classified by age, as proposed by Castillo-Gómez (2011). With the frequencies of each age, static life tables were generated for each population group to describe survival and mortality in each age class based on different attributes: l_x, the proportion of surviving individuals entering class x, with respect to the initial number of individuals; q_x, mortality rate as the proportion of dead individuals during the time interval from class x to x + 1, with respect to the original number of individuals; E_x, life expectancy as the expected life time of an individual of class x; R_0, net reproductive rate of each generation. The intrinsic population growth rate was calculated as $\lambda = e^r$, where e = Avogadro's constant of 2.71828 and r = population growth rate per capita. When $\lambda = 1$, population size is constant; $\lambda > 1$ indicates increase in population size per generation, while $\lambda \leq 1$ indicates a decrease (Caswell 2001, Castillo-Lara et al. 2017, Rubio-Méndez et al. 2018a). 2) Population structure: the population structure was estimated from the Gini asymmetry coefficient (*g1*) with the measurements of the base diameter, neck diameter (where the base narrows), and total plant height (Bendel et al. 1989, Palacios-Wassenaar et al. 2016), as described in Rubio-Méndez et al. (2018a); we used base diameter and neck diameter because they are suitable to describe size in this genus. 3) Sex ratio: the sex of adult individuals was recorded based on the presence of reproductive structures (Castillo-Gómez 2011, Hernández-Sandoval et al. 2012). 4) Phenology: two phenophases, flowering and fruiting, were identified. The periods in which each phase occurs were established based on the review of herbarium specimens and collections during field visits. 5) Seed germination: the previously disinfected seeds were placed on moist filter paper in Petri dishes covered with newspaper. 240 seeds collected in six sites (n = 40) were used (Cardel et al. 1997, Castillo-Gómez 2011). 6) Genetics: To estimate genetic diversity, seven populations were analyzed using microsatellite-type molecular markers (SSR: Short Sequence Repeat) as described below. Genetic diversity was evaluated with a sample of 74 individuals from seven populations (El Sabinito, Estación Micos, Grutas de Quintero, La Chaca, RBSAT, San Geronimo, and Sótano del Arroyo).

Genomic DNA was extracted from *Beaucarnea inermis* leaf tissue by the modified CTAB method, adding 1% polyvinyl pyrrolidone (PVP) and 0.1% sodium metabisulfite. PCR reactions were performed by cross-amplification using six previously designed SSR molecular marker primers for species of the phylogenetically close genera *Dracaena cambodiana* and *Asparagus conchinchinensis* (Table 1), 2.5 µl of 10x Taq Buffer + $(NH_4)_2SO_4$ (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.4 µl of 100 mM DNTP's (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 2/1.5 µl of 25 mM MgCl2, 0.5 µl of each primer (Fwd and Rev) 10 mM, 0.1 µl of Taq 500 u (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 50 ng of DNA and ultrapure water (Laboratorio PiSA, México) up to a volume of 25 µl, in a thermocycler T100 Thermal Cycler (Bio Rad, Hercules, California, USA) under the following program: 94 °C for 5' followed by 40 cycles of 94 °C for 30 mins., Ta for 30 mins., 72 °C for 1 min. and a final extension at 72 °C for 8 mins. The PCR products were separated on a 12% polyacrylamide gel stained with EtBr and visualized in a Benchtop 2uv Biodoc-It Imaging System photodocumenter (UVP Laboratory System, Upland, California, USA), using a 50 bp molecular weight marker (ThermoFisher Scientific, Waltham, Massachusetts,

Table 1. Microsatellite molecular markers (SSR: Short Sequence Repeat) used to evaluate the genetic diversity of *Beaucarnea inermis*.

Locus	Primer sequence	Annealing temperature (ºC)	Species	Reference of primer
DC003	F: AGAAAGGGAGGTGACAGG	54	Dracaena cambodiana	Zhang & Li (2010)
DC003	R: GTCAAAGAGCCCAAACAA	54	Dracaena cambodiana	Zhang & Li (2010)
DC006	F: GTTTCTAGTTCAAGAACCCAA	54	Dracaena cambodiana	Zhang & Li (2010)
DC006	R: TTCCTCCTCTTTCTCATCCT	54	Dracaena cambodiana	Zhang & Li (2010)
DC465	F: TCCCATAAATGCTCCTCA	48	Dracaena cambodiana	Zhang & Li (2010)
DC465	R: TCAAGCTATGCATCCAAC	48	Dracaena cambodiana	Zhang & Li (2010)
DC522	F: GTAAGAAGAAAAGAGGAAGA	52	Dracaena cambodiana	Zhang & Li (2010)
DC522	R: AGGGAATCTGTCACTTGT	52	Dracaena cambodiana	Zhang & Li (2010)
AC011	F: TGTGCGGTCGACTGAATTGA	55	Asparagus cochinchinensis	Kim et al. (2017)
AC011	R: GAGGCTACACACTCCCAAGG	55	Asparagus cochinchinensis	Kim et al. (2017)
AC079	F: GCTTTCGGAGGGGGAAGAAA	55	Asparagus cochinchinensis	Kim et al. (2017)
AC079	R: GAAGCGGCGAGAGAGAGTAC	55	Asparagus cochinchinensis	Kim et al. (2017)

USA). Genotyping was performed using Gel Analyzer V. 2010a software (http://www.gelanalyzer.com/index.html). The number of different alleles, the expected heterozygosity, the observed heterozygosity, the F_{IS} fixation index (Peakall & Smouse 2012), and the S self-fertilization index were estimated. Data analyzes were performed using Arlequin V.3.5.2.2 (Excoffier & Lischer 2010) and GenAlex V.6.5 (Peakall & Smouse 2012).

2. *Anthropogenic impact index*

Criterion D) Impact of human activity. Potential risk factors of anthropogenic origin for *Beaucarnea inermis* were identified in the study sites, using the Chronic Disturbance Index (CDI) developed by Martorell and Peters (2005, 2009), which combined 14 parameters grouped into three disturbance agents: livestock raising, human activities, and habitat degradation (Martorell & Peters 2005, 2009). This index was previously applied to *B. inermis* by Rubio-Méndez et al. (2018a). For the calculation of the CDI, values were assigned to 14 variables clustered in three disturbance categories (livestock raising, human activities, and land degradation) at the sites during the field sampling according to the magnitude of the impact considered for each one (Rubio-Méndez et al. 2018a). Data were standardized and combined through a principal component analysis (PCA), discarding those variables with no variation for the sites as recommended by Hernández-Oria et al. (2006) and as it is described in Rubio-Méndez et al. (2018a). Additionally, information on confiscations of *B. inermis* specimens in the state of San Luis Potosí was requested from the Federal Environmental Protection Agency in Mexico (PROFEPA).

Results

1. *Rarity index*

Criterion A) Characteristics of geographical distribution. The known populations of *Beaucarnea inermis* occur in three regions with geomorphologically distinct characteristics: the Sierra Madre Oriental, the Sierra de Tamaulipas, and the Gulf Coastal Plain semi-flat lands, with a discontinuous distribution in isolated patches, although in some cases it is distributed in relatively large areas such as in the SATBR and the Sierra de Tamalave. The potential distribution generated (Figure 3) showed high values of CBI (0.85) and a high probability of prevalence (>75%) coinciding with the current known distribution and some nearby areas with an approximate surface of 102 531.42 ha in San Luis Potosí, which represents only 1.6% of the state territory, while for Tamaulipas the approximate area is 55 338.48 ha, 0.68% of the state territory. The total area that *Beaucarnea inermis* could potentially occupy is 157 869.90 ha, barely 0.08% of the national territory. Of this potential area, only 22.3% is located within some protected natural area, which includes the ECBR, the SATBR, and the STBR, representing 0.01% of the national territory.

Criterion B) Characteristics of the habitat. The habitat of the analyzed populations presents a high percentage of limestone outcrops and shallow soil, from semi-flat terrain to slight slopes. These sites present semi-warm humid and warm sub-humid tropical climates according to the modified Köepen classification (García 2004), with two well-defined climatic seasons, a rainy season between June and September and a dry season between November and April. The populations in the localities of San Gerónimo and Ocampo present the most humid climates with annual precipitation greater than 1 500 mm, while the populations in the localities of El Abra, Grutas de Quintero, San Dieguito, SATBR, and Sótano del Arroyo, present drier conditions, with annual precipitation ranging between 900 mm and 1 000 mm. The types of vegetation present in the study sites correspond to variants of seasonally dry tropical forests, which have been recognized by Rzedowski (1978) as tropical deciduous forest (TDC) and tropical semi-deciduous forest (TSC). In all sites *Beaucarnea inermis* was found in association with *Bursera simaruba* (L.) Sgt. and *Pseudobombax ellipticum* (Kunth) Dugand, as well as *Bromelia pinguin* L. and *Dioon edule* Lindl. The population within the SATBR presents the widest and most continuous distribution for the species. The highest canopy is found in San Gerónimo, with trees up to 18 m tall. In San Dieguito there is secondary vegetation with an abundance of *B. pinguin* and *Sabal mexicana* Mart. and, in both places, the undergrowth is very closed. Ocampo is located near an active mining bank. The populations of El Abra and Sótano del Arroyo have a more open shrub layer and an herbaceous layer with an abundance of *B. pinguin* and *Hechtia* spp. In Grutas de Quintero, the shrubby and herbaceous strata are more open.

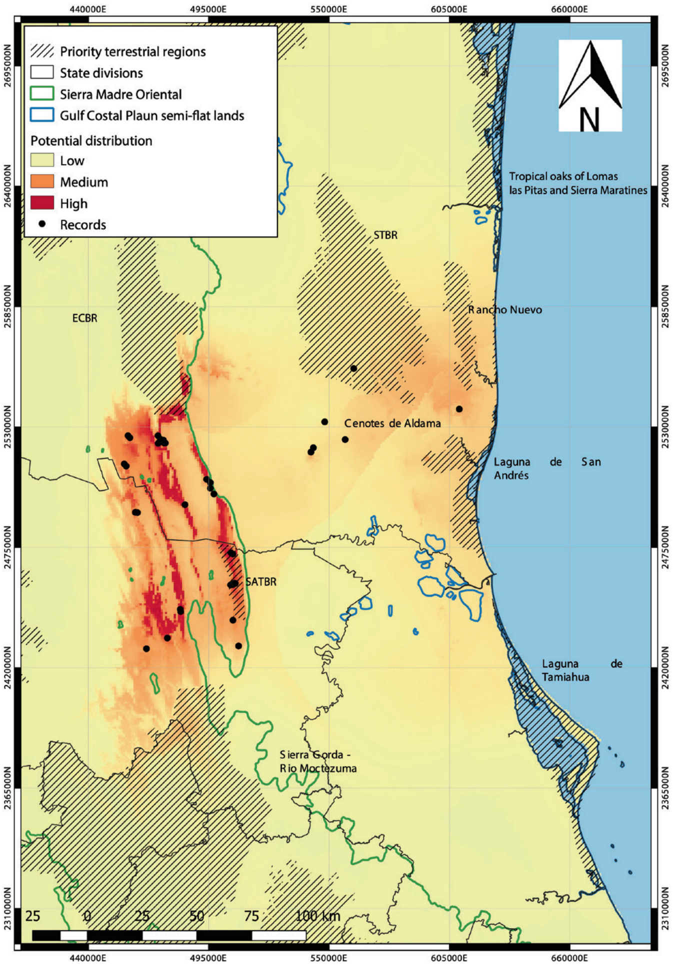

Figure 3. Niche-based distribution model of *Beaucarnea inermis*.

Criterion C) Intrinsic biological vulnerability. The average population density of *Beaucarnea inermis* is 236.9 ind/ha (Rubio-Méndez et al. 2018a), with higher values within the SATBR (280 ind/ha).

The asymmetry index *g1*, previously reported by Rubio-Méndez et al. (2018a), indicates that the populations are dominated by large-sized adult individuals (average *g1* of –0.61 within the SATBR and –0.75 for the other populations). According to the life tables previously reported by Rubio-Méndez et al. (2018a) two mortality events are detected: the transition from seed to seedling, which occurs inside and outside the SATBR, and the transition from seedling to juvenile present in all populations except the SATBR (Table 2). Adult individuals show the highest survival rate in all populations except in El Abra where the dominance corresponds to seedlings. The net reproductive rate (R_0) is less than 1 in all populations, however, the life expectancy for seedlings and juveniles within the SATBR is greater than for the other populations. The proportion of individuals in the reproductive phase during the samplings carried out was approximately 50%. The sex ratio (m:f) was 0.93:1 within the SATBR and 0.76:1 for the other populations. Flowering occurs from February to August and fruiting occurs from June to April of the following year and has two peaks, one in August and the other in January. The percentage of germination in the laboratory is up to 92.5% (Castillo-Gómez 2011), in the field the same probably occurs in favorable times of humidity and temperature, however, only a small proportion manages to establish itself as juvenile; once the adult stage is reached, the populations seem to remain stable. The genetic diversity evaluated with the six SSR markers indicates that the observed heterozygosity for all analyzed populations was lower than expected, indicating a significant heterozygous deficit (average $Ho = 0.39$, average $He = 0.74$, Table 3) according to the Hardy-Weinberg balance test ($\chi^2 = 111.53$, $p < 0.0002$). Likewise, the populations present high inbreeding $F_{IS} = 0.56$, with an index $S = 0.71$, which indicates that 71% of the progeny of the species might be produced by self-fertilization.

2. *Anthropogenic impact index*

Criterion D) Impact of human activity. According to the CDI values (Table 4), the main risk factors identified were human activities, mainly the incidence of fires and the proximity to towns and centers of human activity. These activities mainly affect survival and mortality in the seedling and juvenile stages, which affects the establishment of new individuals and alters the sex ratio, resulting in a decrease in the reproductive rate. According to PROFEPA, in San Luis Potosí there is a record of confiscation of illegal *Beaucarnea inermis* specimens in the last decade; one of the most numerous was recorded in 2012, where 346 plants were confiscated in the municipality of Huehuetlán and 29 in Tancanhuitz (Rubio-Méndez et al. 2018b).

3. *MER results*

The summing results of the MER ratings for each of the evaluated criteria (Appendix I) were A = 0.45 (width of distribution), B = 0.33 (habitat status), C = 0.43 (intrinsic biological vulnerability), and D = 0.7 (impact of human activity), which in total add up to a value of 1.91.

Table 2. Life table for *Beaucarnea inermis* at each disturbance level.

Stage	Level of disturbance	lx	qx	ex
Seedling	Low	9,40E-07	–3	65,83
	Medium	5,48E-05	0,89	1,82
	High	5,04E-05	0,68	1,54
	Mean (SD)f	3.54E-05 (1.73E-05)	–0.47 (1.265)	23.06 (21.38)
Juvenile	Low	3,76E-06	–14,33	15,83
	Medium	5,60E-06	–11	12,5
	High	1,56E-05	–1,35	2,85
	Mean (SD)f	8.35E-06 (3.71E-06)	–8.89 (3.89)	10.39 (3.89)
Adult	Low	5,76E-05	1	0,5
	Medium	6,72E-05	1	0,5
	High	3,69E-05	1	0,5
	Mean (SD)f	5.39E-05 (8.93E-06)	–	–
R0	Low	3,06E-04		
	Medium	2,11E-02		
	High	1,10E-02		
	Mean (SD)f	3.19E-02 (1.43E-02)		
λe	Low	0,66		
	Medium	0,82		
	High	0,79		
	Mean (SD)f	0.83 (2.31E-02)		

Table 3. Genetic diversity for seven populations of *Beaucarnea inermis*. N: Sample Size, Na: Number of alleles, Ne: Number of effective alleles; Ho: Observed heterozygosity, He: Expected heterozygosity, F_{IS}: Fixation index, S: Self-fertilization index, D: Index of chronic disturbance.

Population	N	Na	Ne	Ho	He	F_{IS}	S	D
SATBR	22,83	19,83	14,81	0,4	0,93	0,58	0,74	Low
El Sabinito	4,5	4,66	3,95	0,42	0,61	0,44	0,61	Medium
Estación Micos	3,5	4,5	4,14	0,32	0,74	0,68	0,81	Medium
Sotano del Arroyo	5,67	6,33	5,62	0,33	0,81	0,65	0,79	Medium
Grutas de Quintero	3,17	4	3,61	0,36	0,67	0,61	0,76	Medium
San Gerónimo	3,67	5,16	4,45	0,49	0,66	0,37	0,54	High
La Chaca	5,17	5,5	4,59	0,38	0,77	0,59	0,74	–
Mean	6,93	7,14	5,88	0,39	0,74	0,56	0,71	–

Table 4. Values of the CDI for nine populations of *Beaucarnea inermis* and their disturbance level.

Population	Livestock raising	Human activities	Land degradation	CDI	Level
SATBR	1	0,45	0	0,3	Low
El Abra	1	1,59	0	0,69	Medium
Grutas de Quintero	1	1,71	0,1	0,7	Medium
Sótano del Arroyo	1	2,08	0	0,73	Medium
Estación Micos	1	2	0	0,75	Medium
El Sabinito	1,4	2,4	0	0,81	Medium
Ocampo	1	2,64	0,1	1,33	High
San Dieguito	1,3	2,76	0	1,36	High
San Gerónimo	1,48	2,75	0	1,37	High

According to Normative Annex II of NOM-059-SEMARNAT-2010, *Beaucarnea inermis* remains in the Threatened (A) category.

Discussion

Since its first publication in 1994, NOM-059-SEMARNAT has been used to assess the conservation status of the different biological groups distributed in Mexico for the development and application of conservation policies (García-Aguilar et al. 2017). Despite being questioned due to the apparent lack of objective and scientific criteria in the classification system and the use of subjective terms in the risk categories (De Grammont & Cuarón 2006, Cuarón & De Grammont 2007, Soberón & Medellín 2007, García-Aguilar et al. 2017) this Norm represents the only legal normative instrument in Mexico for the protection of biodiversity and it has been modified over the years in order to constitute a coherent and dynamic element of regulatory consultation with the participation of experts in the different taxonomic groups of Mexico (Tambutti et al. 2001, Sánchez-Salas et al. 2013). It has been highlighted that extinction is a demographic and evolutionary process in which the populations of a species have difficulties replacing their generations until they lose all their individuals (Lacy 1988, Lande 1988, Allendorf & Luikart 2007). In this sense, having a protocol such as the MER, which estimates the risk of extinction of a species based on rigorously weighted and systematized available information, can allow the long-term conservation of some species, at least on the legal normative plane (Tambutti et al. 2001, SEMARNAT 2010, Castillo-Gómez 2011).

The application of the MER for the case of *Beaucarnea inermis*, with updated information, indicates that the species should be kept in the Threatened (A) category, as a species that could become endangered in the short and medium term if the factors that negatively affect its viability continue, particularly the deterioration or modification of its habitat and the direct reduction of the effective population size. Fragmentation and habitat loss, caused by anthropic disturbance, are the main causes of species extinction due to their negative effects on effective population sizes and genetic diversity (Gurrutxaga-San Vicente & Lozano-Valencia 2006). It has been previously indicated that the main threat faced by *B. inermis*, in addition to the individual's illegal extraction, is the loss of habitat caused by anthropic disturbance that affects its demographic dynamics, particularly the mortality of seedlings and juveniles (Rubio-Méndez et al. 2018a, 2018b). The most vulnerable stages are from seedling to juvenile because the pressures of disturbance mainly affect the recruitment. The high mortality at the seedling stage may act as a strong selective filter on seed traits and seedling traits like in other plant species (Kitajima & Fenner 2000), where traits can be interpreted as "gap-detection mechanisms" in seeds restrict germination in time and space, enhancing the likelihood of seedling survival and growth (Rubio-Méndez et al. 2018a). In general, disturbance also affects the sex ratio, which decreases the net reproductive rate of a population in the long term (Pérez-Farrera et al. 2006, Octavio-Aguilar et al. 2018).

Regarding the intrinsic vulnerability of the taxon, the average density recorded for *Beaucarnea inermis* (236.9 ind/ha) is higher than that reported for other species such as *B. gracilis* (16.9 ind/ha) and *B. recurvata* (130 ind/ha; 20.1 ind/ha; Cardel et al. 1997, Hernández-Sandoval et al. 2012, Rubio-Méndez et al. 2018a, Espinoza-Cruz 2019). Although populations of *B. inermis* outside protected natural areas show a higher density of seedlings, they have a higher mortality rate than juveniles and adults (Rubio-Méndez et al. 2018a). Apparently, the disturbance favors the germination and establishment of new individuals for the species, but the microclimate generated by the decrease in canopy cover, with higher temperature and direct incidence of sunlight and lower soil moisture, has a negative effect on the seedlings avoiding they join the population as reproductive individuals (Augspurger 1984, Augspurger & Kelly 1984, Kitajima & Fenner 2000, Flores 2003, Flores et al. 2004). The protected area category in which populations of the species occur allows some of their territories to be under sustainable natural resource management, and juveniles are affected by illegal poaching, threatening their long-term conservation (Rubio-Méndez et al. 2018a, 2018b). The heterozygosity deficit in the analyzed populations of *B. inermis* could imply an interruption in the genetic flow as the result of the disturbance and fragmentation of the habitat that the populations of the species present. In recent decades, the fragmentation of the habitat in the Huasteca region has reached such a magnitude that in some areas with little inclination, the forests and jungles have been eliminated, and only relicts remain on hills with limestone outcrops that are difficult for humans to access (Errejón-Gómez et al. 2018). For genetic diversity in the long term, such fragmentation can lead to alleles being fixed or lost in *B. inermis* populations, leading to possible extinction. However, additional information on genetic diversity and structure is needed to assess the levels of gene flow between populations and their structure to have a better overview of the conservation status of *B. inermis* populations.

Conclusions

The main risk factor of extinction for *Beaucarnea inermis* is anthropogenic activities, such as changes in land use and extractive activities, so we propose to apply management plans and conservation programs focused on awareness, sustainable use, and assisted legal propagation of the species, which allow reducing the effects of anthropic disturbance on the populations and thus achieve their long-term conservation. The protected natural areas ECBR, SATBR, and STBR represent the core zones for *B. inermis* habitat since they buffer the effects of anthropic disturbance by allowing individuals to reach reproductive age. However, the area occupied by *B. inermis* within these reserves may not be sufficient to ensure the protection and quality of the species' habitat in long term. Without the presence of biological corridors that connect them, these areas would remain isolated, favoring the loss of genetic diversity and the local extinction of populations. According to our results, the status of *Beaucarnea inermis* as a Threatened species is confirmed according to the criteria of the MER-Plants, however, this could change for the worse if the intensity of the impacts detected does not decrease, putting *B. inermis* in critical danger of extinction.

Supplementary Material

The following online material is available for this article:

Appendix I - Ratings for each of the evaluated criteria MER-Plantas for Beaucarnea inermis according to Normative Annex II of NOM-059-SEMARNAT-2010.

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Ricardo González Trujillo: results interpretation, manuscript preparation, and critical revision, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

Supporting data are available at <https://doi.org/10.48331/scielodata.TTBDRB>

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Inventory

Occurrence and characterization of insect galls in two reserves of the Peruvian Amazon

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Abstract: An insect gall inventory was carried out in two reserves of the Peruvian Amazon, Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, both situated in Iquitos, northeastern Peru. Four vegetation types were surveyed between December, 2021 and December, 2022: *terra firme* forest, white-sand wet forest, and white-sand dry forest in Allpahuayo-Mishana National Reserve, and palm swamp forest in Quistococha Regional Reserve. Overall, we found 262 gall morphotypes, distributed across 75 host species representing 66 plant genera and 30 families. Fabaceae was the plant family with the greatest number of gall morphotypes (n = 48), followed by Calophyllaceae (n = 21) and Euphorbiaceae (n = 20). The plant genera that supported the highest diversity of galls were *Caraipa* (n = 17), *Eschweilera* (n = 16), *Tapirira* (n = 16), *Micrandra* (n = 14), and *Iryanthera* (n = 10). The plant species *Tapirira guianensis* (n = 16), *Caraipa utilis* (n = 14), *Micrandra elata* (n = 14), *Eschweilera coriacea* (n = 11), and *Sloanea parvifructa* (n = 10) exhibited the highest richness of galls. Among the host plants, *C. utilis* stands alone as the only species noted as both endemic to the Amazonian region and bearing a Vulnerable (VU) conservation status. The leaves were the most attacked organs (90% of all galls). Most morphotypes are glabrous (89%), green (67%), globoid (53%), and one-chambered (91%). We found galling insects belonging to the orders Diptera, Thysanoptera, Lepidoptera, and Hemiptera. The galling insects of Cecidomyiidae (Diptera) were the most common, inducing 22% of the gall morphotypes. In addition to the gallers, we also observed the presence of successors, cecidophages, and parasitoids. Among the sampled vegetation types, the *terra firme* forest presented the highest richness of gall morphotypes and host plant species. This is the first systematic inventory of insect galls in this part of the Peruvian Amazon.

Keywords: *Cecidomyiidae; Fabaceae; galls; host plant.*

Ocorrência e caracterização de galhas de insetos em duas reservas da Amazônia peruana

Resumo: Um inventário de galhas de insetos foi realizado em duas reservas da Amazônia peruana, Reserva Nacional Allpahuayo-Mishana e Reserva Regional Quistococha, ambas situadas em Iquitos, nordeste do Peru. Quatro tipos de vegetação foram pesquisados entre dezembro de 2021 e dezembro de 2022: floresta de *terra firme*, floresta úmida de areia branca e floresta seca de areia branca na Reserva Nacional Allpahuayo-Mishana, e floresta de pântano de palmeiras na Reserva Regional Quistococha. No total, encontramos 262 morfotipos de galhas, distribuídos em 75 espécies hospedeiras representando 66 gêneros de plantas e 30 famílias. Fabaceae foi a família de plantas com o maior número de morfotipos de galhas (n = 48), seguida por Calophyllaceae (n = 21) e Euphorbiaceae (n = 20). Os gêneros de plantas que apresentaram a maior diversidade de galhas foram *Caraipa* (n = 17), *Eschweilera* (n = 16), *Tapirira* (n = 16), *Micrandra* (n = 14) e *Iryanthera* (n = 10). As espécies de plantas *Tapirira guianensis* (n = 16), *Caraipa utilis* (n = 14), *Micrandra elata* (n = 14), *Eschweilera coriacea* (n = 11) e *Sloanea parvifructa* (n = 10) apresentaram a maior riqueza de galhas. Dentre as plantas hospedeiras, *C. utilis* destaca-se como a única espécie listada como endêmica da região amazônica e com um status de conservação Vulnerável (VU). As folhas foram os órgãos mais atacados (90% de todas as galhas). A maioria dos morfotipos é glabra (89%), verde (67%), globoide (53%) e possui apenas uma câmara interna (91%). Encontramos insetos galhadores pertencentes às ordens Diptera, Thysanoptera, Lepidoptera e Hemiptera. Os insetos galhadores da família Cecidomyiidae (Diptera) foram os mais comuns, induzindo 22% dos morfotipos de galhas. Além dos galhadores, também observamos a presença de sucessores, cecidófagos e parasitoides. Entre os tipos de vegetação amostrados, a floresta de *terra firme* apresentou a maior riqueza de morfotipos de galhas e espécies de plantas hospedeiras. Este é o primeiro inventário sistemático de galhas de insetos nesta região da Amazônia peruana.

Palavras-chave: *Cecidomyiidae; Fabaceae; galha; planta hospedeira.*

Introduction

Peru is a mega-diverse country in terms of species richness, and comprises a large part of the Amazon forest, which constitutes over 70% of the national territory (Gagliardi-Urrutia et al. 2022). Estimates based on floristic inventories in the Peruvian Amazon suggest that there are approximately 5,000 plant species (Cardoso et al. 2017), almost 756 (13%) of which are endemic (Leon et al. 2006). This high number of plant species represents a great diversity of potential niches for gall-inducing insects (Mendonça 2007). Galling insects are very specialized herbivores and tend to be species-specific with respect to host plants (Stone & Schönrogge 2003, Carneiro et al. 2009). These insects are also very diverse, with some estimates indicating that there could be as many as 132,000 species worldwide (Espírito-Santo & Fernandes 2007), and 3,090 in Peru (Grandez-Rios et al. 2015).

Taxonomic knowledge of gall-inducing insects in the Amazon forest is scarce, largely because of taxonomic uncertainties and difficulty of identification, or else because the species have yet to be described (review in Grandez-Rios et al. 2015). Most inventories of galling insects therefore identify taxa only to the order or family level (Yukawa et al. 2001, Almada & Fernandes 2011), or rarely to genus (Maia 2011). Galling insects recorded in the Amazon forest belong to Diptera, Hemiptera, Coleoptera, Lepidoptera and Hymenoptera, with the Dipteran family Cecidomyiidae particularly well represented (Julião et al. 2014b). Cecidomyiidae comprise more than 6,500 described species worldwide (Gagné & Jaschhof 2021), and achieves its greatest diversity in Amazonia (Grandez-Rios et al. 2015). Insect gall inventories in Amazonia have found that Fabaceae is the most important host plant family (Almada & Fernandes 2011, Maia 2011, Silva et al. 2011, Araújo et al. 2012, Julião et al. 2017). Other host plant families include Burseraceae, Chrysobalanaceae, Sapotaceae and Lecythidaceae (Julião et al. 2014b). Each of these families is notably species-rich in Amazonian forests.

Studies carried out in the Amazonian region have also show that the diversity of gall-inducing insects is highest in *terra firme* forests in comparison with other vegetation types (Julião et al. 2014a). The reason behind this greater diversity of gall-inducing insects in *terra firme* forest lies in the unique characteristics of this habitat (e.g., plant diversity and soil fertility) (Julião et al. 2014b). Furthermore, *terra firme* forest exhibits a remarkable diversity array of plants, providing a wide range availability of ecological niches and an abundant source of resources when compared to the more limited environments, such as white-sand and palm swamp forests (Draper et al. 2018). In this context, the *terra firme* forest is expected to have a higher diversity of gall-inducing insect than other Amazonian vegetation.

Over the last 30 years, several insect gall inventories have been carried out in different phytogeographic domains of the Neotropics, including in the Amazon, although it is one of the least studied (Araújo 2018). In fact, there is a significant bias for studies in Southeast Brazil, where the most important Brazilian research centers regarding galls and galling insects are located, as shown by Araújo (2018). Araújo et al. (2019) compiled data of four inventories performed in the Brazilian Amazon. Despite this small number of published inventories, the galling species richness was greater than in other Brazilian biomes, such as Cerrado. These results show that Amazonian forests host a high galling insect diversity, likely due to the high specialization associated with the remarkable richness of host plants. However, in other parts of the Amazon, such as Peru, insect gall inventories are lacking, and little is known about host plant associations. The objective of the present study was to inventory the galling insects, associated fauna, and their host plants in the Peruvian Amazon.

Materials and Methods

1. Study area

The study was realized southwest of the city of Iquitos, in the province of Loreto in the Peruvian Amazon. The area exhibits a warm and moist climate with an average annual temperature 26 °C and average annual precipitation of 3,087 mm (Marengo 1998). The site is characterized by a long wet season with monthly precipitations > 300 mm in November, March and April, and a weak dry season in June and September. (Marengo 1998). Humidity is 80–90% all year round.

The research was conducted in three vegetation types in the Allpahuayo-Mishana National Reserve (RNAM): *terra firme* forest (S 03° 57.056′, W 073° 26.002′; S 03° 57.051′, W 073° 26.320′; TF), white-sand wet forest (S 03° 57.062′ W 073° 24.586′; WSw) and white-sand dry forest (S 03° 57.253′, W 073° 25.617′; WSd). In addition, one vegetation type was assessed in the Quistococha Regional Reserve (RRQ): palm swamp forest (S 03° 50.037′, W 073° 19.254′; S 03° 50.082′, W 073° 19.019′; PS) (Figure 1). The Allpahuayo-Mishana National Reserve (58,069.90 ha.), exhibits a high degree of both endemism and biodiversity, largely owing to a complex mosaic of habitats—ranging from white sand forest patch growing on nutrient-poor soils, to seasonally-inundated floodplain forests, and *terra firme* forests growing on nutrient-rich clay soils (Salo & Pyhälä 2007). Meanwhile, Quistococha Regional Reserve (369 ha), is characterized by palm swamp forests, with poorly drained soils that are often flooded (Encarnación 1985). These areas are dominated by the palms *Mauritia flexuosa* and *Mauritiella armata* (both Arecaceae), and by the broadleaf tree *Tabebuia insignis* (Bignoniaceae) (Roucoux et al. 2013).

2. *Sampling of galling insects and host plants*

Sampling in the different vegetation types was done bi-monthly between December 2021 and December 2022, with 8 plots of 5 × 20 m each, separated from one another by 20 m, totaling 800 m^2 sampled for each site, in accord with the methodology proposed by Julião et al. (2014a). In each plot, only woody plants with a diameter at breast height (dbh, 1.3 m) equal to or greater than 10 cm were measured and collected, using the standard methods described in the RAINFOR protocol (Phillips et al. 2009). Altogether 32 plots were established, being 24 plots for RNAM (8 plots in *terra firme* forest; 8 plots in white sand wet forest and 8 plots in white sand dry forest) and 8 plots for RRQ (all in palm swamp forest). The woody plant species sampled in the plots were already identified in species as part of a previous floristic project, when each individual received a permanent metal tag, whose related data were downloaded via ForestPlots.net online database (Lopez-Gonzalez et al. 2011, ForestPlots.net et al. 2021). Data on the conservation status and endemism of each host plant species were retrieved from the IUCN Red List of Threatened Species (IUCN 2023) and Leon et al. (2006).

All the plots were established in the understory vegetation and then visually projected onto the canopy, delimiting the sampling area to collect the tree crown above each individual. Ten terminal units of the plant were randomly collected by climbing, and with a 10 m telescoping aluminum pole pruner. In the field, all gall morphotypes were characterized with respect to external morphology, and by shape, color, presence of trichomes, number of chambers, and the host plant organ attacked, as proposed by Isaias et al. (2014).

Galled branches were photographed, collected and transported to the Natural Products Chemistry Laboratory of the Peruvian Amazon Research Institute (IIAP) in closed and labeled transparent plastic bags. Some specimens of each gall morphotype were dissected using a stylet under a Leica Wild M3C stereoscopic microscope, to observe the number of internal chambers and obtain the immature insects (larvae and pupae). These immatures were initially preserved in 70% alcohol in labeled microtubes. The remainder of the sample was used

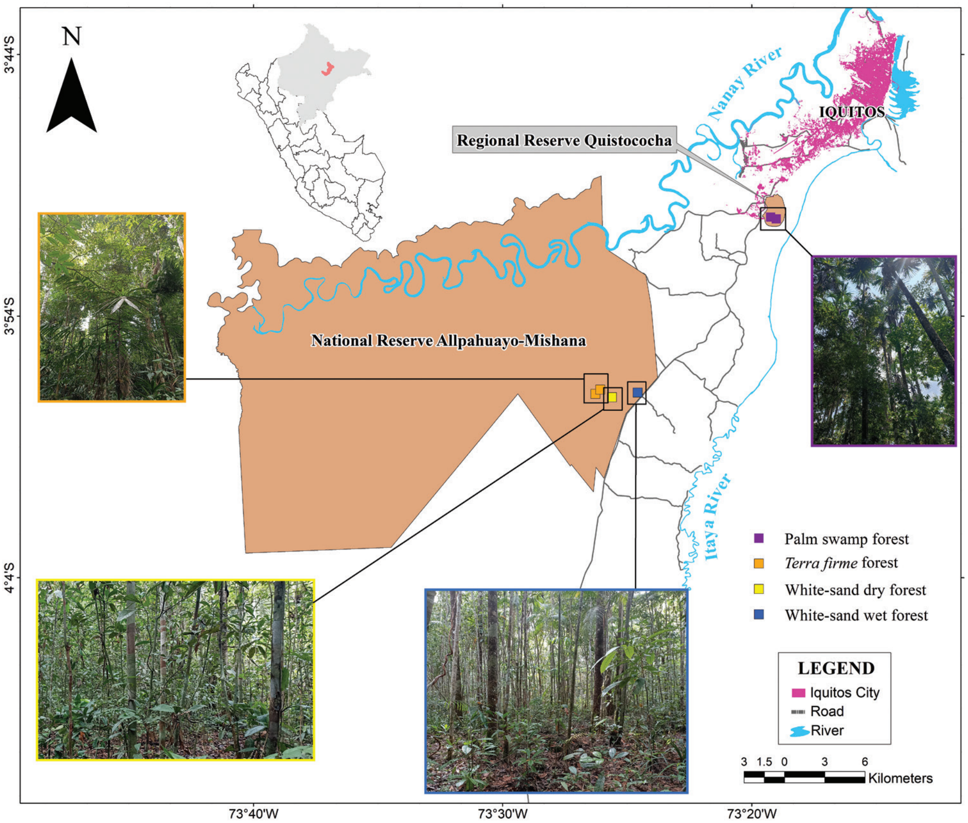

Figure 1. Map of the distribution of sampling areas in four different types of vegetation in the National Reserve Allpahuayo-Mishana and Regional Reserve Quistococha, Iquitos, Peru.

for rearing galling insects and associated fauna (successor, inquilines, cecidophages, and parasitoids). To distinguish these guilds in this study, we used the interaction parameters proposed by Luz & Mendonça Júnior (2019). Successors are organisms that utilize the gall after the inducer departs (Mani 1964), and they use gall tissues for both food and shelter. Inquilines and cecidophages utilize galls alongside the gall inducer. Both feed on plant tissues and consume gall tissues, but only the inquilines stimulate the growth of new tissues (Luz & Mendonça Júnior 2019). Parasitoids are organisms that closely associate with the host, exploiting the host's resources, eventually leading to the host's demise upon the completion of the parasitoid development (Maia 2022). Furthermore, each gall morphotype was placed in a closed transparent plastic pot, labeled and with moistened paper at the bottom. All pots were checked daily for adult emergence.

All insects were preserved in 70% alcohol. The gall midges (Diptera: Cecidomyiidae) were later mounted on slides for microscope, following the methodology outlined in Gagné (1994). Cecidomyiidae genera were determined based on the keys of Gagné available for the Neotropical region (1994). Micro-Hymenoptera were identified to the family and genus levels using Gibson's key (Gibson et al. 1997). Hymenoptera obtained only in the larval or pupal stage remained identified only to order. All the specimens were deposited in the Diptera collection of Museu Nacional (MNNRJ)/ Universidade Federal do Rio de Janeiro, and at the Peruvian Amazon Research Institute (IIAP). The other insects were sent to specialists for identification.

Results

We found 262 gall morphotypes, distributed across 30 botanical families and comprising 66 genera and 75 species (Table 1, Figures 2–12). The average number of gall morphotypes per host plant species was 3.5. The plant families that showed the greatest richness of insect galls were Fabaceae with 48 morphotypes, Calophyllaceae with 21, Euphorbiaceae with 20, Lecythidaceae with 18, Lauraceae with 17, Anacardiaceae with 16, Sapotaceae with 14, Malvaceae and Myristicaceae with 12, Phyllanthaceae with 11, and Elaeocarpaceae with 10 morphotypes. The remaining families had less than 10 insect gall morphotypes each. The plant genera *Caraipa* Aubl. (Calophyllaceae), *Eschweilera* Mart. ex DC. (Lecythidaceae), *Tapirira* Aubl. (Anacardiaceae), *Micrandra* Benth. (Euphorbiaceae) and *Iryanthera* (A. DC.) Warb. (Myristicaceae) hosted the highest numbers of insect gall morphotypes (17, 16, 16, 14 and 10, respectively). The most important host plant species were *T. guianensis* Aubl., *C. utilis* Vásquez, *M. elata* (Didr.) Müll. Arg., *E. coriacea* (DC.) S.A. Mori, and *Sloanea parvifructa* Steyerm., hosting 16, 14, 14, 11 and 10 insect gall morphotypes, respectively (Table 2). On the other hand, among all host plant species, only *C. utilis* is mentioned as endemic to the Amazonian region with a Vulnerable (VU) conservation status, while *T. insignis* (Miq.) Sandwith was categorized as Near Threatened (NT). The rest of the plant species were categorized as Least Concern (LC), with some not being classified within the IUCN list (Table 2).

Galls were found in leaves (n = 235), stems (n = 21), buds (n = 3) and fruits (n = 2), with 90% of all galls occurring on leaves. All morphotypes occurred in just one plant organ, except one morphotype that was found in both leaves and petioles of *E. coriacea.*

Table 1. Number of host plant species and insect gall morphotypes per host plant family in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru.

Host family	Number of plant genera	Number of plant species	Number of gall morphotypes
Anacardiaceae	1	1	16
Annonaceae	3	4	7
Apocynaceae	2	2	5
Araliaceae	1	1	3
Bignoniaceae	1	1	5
Burseraceae	1	1	1
Calophyllaceae	2	3	21
Chrysobalanaceae	1	1	3
Elaeocarpaceae	1	1	10
Euphorbiaceae	4	4	20
Fabaceae	11	12	48
Humiriaceae	1	1	2
Lauraceae	5	5	17
Lecythidaceae	3	4	18
Lepidobotryaceae	1	1	1
Malpighiaceae	1	1	2
Malvaceae	3	3	12
Melastomataceae	1	1	2
Meliaceae	1	1	3
Metteniusaceae	1	1	2
Moraceae	3	3	3
Myristicaceae	2	4	12
Myrtaceae	3	3	6
Nyctaginaceae	1	2	5
Olacaceae	1	1	2
Phyllanthaceae	3	3	11
Salicaceae	1	1	3
Sapindaceae	2	3	5
Sapotaceae	3	4	14
Urticaceae	2	2	3
Total	66	75	262

Ten distinct gall shapes were found: globoid, lenticular, fusiform, marginal roll, conical, clavate, amorphous, cup, pocket, and rosette. The globoid, lenticular and fusiform shapes were the most frequent ones, with 130, 58 and 39 morphoytpes, respectively, followed by marginal roll (n = 15), conical (n = 14), clavate (n = 2), and others (n = 1). The gall morphotypes included green, brown, black, and yellow coloration with green color predominant (67%), followed by brown (32%). The majority of galls were glabrous, with 232 morphotypes (89%); the remainder were hairy, with 30 morphotypes (11%). About 91% (238 morphotypes) of galls were one-chambered, often occupied by a single larva; just 9% (24 morphotypes) were multi-chambered.

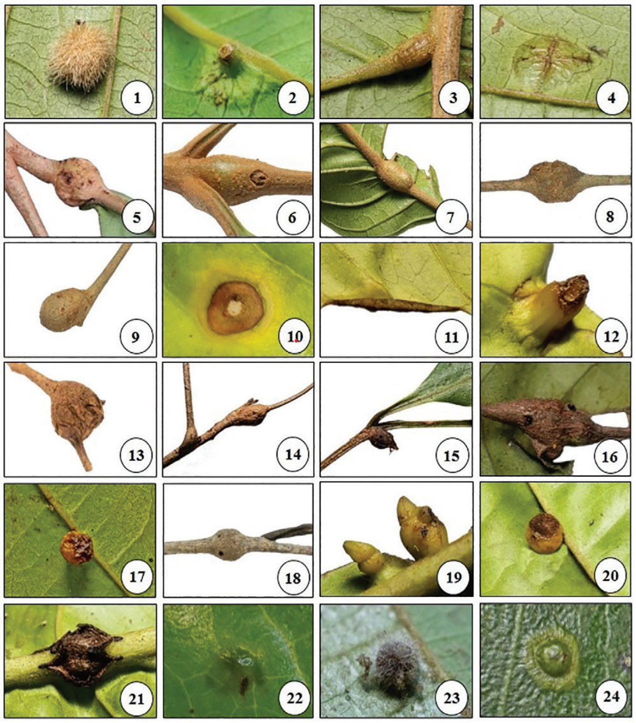

Figure 2. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 1–16; *Tapirira guianensis* (1. globoid-leaf, 2. conical-leaf, 3. fusiform-leaf, 4. lenticular-leaf, 5. globoid-leaf, 6. globoid-leaf (rachis), 7. fusiform-leaf, 8. globoid-leaf (petiole), 9. globoid-leaf, 10. lenticular-leaf, 11. marginal roll-leaf, 12. conical-leaf, 13. globoid-stem, 14. globoid-leaf (rachis), 15. conical-leaf (rachis), 16. fusiform-leaf), 17–18; *Diclinanona tessmannii* (17. globoid-leaf, 18. globoid-stem), 19–21; *Guatteria megalophylla* (19. conical-leaf, 20. globoid-leaf, 21. globoid-leaf), 22; *Porcelia nitidifolia* (22. globoid-leaf), 23; *Unonopsis spectabilis* (23. globoid-leaf), 24; *Aspidosperma excelsum* (24. lenticular-leaf).

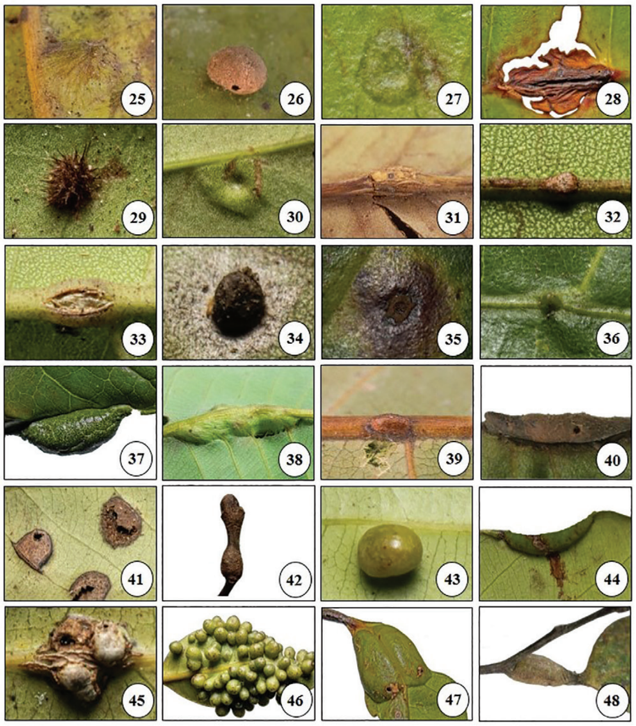

Figure 3. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 25–26; *Aspidosperma excelsum* (25. lenticular-leaf, 26. globoid-leaf), 27–28; *Parahancornia peruviana* (27. lenticular-leaf, 28. fusiform-leaf), 29–31; *Dendropanax umbellatus* (29. globoid-leaf, 30. lenticular-leaf, 31. fusiform-leaf), 32–36; *Tabebuia insignis* (32. globoid-leaf, 33. fusiform-leaf, 34. globoid-leaf, 35. lenticular-leaf, 36. globoid-leaf), 37; *Protium crassipetalum* (37. marginal roll-leaf), 38–40; *Caraipa tereticaulis* (38. fusiform-leaf, 39. Globoid-leaf, 40. marginal roll-leaf), 41–48; *Caraipa utilis* (41. lenticular-leaf, 42. globoid-stem, 43. globoid-leaf, 44. marginal roll-leaf, 45. globoid-leaf, 46. globoid-leaf, 47. globoid-leaf, 48. globoid-leaf (petiole)).

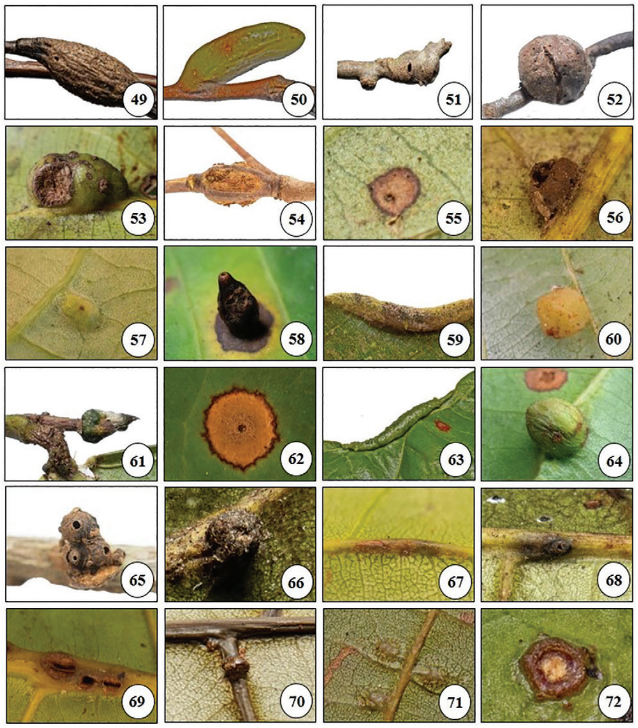

Figure 4. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 49–54; *Caraipa utilis* (49. globoid-leaf (petiole), 50. globoid-leaf, 51. globoid-stem, 52. globoid-stem, 53. globoid-leaf, 54. globoid-stem), 55–58; *Haploclathra cordata* (55. lenticular-leaf, 56. globoid-leaf, 57. globoid-leaf, 58. conical-leaf), 59–61; *Couepia williamsii* (59. marginal roll-leaf, 60. globoid-leaf, 61. globoid-bud), 62–71; *Sloanea parvifructa* (62. lenticular-leaf, 63. marginal roll-leaf, 64. globoid-leaf, 65. globoid-stem, 66. globoid-leaf, 67. fusiform-leaf, 68. fusiform-leaf, 69. globoid-leaf, 70. globoid-leaf, 71. lenticular-leaf), 72; *Alchorneopsis floribunda* (72. lenticular-leaf).

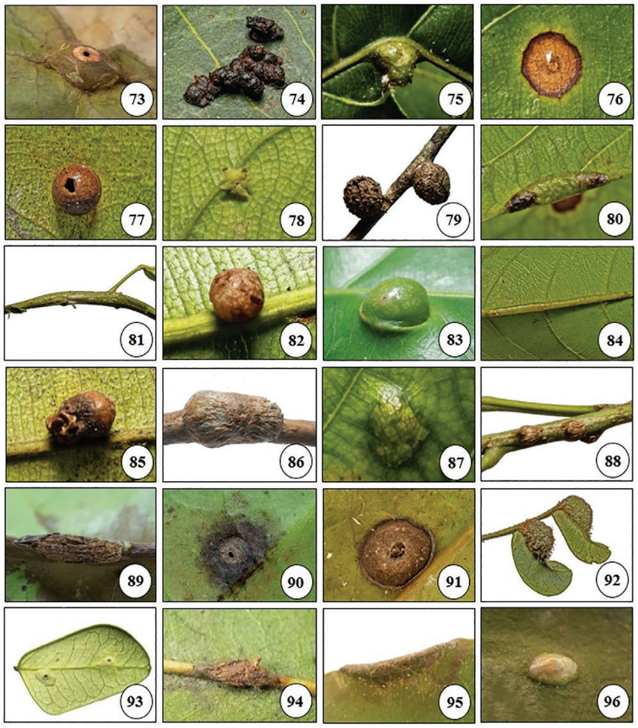

Figure 5. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 73; *Alchorneopsis floribunda* (73. globoid-leaf), 74; *Hevea guianensis* (74. globoid-leaf), 75–88; *Micrandra elata* (75. globoid-leaf, 76. lenticular-leaf, 77. globoid-leaf, 78. amorphous-leaf, 79. globoid-stem, 80. marginal roll-leaf, 81. fusiform-stem, 82. globoid-leaf, 83. globoid-leaf, 84. fusiform-leaf, 85. globoid-leaf, 86. globoid-stem, 87. globoid-leaf, 88. globoid-stem), 89–91; *Nealchornea yapurensis* (89. fusiform-leaf, 90. lenticular-leaf, 91. lenticular-leaf), 92–93; *Abarema auriculata* (92. globoid-leaf, 93. lenticular-leaf), 94–96; *Cynometra bauhiniifolia* (94. fusiform-leaf, 95. marginal roll-leafl, 96. globoid-leaf).

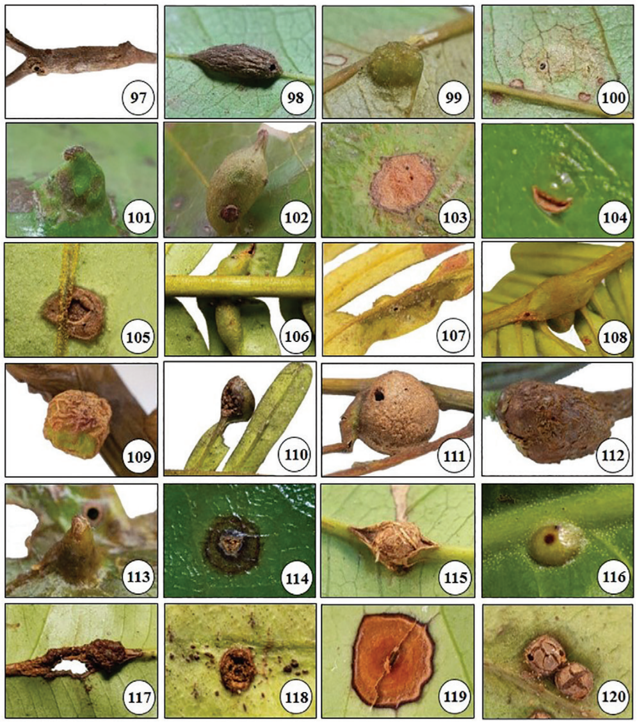

Figure 6. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 97; *Cynometra bauhiniifolia* (97. fusiform-stem), 98–101; *Dialium guianense* (98. fusiform-leaf, 99. globoid-leaf, 100. lenticular-leaf, 101. conical-leaf), 102–103; *Dicymbe uaiparuensis* (102. conical-leaf, 103. lenticular-leaf), 104–111; *Dimorphandra macrostachya* (104. globoid-leaf, 105. lenticular-leaf, 106. globoid-leaf, 107. pocket-leaf, 108. globoid-leaf, 109. globoid-leaf, 110. globoid-leaf, 111. globoid-leaf (rachis)), 112–114; *Hymenaea courbaril* (112. globoid-stem, 113. conical-leaf, 114. lenticular-leaf), 115; *Inga nobilis* (115. globoid-leaf), 116–119; *Macrolobium limbatum* (116. globoid-leaf, 117. fusiform-leaf, 118. lenticular-fruit, 119. lenticular-leaf), 120; *Macrolobium microcalyx* (120. globoid-leaf).

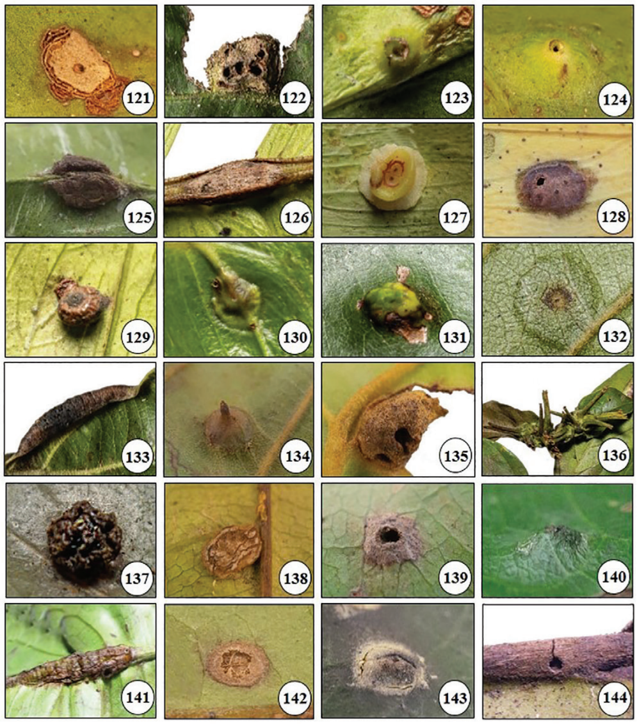

Figure 7. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 121–125; *Macrolobium microcalyx* (121. lenticular-leaf, 122. globoid-leaf, 123. conical-leaf, 124. globoid-leaf, 125. fusiform-leaf), 126–130; *Parkia igneiflora* (126. fusiform-leaf, 127. cup-leaf, 128. lenticular-leaf, 129. globoid-leaf, 130. globoid-leaf), 131–136; *Tachigali loretensis* (131. globoid-leaf, 132. lenticular-leaf, 133. marginal roll-leaf, 134. globoid-leaf, 135. globoid-leaf, 136. rosette-leaf), 137–139; *Vatairea erythrocarpa* (137. globoid-leaf, 138. globoid-leaf, 139. lenticular-leaf), 140–141; *Vantanea parviflora* (140. globoid-leaf, 141. fusiform-leaf), 142–144; *Aniba taubertiana* (142. lenticular-leaf, 143. globoid-leaf, 144. fusiform-leaf).

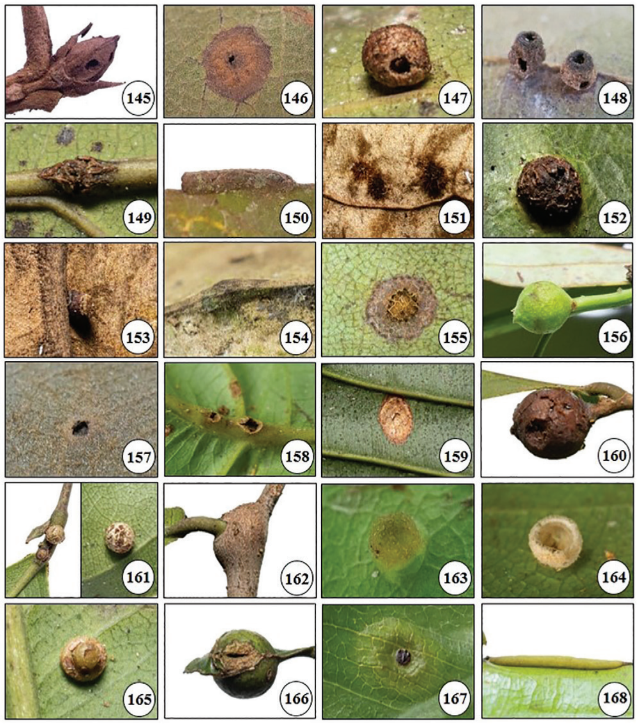

Figure 8. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 145; *Aniba taubertiana* (145. globoid-bud), 146–150; *Nectandra longifolia* (146. lenticular-leaf, 147, globoid-leaf, 148. globoid-leaf, 149. fusiform-leaf, 150. marginal roll-leaf), 151–154; *Ocotea argyrophylla* (151. globoid-leaf, 152. globoid-leaf, 153. conical-leaf, 154. fusiform-leaf), 155–156; *Persea boliviensis* (155. lenticular-leaf, 156. globoid-bud), 157–158; *Pleurothyrium cuneifolium* (157. lenticular-stem, 158. fusiform-leaf), 159; *Allantoma pluriflora* (159. lenticular-leaf), 160–168; *Eschweilera coriacea* (160. globoid-leaf, 161. globoid-petiole/leaf, 162. globoid-stem, 163. globoid-leaf, 164. globoid-leaf, 165. globoid-leaf, 166. globoid-leaf, 167. lenticular-leaf, 168. marginal roll-leaf).

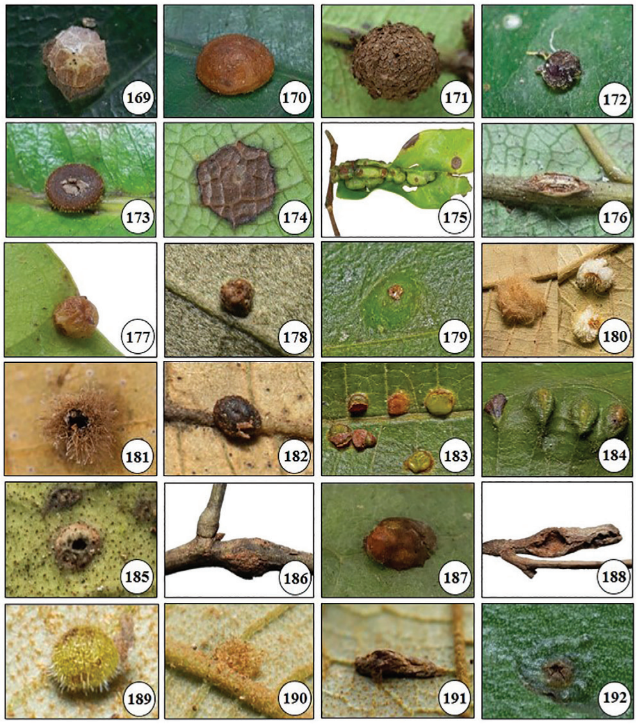

Figure 9. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 169–170; *Eschweilera coriacea* (169. globoid-leaf, 170. globoid-leaf), 171–175; *Eschweilera rufifolia* (171. globoid-leaf, 172, globoid-leaf, 173. lenticular-leaf, 174. lenticular-leaf, 175. globoid-leaf), 176; *Gustavia longifolia* (176. fusiform-leaf), 177; *Ruptiliocarpon caracolito* (177. globoid-leaf), 178–179; *Bunchosia argentea* (178. globoid-leaf, 179. globoid-leaf), 180–184; *Luheopsis hoehnei* (180. globoid-leaf, 181. globoid-leaf, 182. 183. globoid-leaf, 184. fusiform-leaf), 185–188; *Pachira brevipes* (185. lenticular-leaf, 186. globoid-stem, 187. globoid-leaf, 188. fusiform-stem), 189–191; *Theobroma subincanum* (189. globoid-leaf, 190. globoid-leaf, 191. fusiform-leaf), 192; *Mouriri vernicosa* (192. lenticular-leaf).

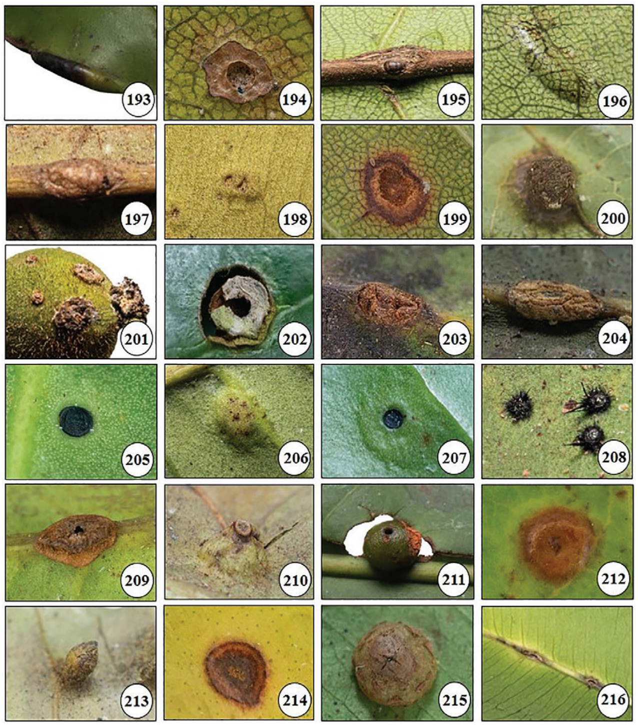

Figure 10. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 193; *Mouriri vernicosa* (193. Marginal roll-leaf), 194–196; *Carapa procera* (194. lenticular-leaf, 195, fusiform-leaf, 196. globoid-leaf), 197–198; *Emmotum floribundum* (197. fusiform-leaf, 198. lenticular-leaf), 199; *Brosimum utile* (199. lenticular-leaf), 200; *Perebea xanthochyma* (200. globoid-leaf), 201; *Trymatococcus amazonicus* (201. lenticular-fruit), 202–203; *Iryanthera juruensis* (202. lenticular-leaf, 203. fusiform-leaf), 204–205; *Iryanthera laevis* (204. fusiform-leaf, 205. lenticular-leaf), 206–211; *Iryanthera lancifolia* (206. lenticular-leaf, 207. lenticular-leaf, 208. lenticular-leaf, 209. fusiform-leaf, 210. conical-leaf, 211. globoid-leaf), 212–213; *Virola pavonis* (212. lenticular-leaf, 213. Conical-leaf), 214; *Blepharocalyx eggersii* (214. lenticular-leaf); 215–216; *Eugenia riparia* (215. globoid-leaf, 216. fusiform-leaf).

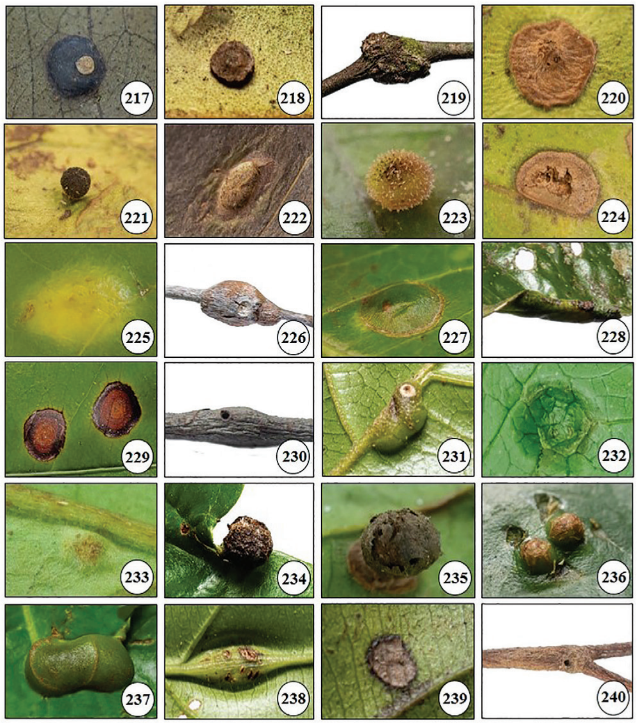

Figure 11. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 217; *Eugenia riparia* (217. globoid-leaf), 218–219; *Myrcia cebra* (218. lenticular-leaf, 219, globoid-stem), 220–222; *Neea spruceana* (220. lenticular-leaf, 221. globoid-leaf, 222. lenticular-leaf), 223–224; *Neea verticillata* (223. globoid-leaf, 224. lenticular-leaf), 225–226; *Cathedra acuminata* (225. lenticular-leaf, 226. globoid-stem), 227–230; *Amanoa guianensis* (227. lenticular-leaf, 228. marginal roll-leaf, 229. lenticular-leaf, 230. globoid-stem), 231–232; *Hieronyma oblonga* (231. globoid-leaf, 232. globoid-leaf), 233–237; *Richeria grandis* (233. globoid-leaf, 234. globoid-leaf, 235. globoid-leaf, 236. globoid-leaf, 237. globoid-leaf), 238–240; *Casearia arborea* (238. fusiform-leaf, 239. lenticular-leaf, 240. fusiform-stem).

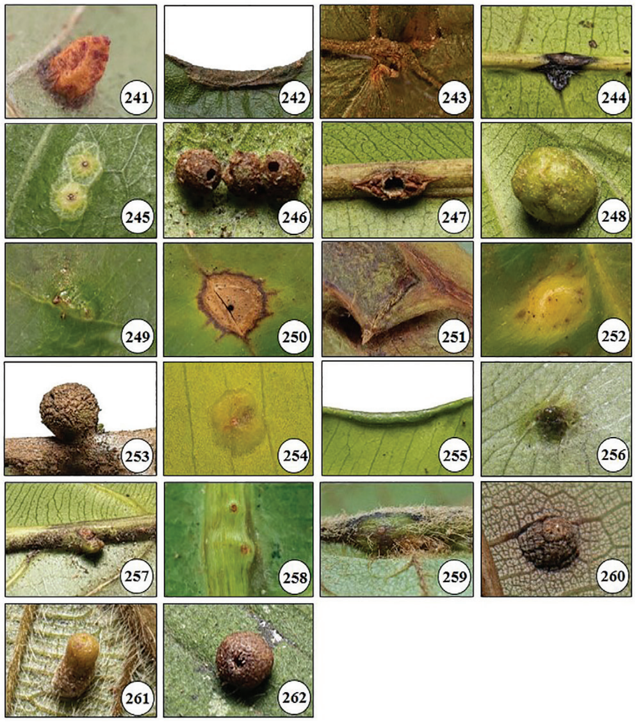

Figure 12. Galls found in four vegetation types in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. 241; *Cupania diphylla* (241. conical-leaf), 242–243; *Cupania latifolia* (242. Marginal roll-leaf, 243, fusiform-leaf), 244–245; *Talisia firma* (244. fusiform-leaf, 245. lenticular-leaf), 246–251; *Chrysophyllum sanguinolentum* (246. globoid-leaf, 247. fusiform-leaf, 248. globoid-leaf, 249. globoid-leaf, 250. lenticular-leaf, 251. Fusiform-leaf), 252–255; *Manilkara bidentata* (252. globoid-leaf, 253. globoid-stem, 254. lenticular-leaf, 255. Marginal roll-leaf), 256–258; *Pouteria subrotata* (256. conical-leaf, 257. clavate-leaf, 258. globoid-leaf), 259; *Pouteria torta* (259. fusiform-leaf), 260; *Cecropia distachya* (260. globoid-leaf), 261–262; *Pouroma bicolor* (261. clavate-leaf, 262. globoid-leaf).

The galling insects were distributed across four orders: Diptera (n = 58), Thysanoptera (n = 2), Lepidoptera (n = 2), and Hemiptera (n = 1) (Table 3). The Cecidomyiidae (Diptera) was responsible for 22% of all morphotypes. In 199 (76%) of the gall morphotypes we did not find the gall-inducing insect, either because the galls were empty, contained parasitoids, or were occupied by multiple potentially gall-inducing taxa. Eigtheen species of gall midges were identified, in two genera: *Contarinia* Rondani, 1860 with five morphospecies and *Bruggmanniella* Tavares, 1909 with one morphospecies. The tribes: Asphondyliini and Lasiopteridi both contained two morphospecies, while Clinodiplosini and Lopesiini had three and five morphospecies, respectively. This is due to scarcity of material, either because one or both sexes were absent, or because no immature specimens were observed. Additionally, the inducer of two gall morphotypes was identified as *Plagiothrips* sp. 2 (Thysanoptera).

The associated fauna comprised successors, cecidophages and parasitoids. Successors, represented by Thysanoptera (*Adraneothrips* sp., *Holothrips* sp. 1, and *Plagiothrips* sp. 1), Hymenoptera (Formicidae and Apidae), Collembola and Acari, were found in 8, 8, 5 and 3 morphotypes, respectively. Cecidophages, Curculionidae (Coleoptera) and Sciaridae (Diptera) were found in only one morphotype. The parasitoids obtained from 55 morphotypes (21% of the total) represented 11 families and 23 genera of Hymenoptera: Aphelinidae (*Coccobius* sp.), Braconidae (*Allorhogas* sp. 1, 2), Encyrtidae (*Metaphycus* sp.), Eulophidae (*Aranobroter* sp.; *Baryscapus* sp.; *Chrysonotomyia* sp.; *Euderus* sp.; *Euderomphale* sp.; *Galeopsomyia* sp.; *Horismenus* sp.; *Neopomphale* sp. 1, 2), Eupelmidae (*Lambdobregma* sp.), Eurytomidae (*Calorileya* sp.; *Phylloxeroxenus* sp.; *Platyrileya* sp. 1; *Prodecatoma* sp.; *Tenuipetiolus* sp.), Mymaridae (*Polynema* sp.), Torymidae (*Torymus* sp. 1, 2), Platygastridae (*Magellanium* sp. 1, 2), Pteromalidae (*Cerocephala* sp.; *Euteloidea* sp.), and Scelionidae (*Baryconus* sp.).

In the Allpahuayo-Mishana National Reserve, a total of 240 gall morphotypes were found across 68 plant species, representing 29 botanical families and 59 genera. Meanwhile, in the Quistococha Regional Reserve, 22 gall morphotypes were found, distributed among 8 families, 8 genera, and 8 species. These data were also analyzed based on the type of vegetation present in each reserve.

The *terra firme* forest showed the greatest diversity of galls and host plants, with 111 different morphotypes found on 39 host plant species, followed by white-sand wet forest, white-sand dry forest and palm swamp forest, which had 73, 56 and 22 morphotypes, among 16, 16 and 8 host plant species respectively (Table 2). In *terra firme* forest, leaves (n = 104) were the most commonly galled plant organ; the most common gall morphologies included the globoid shape (n = 51), green color (n = 70), glabrous surface (n = 94) and single internal chamber (n = 104). In white-sand wet forest, leaves are most commonly attacked (n = 65), and morphologies are mostly globoid (n = 41), green (n = 52), glabrous (n = 67) and with a single inner chamber (n = 66). In white-sand dry forest: leaves (n = 45), globoid (n = 29), green (n = 35), glabrous (n = 52), and a single inner chamber (n = 48). For palm swamp forest: leaves (n = 21), globoid (n = 9), green (n = 18), glabrous (n = 19), and a single inner chamber (n = 20) (Table 2).

The *terra firme* forest also showed the richest gall-inducing insect fauna, represented by Diptera (n = 18), Thysanoptera (n = 2),

Table 2. Characterization of insect galls in four vegetation type in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru. Legends: P = Parasitoid, S = Successor, C = Cecidophages; TF = Terra firme forest, WSd = White-sand dry forest, WSw= White-sand wet forest, and PS = Palm swamp forest.

Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
Anacardiaceae	*Tapirira guianensis* Aubl.	Leaf	Globoid	Brown	Yes	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 1
		Leaf	Conical	Green	Yes	One-chambered	TF	Unidentified	Hymenoptera (P)		Fig. 2
		Leaf	Fusiform	Brown	Yes	One-chambered	TF	Unidentified			Fig. 3
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 4
		Leaf	Globoid	Brown	Yes	One-chambered	TF	Unidentified			Fig. 5
		Leaf	Globoid	Brown	Yes	One-chambered	TF	Unidentified			Fig. 6
		Leaf	Fusiform	Brown	No	One-chambered	TF	Thysanoptera (*Plagiothrips* sp. 2)	Hymenoptera (P)		Fig. 7
		Leaf	Globoid	Brown	No	One-chambered	TF	Thysanoptera (*Plagiothrips* sp. 2)			Fig. 8
		Leaf	Globoid	Brown	No	One-chambered	TF	Cecidomyiidae	Hymenoptera (P)		Fig. 9
		Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified			Fig. 10
		Leaf	Marginal roll	Green	No	One-chambered	WSd	Unidentified			Fig. 11
		Leaf	Conical	Green	No	One-chambered	WSd	Unidentified			Fig. 12
		Stem	Globoid	Brown	No	One-chambered	WSd	Cecidomyiidae			Fig. 13
		Leaf	Globoid	Brown	No	One-chambered	WSd	Cecidomyiidae	*Allohorgas* sp. 2 (P) (Hymenoptera)		Fig. 14
		Leaf	Conical	Brown	No	One-chambered	WSd	Unidentified			Fig. 15
		Leaf	Fusiform	Brown	No	Multilocular	WSd	Cecidomyiidae	Hymenoptera (P)		Fig. 16
Annonaceae	*Diclinanona tessmannii* Diels	Leaf	Globoid	Brown	No	One-chambered	WSd	Unidentified		Least Concern (LC)	Fig. 17
		Stem	Globoid	Brown	No	One-chambered	WSd	Unidentified			Fig. 18
	Guatteria megalophylla Diels	Leaf	Conical	Green	No	One-chambered	WSd	Unidentified		Least Concern (LC)	Fig. 19
		Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified	Formicidae (S)		Fig. 20
		Leaf	Globoid	Brown	No	One-chambered	WSd	Unidentified			Fig. 21
	Porcelia nitidifolia Ruiz & Pav.	Leaf	Globoid	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 22
	Unonopsis spectabilis Diels	Leaf	Globoid	Green	Yes	One-chambered	TF	Cecidomyiidae		Least Concern (LC)	Fig. 23

Continue...

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
Apocynaceae	*Aspidosperma excelsum* Benth.	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 24
		Leaf	Lenticular	Green	No	One-chambered	TF / WSd	Cecidomyiidae	Hymenoptera (P)		Fig. 25
		Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified			Fig. 26
	Parahancornia peruviana Monach.	Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified		-	Fig. 27
		Leaf	Fusiform	Green	No	One-chambered	PS	Unidentified			Fig. 28
Araliaceae	*Dendropanax umbellatus* (Ruiz & Pav.) J.F. Macbr.	Leaf	Globoid	Brown	Yes	One-chambered	WSw	Unidentified		-	Fig. 29
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 30
		Leaf	Fusiform	Green	No	One-chambered	WSw	Cecidomyiidae	Hymenoptera (P)		Fig. 31
Bignoniaceae	*Tabebuia insignis* (Miq.) Sandwith	Leaf	Globoid	Brown	No	One-chambered	PS	Unidentified		Near Threatened (NT)	Fig. 32
		Leaf	Fusiform	Green	No	One-chambered	PS	Unidentified	Polynema sp. (P) (Hymenoptera)		Fig. 33
		Leaf	Globoid	Green	No	One-chambered	PS	Unidentified			Fig. 34
		Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified	*Adraneothrips* sp. (S) (Thysanoptera)		Fig. 35
		Leaf	Globoid	Green	No	One-chambered	PS	Unidentified			Fig. 36
Burseraceae	*Protium crassipetalum* Cuatrec.	Leaf	Marginal roll	Green	No	One-chambered	TF	Cecidomyiidae	Hymenoptera (P) *Holothrips* sp. 1 (S) (Thysanoptera) Formicidae (S)	-	Fig. 37
Calophyllaceae	*Caraipa tereticaulis* Tul.	Leaf	Fusiform	Green	No	Multilocular	WSw	Cecidomyiidae	Hymenoptera (P)	Least Concern (LC)	Fig. 38
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 39
		Leaf	Marginal roll	Green	No	One-chambered	WSw	Cecidomyiidae	Hymenoptera (P)		Fig. 40
	Caraipa utilis Vásquez	Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified		Vulnerable (VU)	Fig. 41
		Stem	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 42

Continue...

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Family	**Species**	**Organ**	**Shape**	**Color**	**Pubescence**	**Chambers**	**Vegetation type**	**Gall-inducers**	**Associated fauna**	**Conservation status (IUCN 2023)**	**Figure number**
		Leaf	Globoid	Green	No	Multilocular	WSw	Cecidomyiidae	*Magellanium* sp. 2 (P) (Hymenoptera) *Holothrips* sp. 1 (S) (Thysanoptera)		Fig. 43
		Leaf	Marginal roll	Green	No	One-chambered	WSw	Cecidomyiidae	*Holothrips* sp. 1 (S) (Thysanoptera) Collembola (S)		Fig. 44
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 45
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 46
		Leaf	Globoid	Green	No	One-chambered	WSw	Cecidomyiidae	Collembola (S) Formicidae (S) Eupelmidae (P) Braconidae (P) (Hymenoptera)		Fig. 47
		Leaf	Globoid	Brown	No	One-chambered	WSw	Cecidomyiidae	*Tenuipetiolus* sp. (P) (Hymenoptera)		Fig. 48
		Leaf	Globoid	Brown	No	One-chambered	WSw	Unidentified	*Phylloxeroxenus* sp. (P) (Hymenoptera)		Fig. 49
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 50
		Stem	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 51
		Stem	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 52
		Leaf	Globoid	Green	No	Multilocular	WSw	Unidentified			Fig. 53
		Stem	Globoid	Brown	No	One-chambered	WSw	Cecidomyiidae	Curculionidae (C) (Coleoptera)		Fig. 54
	Haploclathra cordata Vásquez	Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified		-	Fig. 55
		Leaf	Globoid	Green	No	Multilocular	WSw	Unidentified	Hymenoptera (P)		Fig. 56
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 57
		Leaf	Conical	Green	No	One-chambered	WSw	Unidentified			Fig. 58
Chrysobalanaceae	*Couepia williamsii* J.F. Macbr.	Leaf	Marginal roll	Green	No	One-chambered	WSd	Lopesiini		-	Fig. 59
		Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified	Hymenoptera (P)		Fig. 60

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
		Bud	Globoid	Green	No	One-chambered	WSd	*Contarinia* sp.			Fig. 61
Elaeocarpaceae	*Sloanea parvifructa* Steyerm.	Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified		-	Fig. 62
		Leaf	Marginal roll	Green	No	One-chambered	WSw	Cecidomyiidae	*Horismenus* sp. (P) (Hymenoptera)		Fig. 63
		Leaf	Globoid	Green	No	Multilocular	WSw	Lopesiini	Hymenoptera (P)		Fig. 64
		Stem	Globoid	Green	No	Multilocular	WSw	Asphondyliini	Collembola (S) *Prodecatoma* sp. (P) Platyrileya sp. 1 (P) *Torymus* sp. 1, 2 (P) *Cerocephala* sp. (P) *Lambdobregma* sp. (P) *Allorhogas* sp. 1 (P) (Hymenoptera)		Fig. 65
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 66
		Leaf	Fusiform	Green	No	One-chambered	WSw	Unidentified			Fig. 67
		Leaf	Fusiform	Brown	No	One-chambered	WSw	Unidentified			Fig. 68
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified	Apidae (S) (Hymenoptera)		Fig. 69
		Leaf	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 70
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 71
Euphorbiaceae	*Alchorneopsis floribunda (Benth.)* Müll. Arg.	Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified		Least Concern (LC)	Fig. 72
		Leaf	Globoid	Green	Yes	Multilocular	PS	Unidentified	Hymenoptera (P)		Fig. 73
	Hevea guianensis Aubl.	Leaf	Globoid	Brown	No	One-chambered	WSw	Unidentified		Least Concern (LC)	Fig. 74
	Micrandra elata (Didr.) Müll. Arg.	Leaf	Globoid	Green	No	Multilocular	WSd	Unidentified	Eulophidae (P) (Hymenoptera)	Least Concern (LC)	Fig. 75
		Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified			Fig. 76
		Leaf	Globoid	Green	No	Multilocular	WSd	Lasiopteridi	*Neopomphale* sp. 2 (P) *Calorileya* sp. (P)		Fig. 77

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
		Leaf	Amorphous	Green	No	One-chambered	WSd	Cecidomyiidae	*Coccobius* sp. (P) (Hymenoptera) *Neopomphale* sp. 2 (P) (Hymenoptera)		Fig. 78
		Stem	Globoid	Brown	No	One-chambered	WSd	Cecidomyiidae	Hymenoptera (P)		Fig. 79
		Leaf	Marginal roll	Green	No	One-chambered	WSd	Lopesiini	Hymenoptera (P)		Fig. 80
		Stem	Fusiform	Green	No	One-chambered	WSd	Cecidomyiidae	*Plagiothrips* sp.1 (S) (Thysanoptera) Collembola (S) Formicidae (S)		Fig. 81
		Leaf	Globoid	Green	No	One-chambered	WSd	Cecidomyiidae	*Magellanium* sp.1 (P) (Hymenoptera) Hymenoptera (P) Collembola (S)		Fig. 82
		Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified			Fig. 83
		Leaf	Fusiform	Green	No	One-chambered	WSd	Unidentified			Fig. 84
		Leaf	Globoid	Brown	No	One-chambered	WSd	Unidentified			Fig. 85
		Stem	Globoid	Brown	No	One-chambered	WSd	Cecidomyiidae	Hymenoptera (P)		Fig. 86
		Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified			Fig. 87
		Stem	Globoid	Brown	No	One-chambered	WSd	Unidentified			Fig. 88
	Nealchornea yapurensis Huber	Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 89
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 90
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 91
Fabaceae	*Abarema auriculata* (Benth.) Barneby & J.W. Grimes	Leaf	Globoid	Green	Yes	One-chambered	WSw	Lopesiini	Hymenoptera (P)	Least Concern (LC)	Fig. 92
	Cynometra bauhiniifolia Benth.	Leaf	Lenticular	Green	No	One-chambered	WSw	Cecidomyiidae			Fig. 93
		Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 94
		Leaf	Marginal roll	Green	No	One-chambered	TF	Unidentified			Fig. 95

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
		Leaf	Globoid	Green	No	One-chambered	TF	Lasiopteridi	Acari (S)		Fig. 96
		Stem	Fusiform	Brown	No	One-chambered	TF	Unidentified			Fig. 97
	Dialium guianense (Aubl.) Sandwith	Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 98
		Leaf	Globoid	Green	No	One-chambered	TF	Clinodiplosini	Hymenoptera (P)		Fig. 99
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 100
		Leaf	Conical	Green	Yes	One-chambered	TF	Cecidomyiidae			Fig. 101
	Dicymbe uaiparuensis R.S. Cowan	Leaf	Conical	Green	Yes	One-chambered	WSw	Cecidomyiidae	*Baryscapus* sp. (P) (Hymenoptera)	-	Fig. 102
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 103
	Dimorphandra macrostachya Benth.	Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified		Least Concern (LC)	Fig. 104
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 105
		Leaf	Globoid	Green	Yes	One-chambered	WSw	Cecidomyiidae	Hymenoptera (P)		Fig. 106
		Leaf	Pocket	Green	No	One-chambered	WSw	Cecidomyiidae	*Galeopsomyia* sp. (P) (Hymenoptera)		Fig. 107
		Leaf	Globoid	Green	No	One-chambered	WSw	Clinodiplosini	Hymenoptera (P)		Fig. 108
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 109
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified	Sciaridae (C) (Diptera)		Fig. 110
		Leaf	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 111
	Hymenaea courbaril L.	Stem	Globoid	Brown	No	Multilocular	TF	Asphondyliini	*Euteloidea* sp. (P) (Hymenoptera)	Least Concern (LC)	Fig. 112
		Leaf	Conical	Green	No	Multilocular	TF	*Bruggmanniella* sp.			Fig. 113
		Leaf	Lenticular	Brown	No	One-chambered	TF	Unidentified			Fig. 114
	Inga nobilis Willd.	Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 115
	Macrolobium limbatum Spruce ex Benth.	Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified		Least Concern (LC)	Fig. 116
		Leaf	Fusiform	Brown	No	One-chambered	WSd	Unidentified			Fig. 117

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
		Fruit	Lenticular	Brown	No	One-chambered	WSd	Lepidoptera			Fig. 118
		Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified			Fig. 119
	Macrolobium microcalyx Ducke	Leaf	Globoid	Green	No	Multilocular	WSd	Unidentified		Least Concern (LC)	Fig. 120
		Leaf	Lenticular	Green	No	One-chambered	WSd / WSw	Unidentified			Fig. 121
		Leaf	Globoid	Green	No	Multilocular	WSw	Unidentified			Fig. 122
		Leaf	Conical	Green	No	One-chambered	WSw	Unidentified			Fig. 123
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 124
		Leaf	Fusiform	Brown	No	One-chambered	WSw	Unidentified			Fig. 125
	Parkia igneiflora Ducke	Leaf	Fusiform	Green	No	One-chambered	WSw	Unidentified		Least Concern (LC)	Fig. 126
		Leaf	Cup	Yellow	Yes	One-chambered	WSw	Cecidomyiidae	Braconidae (P) (Hymenoptera)		Fig. 127
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 128
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 129
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified	Hymenoptera (P)		Fig. 130
	Tachigali loretensis van der Werff	Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified		-	Fig. 131
		Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified			Fig. 132
		Leaf	Marginal roll	Green	Yes	One-chambered	WSd	Unidentified	Formicidae (S) *Holothrips* sp. 1 (S) (Thysanoptera)		Fig. 133
		Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified			Fig. 134
		Leaf	Globoid	Green	Yes	Multilocular	WSd	Unidentified			Fig. 135
		Leaf	Rosette	Green	Yes	Multilocular	WSd	Cecidomyiidae	Hymenoptera (P)		Fig. 136
	Vatairea erythrocarpa (Ducke) Ducke	Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 137
		Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified			Fig. 138
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 139
Humiriaceae	*Vantanea parviflora* Lam.	Leaf	Globoid	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 140
		Leaf	Fusiform	Green	No	One-chambered	TF	Lepidoptera			Fig. 141

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
Lauraceae	*Aniba taubertiana* Mez	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 142
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 143
		Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified			Fig. 144
		Bud	Globoid	Brown	Yes	One-chambered	TF	Unidentified			Fig. 145
	Nectandra longifolia (Ruiz & Pav.) Nees	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		-	Fig. 146
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 147
		Leaf	Globoid	Green	No	One-chambered	TF	Cecidomyiidae			Fig. 148
		Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified			Fig. 149
		Leaf	Marginal roll	Green	No	One-chambered	TF	Unidentified			Fig. 150
	Ocotea argyrophylla Ducke	Leaf	Globoid	Brown	Yes	One-chambered	WSd	Unidentified		Least Concern (LC)	Fig. 151
		Leaf	Globoid	Brown	No	One-chambered	WSd	Unidentified	Hymenoptera (P)		Fig. 152
		Leaf	Conical	Brown	No	Multilocular	WSd	Unidentified			Fig. 153
		Leaf	Fusiform	Brown	No	One-chambered	WSd	Unidentified			Fig. 154
	Persea boliviensis Mez & Rusby ex Rusby	Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified		-	Fig. 155
		Bud	Globoid	Green	No	One-chambered	WSd	Cecidomyiidae			Fig. 156
	Pleurothyrium cuneifolium Nees	Leaf	Lenticular	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 157
		Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified			Fig. 158
Lecythidaceae	*Allantoma pluriflora* S.A. Mori, Y.Y. Huang & Prance	Leaf	Lenticular	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 159
	Eschweilera coriacea (DC.) S.A. Mori	Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 160
		Leaf-Petiole	Globoid	Brown	No	One-chambered	TF	Cecidomyiidae	*Holothrips* sp. 1 (S) (Thysanoptera)		Fig. 161

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
		Stem	Globoid	Brown	No	One-chambered	TF	Cecidomyiidae			Fig. 162
		Leaf	Globoid	Green	No	One-chambered	TF	*Contarinia* sp.	Formicidae (S) Baryconus sp. (P) (Hymenoptera)		Fig. 163
		Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified			Fig. 164
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 165
		Leaf	Globoid	Green	No	Multilocular	TF	Cecidomyiidae			Fig. 166
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 167
		Leaf	Marginal roll	Green	No	One-chambered	TF	Unidentified			Fig. 168
		Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified			Fig. 169
		Leaf	Globoid	Brown	No	One-chambered	TF	*Contarinia* sp.			Fig. 170
	Eschweilera rufifolia S.A. Mori	Leaf	Globoid	Brown	No	Multilocular	TF	Unidentified	Formicidae (S) *Plagiothrips* sp.1 (S) (Thysanoptera) Braconidae (P) (Hymenoptera)	-	Fig. 171
		Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified	Hymenoptera (P)		Fig. 172
		Leaf	Lenticular	Brown	Yes	One-chambered	TF	Unidentified			Fig. 173
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 174
		Leaf	Globoid	Green	No	Multilocular	TF	Unidentified			Fig. 175
	Gustavia longifolia Poepp. ex O. Berg	Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified		-	Fig. 176
Lepidobotryaceae	*Ruptiliocarpon caracolito* Hammel & N. Zamora	Leaf	Globoid	Green	No	Multilocular	TF	Unidentified	Eupelmidae (P) (Hymenoptera)	Least Concern (LC)	Fig. 177
Malpighiaceae	*Bunchosia argentea (Jacq.)* DC.	Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 178
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 179
Malvaceae	*Lueheopsis hoehnei* Burret	Leaf	Globoid	Brown	Yes	One-chambered	PS	Unidentified		-	Fig. 180

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
		Leaf	Globoid	Green	Yes	One-chambered	PS	Lopesiini	*Euderus* sp. (P) (Hymenoptera)		Fig. 181
		Leaf	Globoid	Brown	No	One-chambered	PS	Unidentified			Fig. 182
		Leaf	Globoid	Green	No	Multilocular	PS	Unidentified	Chrysonotomyia sp. (P) *Euderomphale* sp. (P) *Metaphycus* sp. (P) (Hymenoptera)		Fig. 183
		Leaf	Fusiform	Green	No	One-chambered	PS	Cecidomyiidae	Hymenoptera (P)		Fig. 184
	Pachira brevipes (A. Robyns) W.S. Alverson		Lenticular	Green	No	One-chambered	WSw	Unidentified		Least Concern (LC)	Fig. 185
		Leaf									
		Stem	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 186
		Leaf	Globoid	Brown	No	One-chambered	WSw	Unidentified			Fig. 187
		Stem	Fusiform	Brown	No	One-chambered	WSw	Unidentified			Fig. 188
	Theobroma subincanum Mart.	Leaf	Globoid	Green	Yes	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 189
		Leaf	Globoid	Brown	Yes	One-chambered	TF	Unidentified			Fig. 190
		Leaf	Fusiform	Brown	Yes	One-chambered	TF	Unidentified			Fig. 191
Melastomataceae	*Mouriri vernicosa* Naudin	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 192
		Leaf	Marginal roll	Green	No	One-chambered	TF	Unidentified	Hymenoptera (P)		Fig. 193
Meliaceae	*Carapa procera* DC.	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 194
		Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified			Fig. 195
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 196
Metteniusaceae	*Emmotum floribundum* R.A. Howard	Leaf	Fusiform	Brown	No	One-chambered	WSw	Unidentified		-	Fig. 197
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 198
Moraceae	*Brosimum utile* (Kunth) Oken	Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified		Least Concern (LC)	Fig. 199

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
	Perebea xanthochyma H. Karst.	Leaf	Globoid	Green	Yes	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 200
	Trymatococcus amazonicus Poepp. & Endl.	Fruit	Lenticular	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 201
Myristicaceae	*Iryanthera juruensis* Warb.	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 202
		Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified			Fig. 203
	Iryanthera laevis Markgr.	Leaf	Fusiform	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 204
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 205
	Iryanthera lancifolia Ducke	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 206
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 207
		Leaf	Lenticular	Black	Yes	One-chambered	TF	Unidentified			Fig. 208
		Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified			Fig. 209
		Leaf	Conical	Green	Yes	One-chambered	TF	Unidentified			Fig. 210
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 211
	Virola pavonis (A. DC.) A.C. Sm.	Leaf	Lenticular	Brown	No	One-chambered	PS / WSd	*Contarinia* sp.	Hymenoptera (P)	Least Concern (LC)	Fig. 212
		Leaf	Conical	Green	No	One-chambered	PS	Cecidomyiidae			Fig. 213
Myrtaceae	*Blepharocalyx eggersii* (Kiaersk.) Landrum	Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified		Least Concern (LC)	Fig. 214
	Eugenia riparia DC.	Leaf	Globoid	Green	No	One-chambered	TF	Unidentified	*Neopomphale* sp. 1 (P) (Hymenoptera)	-	Fig. 215
		Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified			Fig. 216
		Leaf	Globoid	Green	Yes	One-chambered	TF	Unidentified			Fig. 217
	Myrcia crebra (McVaugh) A.R. Lourenço & E. Lucas	Leaf	Lenticular	Brown	No	One-chambered	WSd	Cecidomyiidae		Least Concern (LC)	Fig. 218
		Stem	Globoid	Brown	No	One-chambered	WSd	Unidentified			Fig. 219

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
Nyctaginaceae	*Neea spruceana* Heimerl	Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 220
		Leaf	Globoid	Green	No	One-chambered	TF	Cecidomyiidae			Fig. 221
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 222
	Neea verticillata Ruiz & Pav.	Leaf	Globoid	Green	No	One-chambered	WSd	Unidentified	Hymenoptera (P)	-	Fig. 223
		Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified			Fig. 224
Olacaceae	*Cathedra acuminata* (Benth.) Miers	Leaf	Lenticular	Green	No	One-chambered	WSd	Unidentified	Hymenoptera (P)	Least Concern (LC)	Fig. 225
		Stem	Globoid	Brown	No	Multilocular	WSd	Unidentified			Fig. 226
Phyllanthaceae	*Amanoa guianensis* Aubl.	Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified		Least Concern (LC)	Fig. 227
		Leaf	Marginal roll	Green	No	One-chambered	PS	Cecidomyiidae			Fig. 228
		Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified			Fig. 229
		Stem	Globoid	Brown	No	One-chambered	PS	Unidentified			Fig. 230
	Hieronyma oblonga (Tul.) Müll. Arg.	Leaf	Globoid	Green	No	One-chambered	TF	*Contarinia* sp.	Hymenoptera (P)	Least Concern (LC)	Fig. 231
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified	Acari (S)		Fig. 232
	Richeria grandis Vahl	Leaf	Globoid	Green	No	One-chambered	TF	Unidentified		-	Fig. 233
		Leaf	Globoid	Green	No	Multilocular	TF	Unidentified			Fig. 234
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified	Acari (S)		Fig. 235
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 236
		Leaf	Globoid	Green	No	One-chambered	TF	Hemiptera (Psyllidae)	*Aranobroter* sp. (P) (Hymenoptera)		Fig. 237
Salicaceae	*Casearia arborea* (Rich.) Urb.	Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 238
		Leaf	Lenticular	Green	No	One-chambered	TF	Unidentified			Fig. 239
		Stem	Fusiform	Brown	No	One-chambered	TF	Unidentified			Fig. 240
Sapindaceae	*Cupania diphylla* Vahl	Leaf	Conical	Brown	Yes	One-chambered	WSw	Unidentified		Least Concern (LC)	Fig. 241

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Family	Species	Organ	Shape	Color	Pubescence	Chambers	Vegetation type	Gall-inducers	Associated fauna	Conservation status (IUCN 2023)	Figure number
	Cupania latifolia Kunth	Leaf	Marginal roll	Green	Yes	One-chambered	TF	Clinodiplosini		Least Concern (LC)	Fig. 242
		Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified			Fig. 243
	Talisia firma Radlk.	Leaf	Fusiform	Green	No	One-chambered	PS	Unidentified		Least Concern (LC)	Fig. 244
		Leaf	Lenticular	Green	No	One-chambered	PS	Unidentified			Fig. 245
Sapotaceae	*Chrysophyllum sanguinolentum* (Pierre) Baehni	Leaf	Globoid	Brown	No	One-chambered	WSw	Unidentified		Least Concern (LC)	Fig. 246
		Leaf	Fusiform	Brown	No	One-chambered	WSw	Unidentified			Fig. 247
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 248
		Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified			Fig. 249
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 250
		Leaf	Fusiform	Green	No	One-chambered	WSw	Unidentified			Fig. 251
	Manilkara bidentata (A. DC.) A. Chev.	Leaf	Globoid	Green	No	One-chambered	WSw	Unidentified	Hymenoptera (P)	Least Concern (LC)	Fig. 252
		Stem	Globoid	Brown	No	One-chambered	WSw	Cecidomyiidae			Fig. 253
		Leaf	Lenticular	Green	No	One-chambered	WSw	Unidentified			Fig. 254
		Leaf	Marginal roll	Green	No	One-chambered	WSw	Unidentified			Fig. 255
	Pouteria subrotata Cronquist	Leaf	Conical	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 256
		Leaf	Clavate	Green	No	One-chambered	TF	Unidentified			Fig. 257
		Leaf	Globoid	Green	No	One-chambered	TF	Unidentified			Fig. 258
	Pouteria torta (Mart.) Radlk.	Leaf	Fusiform	Green	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 259
Urticaceae	*Cecropia distachya* Huber	Leaf	Globoid	Green	No	One-chambered	TF	Cecidomyiidae		Least Concern (LC)	Fig. 260
	Pourouma bicolor Mart.	Leaf	Clavate	Brown	No	One-chambered	TF	Unidentified		Least Concern (LC)	Fig. 261
		Leaf	Globoid	Brown	No	One-chambered	TF	Unidentified			Fig. 262

Table 3. Number and percentage of gall morphotypes in the different taxa of galling insects recorded in the Allpahuayo-Mishana National Reserve and Quistococha Regional Reserve, Iquitos, Peru.

Gall-inducing taxa	Gall morphotypes	
	N	%
Diptera (Cecidomyiidae)	58	22.14
Thysanoptera	2	0.76
Lepidoptera	2	0.76
Hemiptera (Psyllidae)	1	0.38
Unidentified	199	75.95
Total	262	100

Hemiptera (n = 1), and Lepidoptera (n = 1) (Table 2); white-sand dry forest was next, with Diptera (n = 15) and Lepidoptera (n = 1) as gall-inducing taxa. White-sand wet forest and palm swamp forest had only Diptera (n = 19 and n = 4, respectively). Additionally, two gall-inducing insects (Diptera) were found in two types of vegetation, one in palm swamp and white-sand dry forests on *Virola pavonis* (A. DC.) A.C. Sm. (Myristicaceae), and other in *terra firme* and white-sand dry forests on *Aspidosperma excelsum* Benth. (Apocynaceae).

The associated fauna was found in 66 gall morphotypes, with the highest frequency of occurrence observed in the white-sand wet forest (n = 23 morphotypes), followed by *terrra firme* forest (n = 18), white-sand dry forest (n = 17), and palm swamp forest (n = 6).

Discussion

We recorded 262 gall morphotypes across our sites in the Peruvian Amazon, a relatively high number compared to other areas with similar vegetation. For example, Almada & Fernandes (2011) reported the highest insect gall diversity (n = 309 insect gall morphogypes) in an Amazonian forest site, FLONA-Saracá Taquera, Brazil. Other studies in the Brazilian Amazon recorded from 27 to 236 morphotypes (Almada & Fernandes 2011, Maia 2011, Silva et al. 2011, Araújo et al. 2012, Julião et al. 2017, Carvalho & Mota 2018). Thus, our study presents the second highest diversity of galling insects recorded in Amazonian forest. We also found that the average number of gall morphotypes per plant species was 3.5, which was high when compared to other locations in the Amazon—with average values of 1.2, 2.0, 1.7, 1.4 (Almada & Fernandes 2011, Maia 2011, Araújo et al. 2012, Julião et al. 2017). Variations in the insect gall richness and number of gall morphotypes per plant species can be explained by differences in sampling method, collecting effort and host plants sampled (Bergamini et al. 2017).

Among the sampled vegetation types, the *terra firme* forest exhibited the greatest richness of gall morphotypes and host species. This can be attributed to the exceptionally diverse and heterogeneous flora found in this forest type, which characterized by nutrient-rich soils and a wide array of niches and ecological resources (Pitman et al. 2001). This finding is consistent with results reported by Julião et al. (2014b), which demonstrated that *terra firme* forests have a greater number of galling insect morphospecies per sampled area than other vegetation types, such as *várzea* forest.

Many inventories of gall diversity in the Amazon forest indicate that Fabaceae is the most important host plant family (Almada & Fernandes 2011, Maia 2011, Araújo et al. 2012, Julião et al. 2017, Carvalho & Mota 2018), a result also observed in the present study. Araújo et al. (2012) suggested that the main explanation for the high insect gall richness hosted by Fabaceae in the Amazon is the large number of species in that family. According to Julião et al. (2014b), Fabaceae stands out as the family with the greatest richness of Cecidomyiidae galls in the Amazon. It is expected that the number of taxa in a host plant family will be reflected in the number of galling associates, with more species-rich families hosting a richer fauna (Gonçalves-Alvim & Fernandes 2001). Consistent with previous work, we showed that the host genus *Eschweilera* (Lecythidaceae) was one of the richest in insect gall morphotypes. Julião et al. (2014b) found that 77% of galling insect morphospecies sampled from Lecythidaceae are associated with this single genus. With respect to the high gall diversity on *T. guianesis*, *C. utilis*, *M. elata*, *E. coriacea* and *S. parvifructa*—the most important host species in the present study—there are no reports in the literature. Among all host plants, only *C. utilis* is endemic to the Amazonian region. This plant hosts 14 gall-inducing species. Since galls result from species-specific interactions, the plant endemism also applies to the inducers. So, these 14 cecidogenous species are endemic too.

Leaves were the most frequently galled plant organ in our study. This pattern was observed in previous studies, including inventories carried out in the Amazon forest (Mani 1964, Almada & Fernandes 2011, Maia 2011, Silva et al. 2011, Araújo et al. 2012, Julião et al. 2017, Carvalho & Mota 2018), and in other biomes (Rodrigues et al. 2014, Bergamini et al. 2017, Marinho et al. 2023). According to Proença & Maia (2023), 81% of galls occurred on leaves in the Amazon forest. This is probably because leaves represent an abundant and renewable resource available to gall inducers (Maia 2011). The globoid shape also was the most common one, together with the green color, glabrous surface and one-chambered galls. These predominant characteristics are also consistent with previous studies in the Amazon (Almada & Fernandes 2011, Maia 2011, Silva et al. 2011, Araújo et al. 2012, Julião et al. 2017, Carvalho & Mota 2018).

Galling insects of the family Cecidomyiidae were the most important in the fauna studied. This result corroborates previous studies that pointed to the dominance of Cecidomyiidae in the Amazon forest (Proença & Maia, 2023). For example, Julião et al. (2014b), showed that Cecidomyiidae was responsible for 72% of gall morphospecies in the Amazon forest. This points to Cecidomyiidae as the family with the greatest diversity of galling insects in the Neotropical region (Gagné 1994).

Regarding the associated fauna, Thysanoptera, Hymenoptera, Acari, and Collembola were found as successors. Additionally, Curculionidae (Coleoptera) and Sciaridae (Diptera) were identified as cecidophages, which is the first documented record of this in Amazonian forest. Hymenoptera was the only insect order represented as a parasitoid. Among the Hymenoptera families, Eulophidae was the most frequent in our study. This pattern aligns with previous findings in the Brazilian Amazon, where Eulophidae has been reported as the predominant parasitoid group (Carvalho & Mota 2018). In restinga areas of the state of Rio de Janeiro (Atlantic forest), Eulophidae were also the most frequent and best represented parasitoids (Maia & Azevedo 2009), as well as in Cerrado (Maia & Silva 2021), showing that this pattern can be widespread in South America.

The taxonomic resolution of gall-inducing insects in our study was low, which was also observed in previous studies in the Amazon. Julião et al. (2014b) recorded 1,549 gall morphotypes in the Brazilian

Amazon, and inducers of only 386 morphotypes were identified to order or family level. In that study, gall-inducing insects were found to belong to the orders Diptera (Cecidomyiidae), Hemiptera, Coleoptera, Lepidoptera, and Hymenoptera, while 24% of the inducers could not be identified to any possible level.

Conclusions

This is the first systematic survey of insect galls undertaken in the Peruvian Amazon. With this study, 262 gall morphotypes and 75 host plant species have been recorded. Fabaceae was the family with the highest number of galled species. *T. guianesis*, *C. utilis*, *M. elata*, *E. coriacea* and *S. parvifructa* were identified as the host plant species with the highest richness of gall-inducing insects. The leaf was the most galled plant organ, and globoid shape was the most common morphology. Among the galler orders, Diptera was the most representative, with Cecidomyiidae being the standout family in terms of species richness. Hymenoptera was identified as the only order of parasitoids, while successors were represented by four orders. Cecidophages were represented by both Coleoptera and Diptera orders. Finally, *terra firme* forest presented the largest richness of gall morphotypes and host plant species.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available at: https://doi.org/10.48331/scielodata.025WUA.

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Article

Social wasps (Vespidae: Polistinae) in Cerrado and Caatinga conservation units, Minas Gerais, Brazil

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Abstract: The Parque Nacional Grande Sertão Veredas, northern Minas Gerais, is located in the Cerrado, a global hotspot, and the literature lacks information about its communities of social wasps, insects that perform numerous services for the functioning of ecosystems. Therefore, this study aimed to conduct an inventory of social wasps (Vespidae: Polistinae) in the Parque Nacional Grande Sertão Veredas, and compare its richness and composition with three other fully protected conservation units in northern Minas Gerais and Vale do Jequitinhonha, Minas Gerais. A total of 41 species of 14 genera of social wasps were recorded, including a new occurrence in the state: *Mischocyttarus campestris* Raw, 1985. The presence of distinct social wasp communities in the Cerrado and Caatinga conservation units analyzed in this study, reinforces and justifies the creation of these fully protected areas, which ensure the conservation of this biological heritage and the environmental services provided by these taxa, besides providing subsidies for the management and sustainable use of the natural resources of these conservation units.
Keywords: *Biodiversity; Dry Forest; Hymenoptera; Mischocyttarus; Veredas.*

Vespas sociais (Vespidae: Polistinae) em unidades de conservação do Cerrado e da Caatinga, Minas Gerais, Brasil

Resumo: O Parque Nacional Grande Sertão Veredas, norte de Minas Gerais, inserido no Cerrado, *hotspot* mundial, carece de informações sobre a comunidade de vespas sociais, insetos que desempenham inúmeros serviços para o funcionamento dos ecossistemas. Portanto, o objetivo deste estudo foi realizar um inventário de vespas sociais (Vespidae: Polistinae) no Parque Nacional Grande Sertão Veredas, além de comparar sua riqueza e composição com à de outras três Unidades de Conservação de proteção integral no Norte e no Vale do Jequitinhonha no estado de Minas Gerais. Foram registradas 41 espécies de 14 gêneros de vespas sociais, com um novo registro de ocorrência para o estado, *Mischocyttarus campestris* Raw, 1985. A presença de comunidades distintas de vespas sociais nas Unidades de Conservação no Cerrado e Caatinga, analisadas no presente estudo, reforça e justifica a criação dessas áreas de proteção integral, que assegura a conservação desse patrimônio biológico, bem como os serviços ambientais prestados por esses táxons, além de fornecer subsídios para o manejo e uso sustentável dos recursos naturais dessas Unidades de Conservação.
Palavras-chave: *Biodiversidade; Mata Seca; Hymenoptera; Mischocyttarus; Veredas.*

Introduction

The Cerrado is one of the world's biodiversity hotspots (Myers et al. 2000; Klink & Machado 2005; Mittemeier et al. 2011), as it is home to a great diversity of species, including endemic (Myers et al. 2000; Silva & Bates 2002; ICMBio 2018) and endangered species (Aquino & Oliveira 2006; ICMBio 2018). Although considered the richest savanna on the planet, covering about 23.9% of the Brazilian territory (MMA 2023), studies show that 55% of its original area was deforested or transformed by human action. In contrast, only 8.21% of its estimated area is protected by conservation units (CUs) (MMA 2023). The state of Minas Gerais has 23 state parks, 12 national parks, nine natural monuments, four ecological stations, and three wildlife refuges, which represent 1.53% of the state's territory and ensure the full protection of about 2.69% of the Cerrado in Minas Gerais (Drummond 2005; MMA 2023).

The Caatinga, a semi-arid climate, occupies 11% of the Brazilian territory, which encompasses the northeastern states and part of the north of Minas Gerais (EMBRAPA 2022). Although not being a hotspot, it shelters a relevant insect biodiversity (Andena & Carpenter 2003), and a high rate of vegetal endemism (Taylor & Zappi 2002; Fernandes et al. 2020). Despite its relevance, only 1.2% of the Caatinga territory is under protection of CUs (Brasil 2016), an alarming fact due to the several anthropic pressures that are causing a growing desertification process (Santos et al. 2022).

CUs are essential for biota conservation in Brazil (Salvio 2017) by ensuring the preservation of natural habitats and providing abiotic and biotic conditions for maintaining viable populations. This preserves different ecosystem services provided by insects such as social wasps (Vespidae: Polistinae), which perform biological control (Prezoto et al. 2019; Lourido et al. 2019) and pollination (Suhs et al. 2009; Bergamo et al. 2021).

The CUs in Minas Gerais meet the objective of conserving these insects, since they are home to more than 90% of the species sampled in the state, including 34 recorded only in CUs (Oliveira et al. 2021). This information comes from inventory studies, which also contribute to the management of CUs, with the assessment of the carrying capacity of the area, for example, which is important for the implementation of ecotourism (Jesus & Selva 2009) and justifies studies to know the occurrence and distribution of species in ecosystems.

The number of studies on the diversity and distribution of social wasps in Brazil increased in the first two decades of the 21st century (Barbosa et al. 2016; Souza et al. 2020a, b), northern Minas Gerais and Vale do Jequitinhonha, Minas Gerais, still have undersampled ecosystems, such as areas of Cerrado and transition to Caatinga (Brunismann et al. 2016; Souza et al. 2020a; Jacques et al. 2023), due to the greater sampling effort in areas of Atlantic Forest (Barbosa et al. 2016; Souza et al. 2017; Souza et al. 2020b), which shows the need to obtain more information about the occurrence and distribution of these insects in these ecosystems, especially in CUs (Oliveira et al. 2021).

Therefore, this study aimed to conduct an inventory of social wasps (Hymenoptera, Vespidae) in the Parque Nacional Grande Sertão Veredas, and compare its richness and composition with three other fully protected conservation units in northern Minas Gerais and Vale do Jequitinhonha.

Material and Methods

This study was performed in the Parque Nacional Grande Sertão Veredas (15°6'S and 45°48'W), in the municipality of Chapada Gaúcha, Minas Gerais, Brazil. The total area of the park is about 230,671 hectares, with an altitude of 600 to 900 m, characterized by sandstone plateaus covered by vegetation of the Cerrado biome. Its phytophysiognomies include Cerrado *sensu stricto*, *Cerradão*, *vereda*, gallery forest, *campo sujo*, and *campo limpo* (FUNATURA 2003; Ribeiro & Walter 2008). The climate of the region is tropical, in sub-humid conditions, with an average annual temperature of about 23°C, with maximums of 37°C to 40°C and minimums of 16°C to 19°C (FUNATURA 2003). The dry season in winter begins in May and lasts until September/October, and the rainy season in summer is from November to March. The average rainfall is 1,400 mm/year (FUNATURA 2003).

For this study, two methodologies were used for sampling social wasps: active and passive (Jacques et al. 2018), since the simultaneous use of different sampling methodologies optimizes the recording of species (Barbosa et al. 2020). Active sampling consisted of capturing social wasps in flight and/or in colonies with entomological nets (Souza & Prezoto 2006) from 9 a.m. to 1 p.m. and 4 p.m. to 6 p.m. (Jacques et al. 2023) for 24 days, six days per season, in September and November 2022 during the dry season, and in February and April 2023 during the rainy season. Four researchers conducted the searches, totaling 144 hours of sampling effort per researcher. They moved along pre-existing trails or roads in areas of gallery forests, *campo sujo*, *campo limpo*, *veredas,* and Cerrado *stricto sensu*.

Passive sampling was performed using attractant traps made with two-liter disposable PET (polyethylene terephthalate) bottles. Three triangular openings were made on the sides of the bottles (2 × 2 × 2 cm), about 10 cm from the base, and 150 ml of attractant were placed in each (Souza & Prezoto 2006). They were distributed in areas of *veredas*, gallery forests, and Cerrado *sensu stricto* by two different transects, totaling 20 traps, 10 with molasses diluted in water in a 50–50 proportion and 10 with passion fruit juice. The traps were positioned 10 m from each other and 1.5 m above the ground (Jacques et al. 2018), and were in the field for six days in each campaign, accounting for 576 hours of sampling per bottle used.

At both collection times, the specimens were sacrificed and stored in 70% alcohol for later identification in the laboratory. The genera and species were identified using dichotomous keys proposed by Richards (1978) and Somavilla & Carpenter (2021), and compared with the biological collection of social wasps (BCSW) of the Instituto Federal do Sul de Minas Gerais (IFSULDEMINAS), where the specimens are deposited (Tumble No. 09573-2022 to 09708-2023). The samples with doubtful taxonomy were sent to Dr. Orlando Tobias Silveira (Museu Emílio Goeldi, Belém, Pará), who collaborated with the identifications.

To evaluate the sampling effort, an accumulation curve was constructed using the observed richness with 95% confidence interval, under Bootstrap 1 estimator in the EstimateS 9.1.0 software (Cowell & Elsensohn 2014). This estimator uses information from all species collected instead of restricting the analysis to rare species (Santos 2003).

The species richness of the area studied was compared to three other CUs in northern Minas Gerais and Vale do Jequitinhonha (Figure 1), which used similar methodologies: the Parque Nacional das Sempre-Vivas (PNSV), an area of Cerrado associated with *campo rupestre*

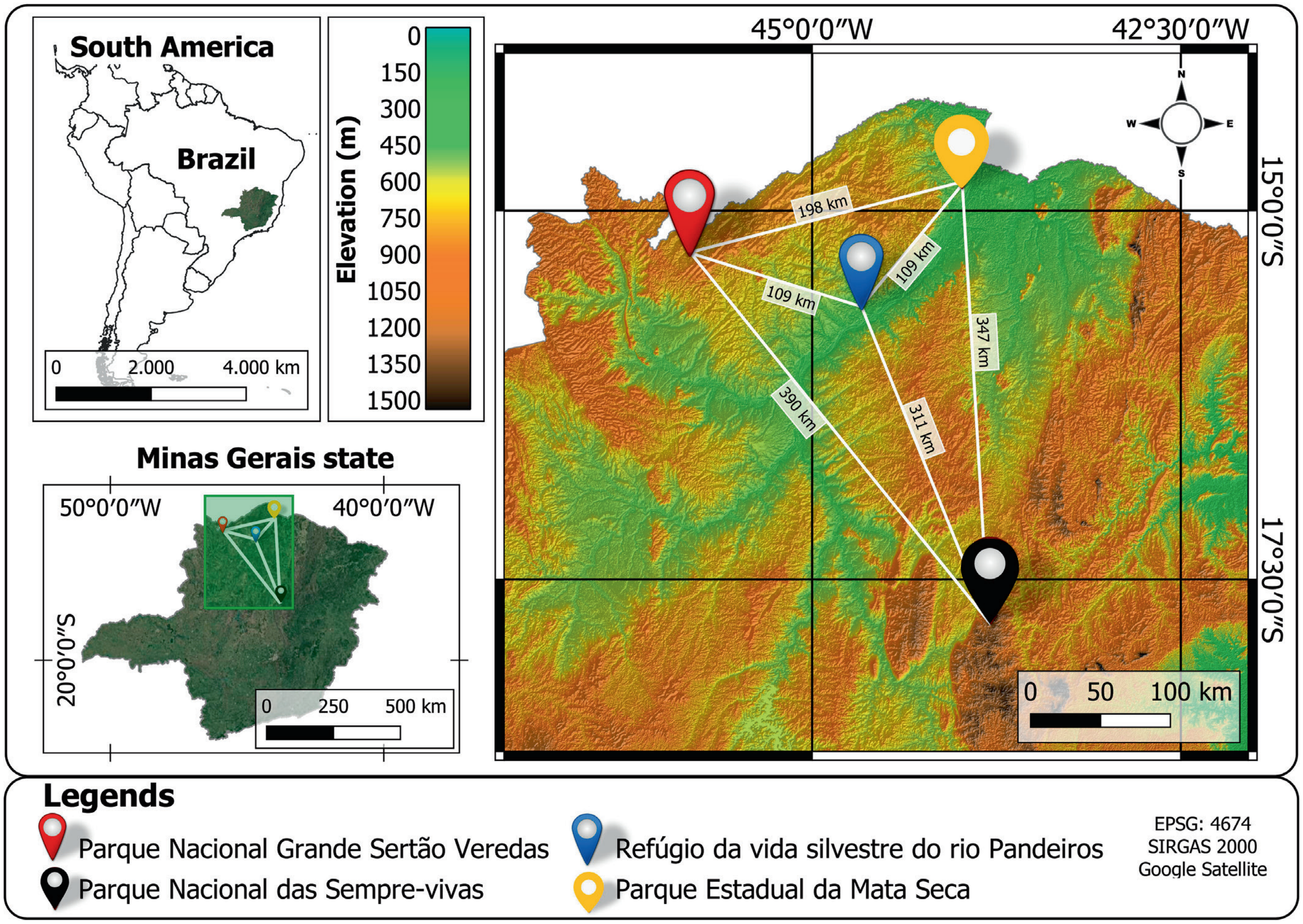

Figure 1. Location of the Parque Nacional Grande Sertão Veredas; approximate distance and elevation of the other conservation units compared with northern Minas Gerais (Parque Estadual da Mata Seca and Refúgio da Vida Silvestre do Rio Pandeiros) and Vale do Jequitinhonha (Parque Nacional das Sempre-Vivas), Minas Gerais, Southeastern Brazil.

(Souza et al. 2020a); the Refúgio da Vida Silvestre do Rio Pandeiros (RVSRP), Cerrado-Caatinga transition zone (Brunismann et al. 2016); and the Parque Estadual da Mata Seca (PEMS), located in an area of Caatinga (Jacques et al. 2023) (Figure 2).

To evaluate the similarity of the species community between the areas, a principal coordinate analysis (PCoA) was performed using Jaccard's index, produced from the presence/absence data of the species. Then, a permutational multivariate analysis of variance (PERMANOVA) was performed using the same software to check for any significant difference in the community between the areas. Moreover, a cluster analysis of diversity among the areas was performed, also using Jaccard's similarity index. All analyses were performed using the Past program (Hammer et al. 2005). Wasps not identified to species level were removed from analyses. The study was authorized by SISBIO license No. 83294-1.

Results

1. *Richness and composition of the social wasp fauna*

We recorded 41 species of 14 genera of social wasps in the Parque Nacional Grande Sertão Veredas (PNGSV) (Table 1; Figure 3), especially *Mischocyttarus campestris* Raw, 1985 (Figure 4), which is a new occurrence in the state of Minas Gerais.

The richness of the PNGSV may be even higher than our records shows, according to the species accumulation curve (Figure 5), which has not reached an asymptote. Moreover, the number of species estimated by BootStrap1 is 44.65, outside the 95% confidence limit.

2. *Composition and similarity of the Polistinae fauna in Cerrado and Caatinga CUs*

PCoA and PERMANOVA (Figure 6) showed a statistical difference between the social wasp fauna of the different studies in CUs in northern Minas Gerais and Vale do Jequitinhonha ($p = 0.0001$).

We observed a low similarity between the CUs — PNGSV and RVSRP (Refúgio da Vida Silvestre do Rio Pandeiros) were the most similar, with 52.38%. The similarity between the PNGSV and the Parque Nacional das Sempre-Vivas (PNSV) was 31.81%. The lowest similarities were between the PEMS (Parque Estadual da Mata Seca) and the PNGSV (16.66%), and the PEMS and the PNSV (20%). The RVSRP was the most similar CU to the PEMS (23.52%) (Figure 7).

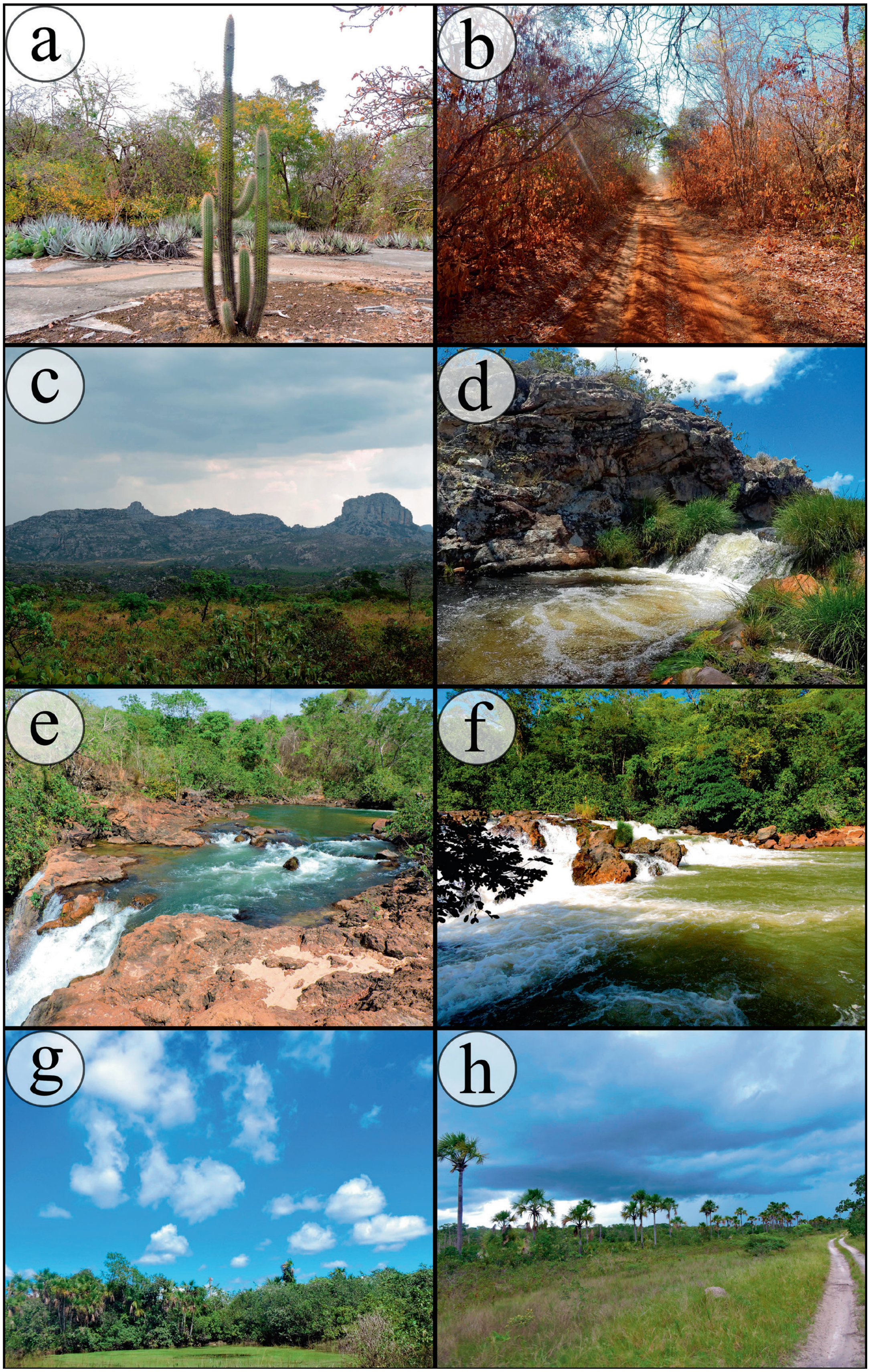

Figure 2. Conservation units where the degree of similarity of social wasp communities in the state of Minas Gerais was evaluated: Caatinga, Parque Estadual da Mata Seca (a–b); Cerrado associated with *campo rupestre*, Parque Nacional das Sempre-Vivas (c–d); Cerrado-Caatinga transition zone, Refúgio da Vida Silvestre do Rio Pandeiros (e–f); Cerrado, Parque Nacional Grande Sertão Veredas (g–h).

Table 1. Social wasp species and richness in the Parque Nacional Grande Sertão Veredas (PNGSV), the Parque Estadual da Mata Seca (PEMS), the Refúgio da Vida Silvestre do Rio Pandeiros (RVSRP), and the Parque Nacional das Sempre-Vivas (PNSV). (1 = presence; 0 = absence).

Species	**PNGSV**	**PEMS**	**RVSRP**	**PNSV**
Agelaia angulata (Fabricius, 1804)	1	0	0	0
Agelaia multipicta (Haliday, 1836)	1	0	1	1
Agelaia pallipes (Olivier, 1792)	1	0	0	0
Agelaia vicina (de Saussure, 1854)	0	0	1	1
Apoica flavissima Van der Vecht, 1972	0	0	0	1
Apoica gelida Van der Vecht, 1973	0	0	1	1
Apoica pallens (Fabricius, 1804)	1	0	1	1
Apoica thoracica du Buysson, 1906	1	0	1	0
Brachygastra augusti (Saussure, 1854)	1	0	1	0
Brachygastra lecheguana (Latreille, 1824)	1	1	1	1
Brachygastra moebiana (de Saussure, 1867)	0	0	1	0
Brachygastra sp.	1	0	0	0
Chartergellus communis Richards, 1978	1	0	1	1
Chartergus globiventris de Saussure, 1854	1	0	1	0
Clypearia angustior Ducke, 1906	0	0	1	0
Metapolybia cingulata (Fabricius, 1804)	1	0	1	0
Mischocyttarus bertonii Ducke, 1918	0	1	1	0
Mischocyttarus campestris Raw, 1985	1	0	0	0
Mischocyttarus cassununga (R. von Ihering, 1903)	1	1	1	1
Mischocyttarus cerberus Ducke, 1918	1	0	1	1
Mischocyttarus consimilis Zikán, 1949	0	1	0	0
Mischocyttarus drewseni de Saussure, 1857	1	1	1	1
Mischocyttarus giffordi Raw, 1985	0	0	0	1
Mischocyttarus ignotus Zikán, 1949	0	0	0	1
Mischocyttarus iheringi Zikán, 1935	0	0	0	1
Mischocyttarus latior (Fox, 1898)	1	0	0	1
Mischocyttarus marginatus (Fox, 1898)	0	0	0	1
Mischocyttarus matogrossoensis Zikán, 1935	1	1	0	1
Mischocyttarus montei Zikán, 1949	0	0	1	0
Mischocyttarus rotundicollis (Cameron, 1912)	1	0	1	1
Mischocyttarus socialis (de Saussure, 1854)	1	0	0	1
Mischocyttarus (*Monogynoecus*) sp.	1	0	0	0
Mischocyttarus sp. 01	1	0	0	0
Mischocyttarus sp. 02	0	0	1	0
Mischocyttarus sp. 03	0	0	1	0
Parachartergus fraternus (Gribodo, 1892)	1	0	1	1
Parachartergus smithii (de Saussure, 1854)	0	0	1	0

Continue...

Continuation

Species	PNGSV	PEMS	RVSRP	PNSV
Polistes billardieri Fabricius, 1804	1	0	0	0
Polistes cinerascens de Saussure, 1854	0	1	0	1
Polistes erythrocephalus Latreille, 1813	1	0	0	0
Polistes ferreri de Saussure, 1853	1	1	0	1
Polistes lanio (Fabricius, 1775)	0	0	0	1
Polistes satan Bequaert, 1940	0	0	0	1
Polistes simillimus Zikán, 1948	1	0	1	1
Polistes subsericeus de Saussure, 1854	1	0	1	1
Polistes versicolor (Olivier, 1791)	1	1	0	1
Polistes sp.	1	0	0	0
Polybia chrysothorax (Lichtenstein, 1796)	0	0	1	1
Polybia fastidiosuscula (de Saussure, 1854)	1	0	0	1
Polybia ignobilis (Haliday, 1836)	1	1	1	1
Polybia jurinei de Saussure, 1854	0	0	1	1
Polybia occidentalis (Olivier, 1791)	1	1	1	1
Polybia paulista (H. von Ihering, 1896)	1	0	0	1
Polybia punctata Buysson, 1908	0	0	1	0
Polybia ruficeps (Schrottky, 1902)	1	0	1	1
Polybia sericea (Olivier, 1791)	1	1	1	1
Polybia striata (Fabricius, 1787)	1	0	0	0
Polybia sp.	1	0	0	0
Protonectarina sylveirae (de Saussure, 1854)	1	1	1	1
Protopolybia exigua (de Saussure, 1854)	0	1	1	1
Protopolybia sedula (de Saussure, 1854)	1	0	1	1
Pseudopolybia vespiceps (de Saussure, 1863)	1	0	0	0
Synoeca cyanea (Fabricius, 1775)	0	0	0	1
Synoeca surinama (Linnaeus, 1767)	1	0	1	1
Species Richness	**41**	**14**	**35**	**39**
Unique species for each Ucs	**12**	**1**	**7**	**8**

Discussion

1. *Richness and composition of the social wasp fauna*

This richness and composition of the community of the PNGSV is expressive compared to other inventories conducted in the Brazilian Cerrado (Santos et al. 2009; Auko et al. 2017; De Castro et al. 2021; Oliveira et al. 2022). The occurrence of 41 species is possibly due to the sum of different biotic, abiotic, and methodological factors.

The PNGSV has several phytophysiognomies, such as Campo Limpo, Cerradão, gallery forests and veredas (Neto et al. 2001), which provides greater diversity of ecological niches for social wasps (Santos et al. 2007), since each phytophysiognomy it forms a distinct environment, with different communities of social wasps (Diniz & Kitawama (1994, 1998). Added to this are three other factors; first, the conservation status of the area (Graça & Somavilla 2018), which may favor species with restricted environments, since there are species that better explore more conserved environments, with lower anthropic pressure, and those in environments with greater anthropic pressure (Souza et al. 2010); second, the mosaic of environments in different stages of ecological succession, which favors greater plant diversity, which provides different substrates for nesting and a greater supply of food (Lawton, 1983; Santos et al. 2007; Souza et al. 2010); third, the

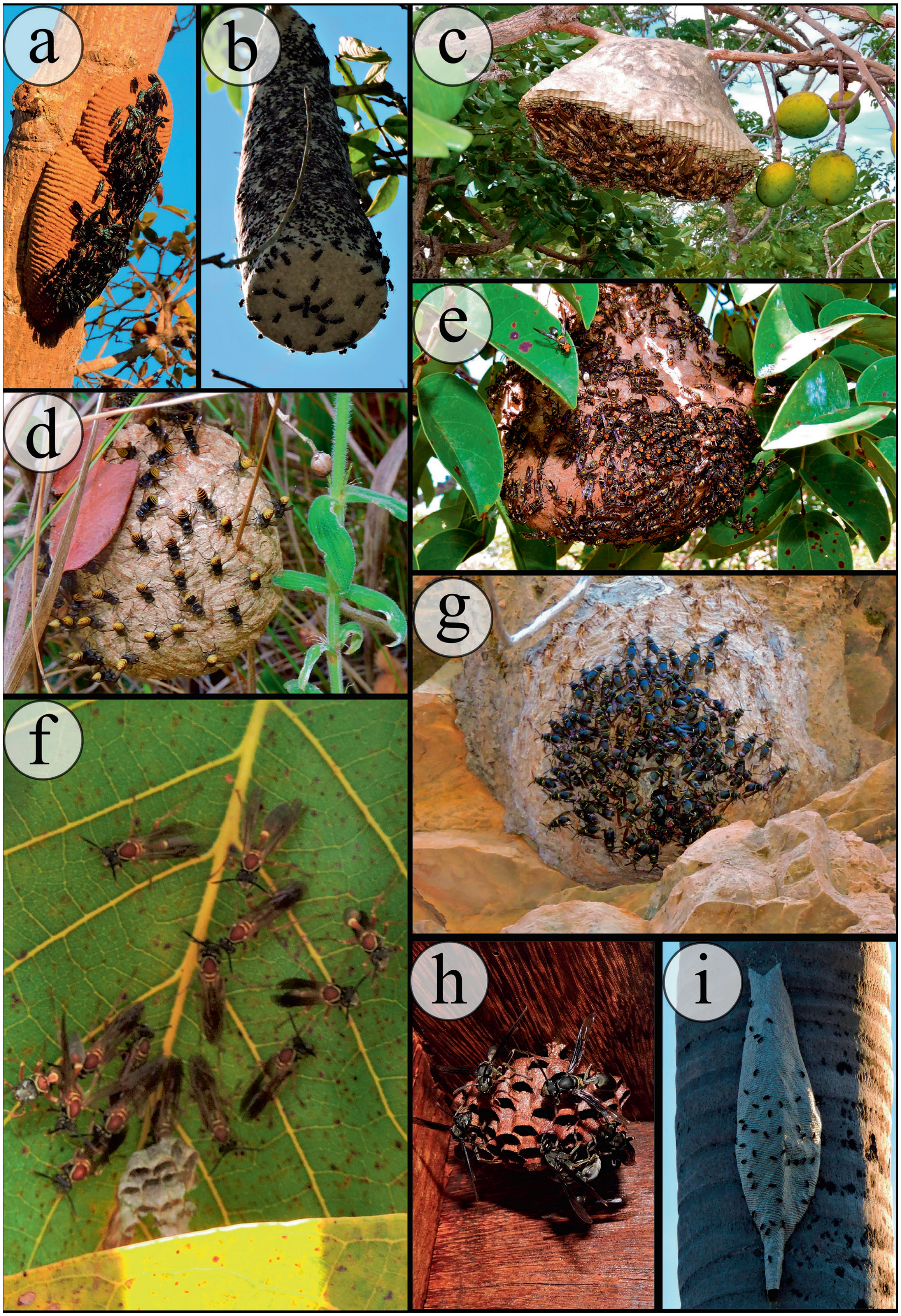

Figure 3. Some species of Polistinae recorded in the Parque Nacional Grande Sertão Veredas, northern Minas Gerais: (a) *Synoeca surinama*; (b) *Chartergus globiventris*; (c) *Apoica pallens*; (d) *Brachygastra augusti*; (e) *Polybia ruficeps*; (f) *Mischocyttarus campestris*; (g) *Chartergellus communis*; (h) *Mischocyttarus latior*; (i) *Parachartergus fraternus*.

Figure 4. *Mischocyttarus campestris* collected in the Parque Nacional Grande Sertão Veredas, northern Minas Gerais.

availability of water (Henriques et al. 1992), present all year round, ensured by the Veredas (Ribeiro & Walter 2008).

In addition to biotic and abiotic factors, the use of two collection methodologies (active search and attractive traps) increases the sampling effort, and the consortium of methodologies is the best way to record the species richness of an area, as the more methods applied, the greater the chances of capturing the largest number of species possible (Barbosa et al. 2020).

Besides the number of species recorded, which highlights the importance of the PNGSV, the presence of the species *M. campestris*, which until then had only been recorded in a Cerrado area in the Federal District (Raw 2016), reinforces the role of the CU in the conservation of the Cerrado Polistinae fauna. However, its occurrence reported in this study may represent a possible restriction of the species to this biome, requiring further study.

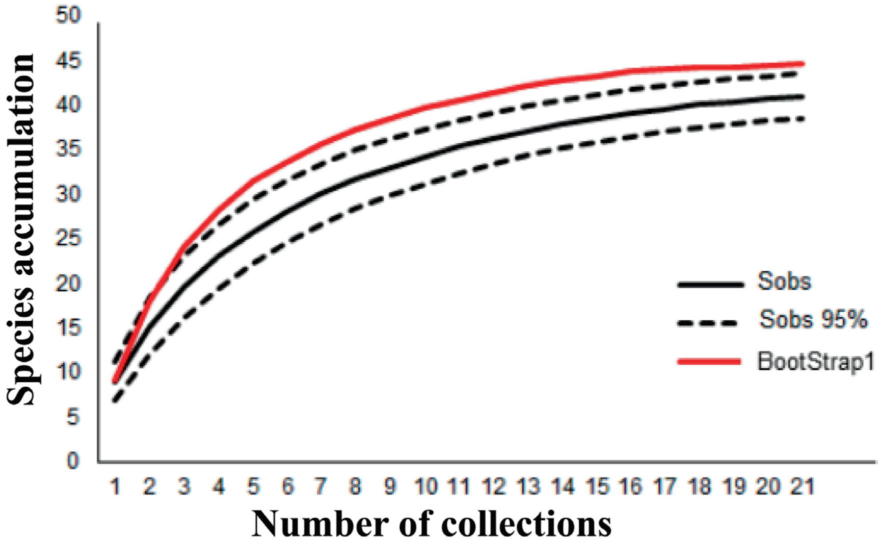

Figure 5. Species accumulation curve for social wasps collected in the Parque Nacional Grande Sertão Veredas using observed species richness, within a 95% confidence interval, and estimated species richness (Bootstrap 1).

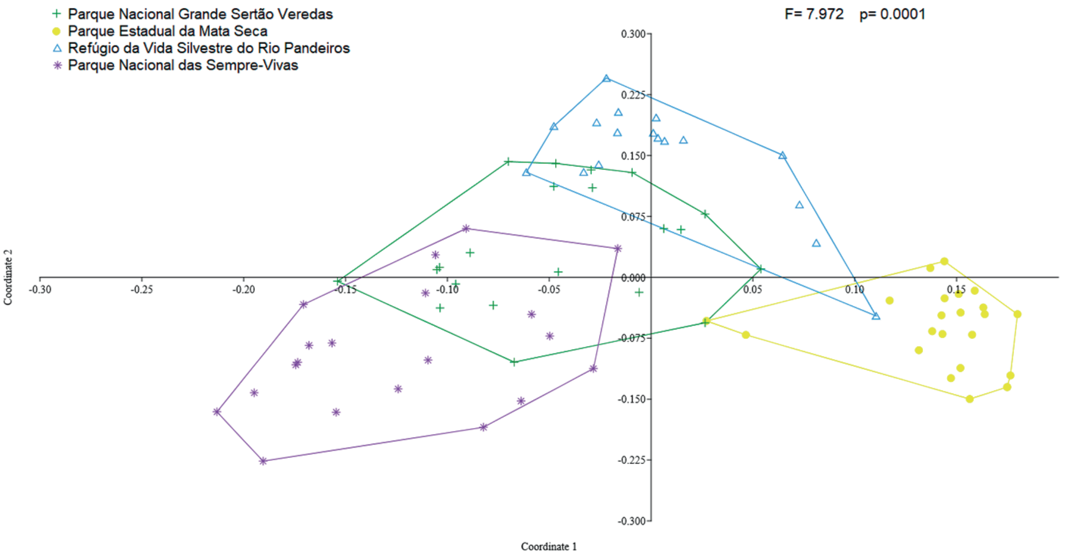

Figure 6. Principal coordinate analysis (PCoA) and Permutational multivariate analysis of variance (PERMANOVA) of the social wasp community. The different colors represent the different conservation units in northern Minas Gerais and Vale do Jequitinhonha, Minas Gerais, Brazil. PERMANOVA results are at the top.

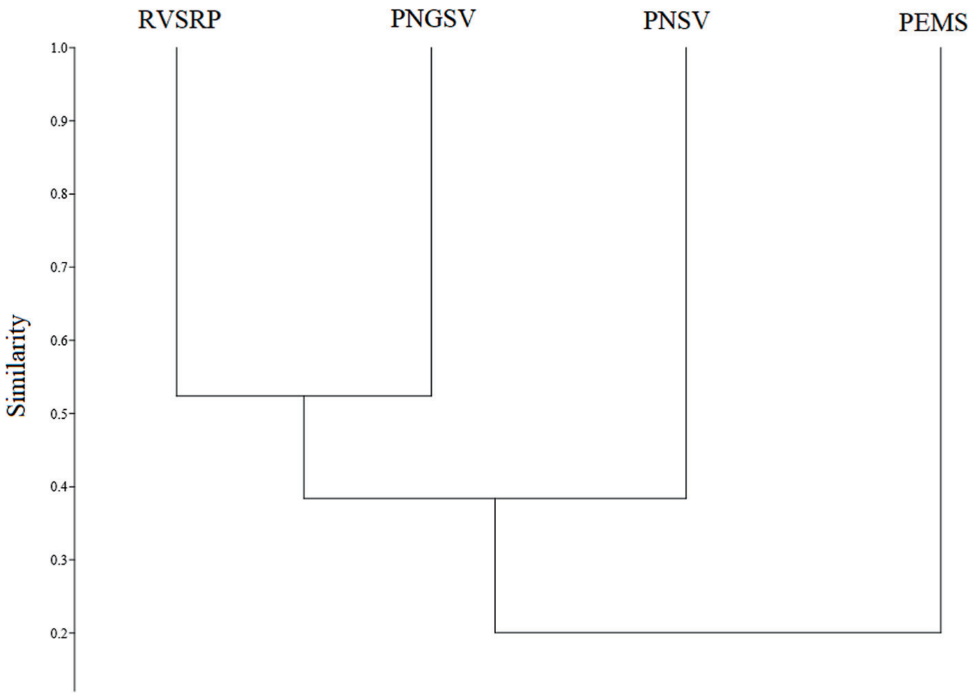

Figure 7. Cluster analysis between social wasp faunas from different conservation units in northern Minas Gerais and Vale do Jequitinhonha, Minas Gerais. RVSRP = Refúgio da Vida Silvestre do Rio Pandeiros; PNGSV = Parque Nacional Grande Sertão Veredas; PNSV = Parque Nacional das Sempre-Vivas; PEMS = Parque Estadual da Mata Seca.

2. *Composition and similarity of the Polistinae fauna in Cerrado and Caatinga CUs*

Although the works in the different UCs have used similar methodologies, mainly due to the use of the active search method, considered more effective for sampling social wasps by different authors (Souza & Prezoto 2006; Silva & Silveira 2009; Jacques et al. 2018), we cannot rule out that the collection methodology of each work may have some influence on the composition of the communities of each UC, however, the low similarity between communities indicates that it is a result of the biological diversity and unique environmental characteristics of each protected area. Different social wasp communities play specific ecological roles in ecosystems, in which some species may be more efficient in controlling certain insect populations (Southon et al. 2019; Pinheiro et al. 2023), while others may have specific interactions with plants or other organisms (Clemente et al. 2012; Barbosa et al. 2021). Therefore, conserving the diversity of social wasp communities directly affects the maintenance of the ecological balance of the ecosystems where they are located.

Social wasps have a close relationship with different habitats, adapting and interacting in specific ways with each of them (Santos et al. 2007). Their interactions with the environment include choosing nest sites, searching for food resources, and defending their territory (Resende et al. 2001; Brito et al. 2018; Ferreira et al. 2022). Besides the different communities in each CU, the presence of 12 unique species in the PNGSV, eight in the PNSV, seven in the RVSRP, and one in the PEMS (Table 1) also reinforces this reality.

PNGSV and RVSRP, although close (109 km), have different phytophysiognomies. The RVSRP has a predominance of Cerrado, including the presence of *veredas*, which justifies a greater similarity with the PNGSV, but with traces of Caatinga, such as a dense seasonal deciduous forest or dry forest, open arboreal Caatinga, and *carrasco* (IEF 2022). This shows the unique environmental characteristics of each protected area and consequently a biological diversity.

The similarity between PNGSV and PNSV is possibly linked to a greater geographic distance between the analyzed units (390 km). Moreover, the PNGSV covers an area ranging from about 600 to 900 m above sea level, with an average annual temperature of 23°C (FUNATURA 2003). In turn, the PNSV is located in the Serra do Espinhaço and has a more pronounced altimetric variation, with a predominance of altitudes of 1,100 to 1,250 m, the presence of *campos rupestres*, which are absent in the PNGSV, and a milder temperature, with an annual average of 19°C (MMA/ICMBio 2016). Several insect species are restricted to high altitudes due to increased climatic severity, reduced area, resources, and primary productivity, absence or reduction of morphophysiological adaptive traits, and even geographic isolation (Janzen 1973; Lawton et al. 1987; Fernandes 2016). This also occurs with social wasps (Souza et al. 2015; Oliveira et al. 2021), since their flight usually requires their body temperature to be higher than that of the environment (Hozumi et al. 2010).

The lowest similarities between PEMS and PNGSV and PEMS and PNSV was expected, as PEMS is located in an area of Caatinga domain, while PNGSV and PNSV are in Cerrado areas. The RVSRP was the most similar CU to the PEMS, since, besides their proximity (109 km), the RVSRP, as aforementioned, has a predominance of Cerrado, but with traces of Caatinga, including a dry forest, just like the PEMS. The dry forest, a deciduous forest, has two well-defined climatic seasons: one rainy followed by another with a long dry period. Its tree layer is predominantly deciduous, and more than 50% of the trees become leafless in the unfavorable season (Belém et al. 2021). The responses of social wasps to dry periods in deciduous forests are variable. Some species of Epiponini, due to their ability to store resources in their nests, such as proteins and carbohydrates (Ihering 1896; Machado & Parra 1984), manage to maintain active colonies during the dry season (Jacques et al. 2023). In turn, in the subfamily Polistini, some species, such as *Polistes versicolor* (Olivier, 1791), form aggregations of queens in a state of hibernation during the period of intense drought (Gonzáles et al. 2002), and as soon as the rains start, with a rapid replacement of leaves, the number of colonies increases (Jacques et al. 2023), showing an adaptation to the dry season.

The presence of distinct social wasp communities in the Cerrado and Caatinga conservation units analyzed in this study, reinforces and justifies the creation of these fully protected areas, which ensure the conservation of this biological heritage and the environmental services provided by these taxa, besides providing subsidies for the management and sustainable use of the natural resources of these CUs.

Conclusion

The presence of distinct social wasp communities in the Cerrado and Caatinga conservation units analyzed in this study, reinforces and justifies the creation of these fully protected areas, which ensure the conservation of this biological heritage and the environmental services provided by these taxa, besides providing subsidies for the management and sustainable use of the natural resources of these CUs

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Conflicts of Interest

The author(s) declare(s) that they have no conflict of interest related to the publication of this manuscript

Data availability

The data collected and generated during this study are available in the SpeciesLink at https://specieslink.net/col/CBVS/. The dataset includes social wasps species used in the analysis and can be accessed at https://specieslink.net/search/records/col/430. The authors confirm that all data necessary for reproducing the study findings are available in the designated dataset.

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Inventory

An updated distribution list of Leptophlebiidae Banks, 1900 (Ephemeroptera: Insecta) for Piauí state

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Abstract: Leptophlebiidae is the second most diverse family within Ephemeroptera, with species distributed across various States in Brazil, but with gaps in distribution records in others. Currently, nine species of Leptophlebiidae are recorded for the state of Piauí. Based on this information gap, the objective of this study was to present an updated species list of the family Leptophlebiidae new occurrence records and distributional sites for the state of Piauí, Brazil. By analyzing 447 specimens, we have significantly expanded our knowledge about the distribution of Leptophlebiidae species in the state of Piauí, increasing the recorded species from nine to 17. We have also added new occurrence records for six species and, for the first time, documented the presence of four genera. It is important to highlight that there is still an extensive area within the Cerrado and Brazilian semiarid regions where the occurrence of Ephemeroptera is unknown, confirming that the diversity in this area is underestimated and that knowledge of Ephemeroptera species and their distributions can expand with increased sampling efforts in the coming years, reducing the Linnean and Wallacean shortfall regarding this group. Our results also demonstrate the urgent need for inventories in the southern part of the state of Piauí, particularly in the sub-basins of the middle and upper Parnaíba river, which are considered suitable for monoculture expansion in Brazil.
Keywords: *Aquatic insect; Freshwater ecosystem; Parnaíba river basin.*

Uma lista de distribuição atualizada de Leptophlebiidae Banks, 1900 (Ephemeroptera: Insecta) para o estado do Piauí

Resumo: Leptophlebiidae é a segunda família mais diversa de Ephemeroptera, com espécies distribuídas em vários estados do Brasil, mas com lacunas no registro de distribuição em outros. Por exemplo, no estado do Piauí tem registrado apenas nove espécies de Leptophlebiidae. Baseada nessa lacuna de informação, o objetivo deste estudo foi apresentar uma lista atualizada de espécies da família Leptophlebiidae, novos registros de ocorrência e sítios de distribuição para o estado do Piauí, Brasil. Ao analisar 447 exemplares, ampliamos significativamente nosso conhecimento sobre a distribuição das espécies de Leptophlebiidae no estado do Piauí, aumentando o número de espécies registradas de nove para 17. Também adicionamos novos registros de ocorrência para seis espécies e, pela primeira vez, documentamos a presença de quatro gêneros. Destacamos que ainda existe uma extensa área do Cerrado e semiárido brasileiro que se desconhece a ocorrência de Ephemeroptera, confirmando que a diversidade nessa área é subestimada e que o conhecimento sobre as espécies de Ephemeroptera e suas distribuições podem se expandir com o aumento do esforço amostral nos próximos anos, diminuindo as lacunas Lineana e Wallaceana sobre esse grupo. Nossos resultados também demonstram a necessidade emergencial de inventários no sul do Estado do Piauí, principalmente nas sub-bacias do médio e alto rio Parnaíba que é considerado adequado para a expansão da monocultura no Brasil.
Palavras-chave: *Inseto aquático; Ecossistema de água doce; Bacia do rio Parnaíba.*

Introduction

Ephemeroptera Hyatt & Arms, 1890 is currently represented by approximately 3500 species, 400 genera, and 42 families (Sartori & Brittain 2015; Jacobus et al. 2021), occupying nearly all freshwater environments except Antarctica (Barber-James et al. 2013). In Brazil, there are 447 species distributed among 83 genera and 10 families: Caenidae, Euthyplociidae, Leptohyphidae, Melanemerellidae, Oligoneuriidae, Coryphoridae, Ephemeridae, Polymitarcyidae, Baetidae, and Leptophlebiidae (Salles et al. 2023). Leptophlebiidae Banks, 1900 is a cosmopolitan and morphologically diverse family (Raimundi et al. 2017), ranking as the second most diverse family in the Neotropical region within the order Ephemeroptera (Barber-James et al. 2013, Domínguez & Dos-Santos 2014). This family comprises seven subfamilies: Castanophlebiinae, Habrophlebinae, Leptophlebiinae, Terpidinae, Atalophlebiinae, Choroterpinae, and Hagenulinae (Monjardim et al. 2020). In Brazil, the family Leptophlebiidae is classified into 30 genera and 146 species, with 95 species being endemic to the country (Salles et al. 2023).

In recent years, there has been an increase in studies on the Leptophlebiidae family in Brazil, especially in the Northeast region (e.g., Mariano 2010, Lima et al. 2012ab, 2013, 2014, 2015, 2016, Costa & Mariano 2013, Almeida et al. 2016, Takiya et al. 2016, Lima 2018, Nascimento et al. 2019, 2020, Campos et al. 2022, Rodrigues et al. 2023). Despite these efforts, there is still an imbalance in the number of distribution records for Leptophlebiidae species among the nine states that make up the Brazilian Northeast region. While Bahia, Maranhão, and Pernambuco have an average distribution of 18 species, the states of Alagoas, Ceará, and Piauí have an average of three species, and there are no recorded species of Leptophlebiidae in Rio Grande do Norte, Paraíba, and Sergipe (Salles et al. 2023).

Currently, the state of Piauí have recorded nine species of Leptophlebiidae: *Farrodes tepui* Domínguez, Molineri & Peters 1996; *Fittkaulus cururuensis* Savage 1986; *Hagenulopsis minuta* Spieth, 1943; *Miroculis botafora* Rodrigues, Nascimento, Raimundi & Lima, 2023; *Miroculis fittkaui* Savage & Peters, 1983; *Simothraulopsis demerara* (Traver, 1947); *Simothraulopsis sinuosus* Lima, 2018; *Thraulodes sternimaculatus* Lima, Mariano & Pinheiro, 2013; and *Ulmeritoides flavopedes* (Spieth, 1943) (Takiya et al. 2016, Nascimento et al. 2017, Boldrini et al. 2018, Lima 2018, Campos et al. 2022, Rodrigues et al. 2023). These records were obtained within a small area of the Parnaíba river basin, underscoring the substantial knowledge gap regarding the distribution of Leptophlebiidae species in the state of Piauí, often referred to as the 'Wallacean shortfall' (Hortal et al. 2015).

The state of Piauí covers an area of 251,755.485 km² (IBGE 2020) and is characterized by a high floristic diversity in the transitional zone (ecotone) between the Cerrado and Caatinga biomes (Souza et al. 2017). The main basin in the state is the Parnaíba River, which has a drainage area of 343,000 km², with approximately 73.1% of its surface located within the territorial limits of Piauí (JICA 1995). The extensive hydrographic network formed by this basin is being heavily impacted by human activities and the deforestation of natural vegetation. In 2022 alone, there was a cumulative deforestation of 1,188.76 km² and 2,282.04 km² in the states of Piauí and Maranhão, respectively (Assis et al. 2019). In this scenario, understanding the distribution of Leptophlebiidae diversity in the state of Piauí becomes essential to comprehend the current threats to biodiversity and proactively prevent damage to the natural ecosystem (Barnosky et al. 2011, Pimm et al. 2014, Steffen et al. 2015, Díaz et al. 2019) and local extinctions of species, particularly in biodiversity hotspots like the Cerrado.

The objective of this study was to present an updated species list of the family Leptophlebiidae Banks 1900 (Ephemeroptera), new occurrence records and distributional sites for the state of Piauí, Brazil.

Materials and Methods

The updated list of Leptophlebiidae for Piauí state was based on individuals collected and deposited in the Entomological Collection of the State University of Piauí, Campus Heróis do Jenipapo (CEHJ), Campo Maior, Piauí, Brazil; and the Invertebrate Collection of the National Institute of Amazonian Research (INPA), Manaus, Amazonas, Brazil. The collections were carried out between April 2017 and June 2023 at 20 sites distributed within the Parnaíba River basin (Table 1).

The nymphs were collected from random samples of all visible substrates at each site using a D-shaped net (34 cm diameter, 250 µm mesh), with an average scanning time of four hours per site. The observed substrate types at the sites included sand, leaf banks, wood, roots, stones, and macrophytes. Some nymphs with well-developed anterior wing pads were separated to observe the transition from the aquatic form to the winged form. The nymphs were individually placed in plastic containers with water, and the opening was covered with a fine mesh fabric to prevent the individual from flying after emergence (Boldrini & Cruz 2013). After the subimago stage, the specimen was transferred to a dry vial until the imaginal ecdysis occurred. Once the transition cycle was complete, the specimen was fixed in 96% ethanol.

Some imagos were collected during the daytime, using a sweep net through the vegetation near the water's edge at the site. During the nighttime, both active and passive collecting methods were employed using a light trap placed near the water. For active collection, a white sheet illuminated with LED lamps was set up perpendicular to the water, capturing all individuals that landed on the sheet between 6:00 pm and 8:00 pm. For passive collection, a Pennsylvania trap was used, which remained deployed for 12 hours.

The identification of adults was performed by removing the wings, which were then mounted dry between a slide and a cover slip, sealed only with colorless enamel. The genitalia were also removed and mounted on permanent or temporary slides. Canada balsam was used to secure the structures on the permanent slides. After mounting the slides, they were placed in an oven with an average temperature of 60 °C for 2 to 3 days to ensure complete drying, following the procedures outlined by Waltz & McCafferty (1987). The identifications were based on Domínguez et al. (2006) and careful comparisons with the original descriptions (e.g., Lima et al. 2013, Salles et al. 2016, Nascimento et al. 2017).

Species distribution was consulted using the Taxonomic Catalog of the Fauna of Brazil database (http://fauna.jbrj.gov.br/), and the conservation status of the species was assessed using the Biodiversity Extinction Risk Assessment System (https://salve.icmbio.gov.br/#/) and the IUCN Red List of Threatened Species (https://www.iucnredlist.org/).

Table 1. Sample sites (S) in the Parnaíba river basin, Piauí state, Brazil. Latitude and longitude represent the specific coordinates of each sample site.

Municipality	Sites	Location	Latitude	Longitude
Pedro II	S1	Cachoeira do Urubu-Rei	−4.32617	−41.46264
Barras	S2	Longá river	−4.20233	−42.23922
Piripiri	S3	Açude Caldeirão	−4.34794	−41.72039
Esperantina	S4	Longá river	−3.91436	−42.11050
Castelo do Piauí	S5	Poti river	−5.18797	−41.70956
Coivaras	S6	Cachoeira da Campeira	−5.19586	−42.25942
Esperantina	S7	Cachoeira do Urubu	−3.91286	−42.11336
Brasileira	S8	Cachoeira do Bota Fora	−4.21419	−41.66714
Novo Santo Antônio	S9	Cachoeira da Coruja	−5.36739	−41.99803
Pedro II	S10	Cachoeira do Buriti	−4.42978	−41.63139
Pedro II	S11	Cachoeira das Tuncas	−4.42458	−41.64311
Alto Longá	S12	Nascente do Frei Pedro	−5.22839	−42.17334
Coivaras	S13	Parque Nacional de Sete Cidades	−5.19374	−42.26281
Pedro II	S14	Samambaia stream	−4.32794	−41.46289
Pedro II	S15	Corrente river	−4.43239	−41.45819
Piracuruca	S16	Parque Nacional de Sete Cidades	−4.09708	−41.68006
Piracuruca	S17	Parque Nacional de Sete Cidades	−4.10947	−41.72746
Floriano	S18	Parnaíba river	−6.763464	−43.022408
Castelo do Piauí	S19	Palmeira stream	−5.20356	−41.63028
Castelo do Piauí	S20	Cais stream	−5.203500	−41.689750

Results

We collected a total of 447 Leptophlebiidae specimens, distributed across 11 genera and 14 species. The inventory resulted in new records for five genera and eight species for Piauí state (Figure 1A). Seven species with new occurrences, and two species with previously known distribution in the state. Table 1 presents the geographical information of the described sites (S) in the examined material. With the exception of *Miroculis botafora*, all other recorded Leptophlebiidae species in Piauí are classified as least concern (LC) according to the Chico Mendes Institute for Biodiversity Conservation (ICMBIO).

Updated checklist and new geographical records

Farrodes carioca Domínguez, Molineri & Peters, 1996

Figure 2A–B

Previous distribution. Bahia (Lima et al. 2016), Goiás (Raimundi 2019), Espírito Santo (Salles et al. 2010), Rio de Janeiro (Domínguez et al. 1996). **New record for Piauí state**.

Material examined. Two ♂ imagos (light trap), S8, 28.x.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ); same data as preceding except four ♂ imagos, 15.xii.2018, L.R.C. Lima col.; same data as preceding except 16 ♂ imagos, 01.vi.2019, L.R.C. Lima col. 29 ♂ imagos (light trap), S11, 29.vi.2019, L.R.C. Lima col. (CEHJ). Two ♂ imagos (light trap), S14, 29.vi.2019, L.R.C. Lima col. (CEHJ). Two ♂ imagos (light trap), S15, 29.vi.2019, L.R.C. Lima col. (CEHJ). Four ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Farrodes tepui Domínguez, Molineri & Peters, 1996

Previous distribution. Ceará (Takiya et al. 2016), Bahia (Lima et al. 2016), Pernambuco (Lima et al. 2012b) and Piauí (Takiya et al. 2016).

Fittkaulus cururuensis Savage, 1986

Previous distribution. Pará (Savage 1986), Roraima (Gama-Neto et al. 2018), Bahia (Costa et al. 2018), Piauí (Takiya et al. 2016), Pernambuco (Lima et al. 2012b), Maranhão (Nascimento et al. 2020), Mato Grosso (Boldrini et al. 2009) and Espírito Santo (Boldrini et al. 2009).

Material examined. One ♀ imago (light trap), S8, 15.xii.2018, L.R.C. Lima col. (CEHJ); same data as preceding except one nymph (D-shaped net), 05.ix.2022. Three nymphs (D-shaped net), S16, 15.viii.2022, L.R.C. Lima col. (CEHJ).

Hagenulopsis minuta Spieth, 1943

Previous distribution. Amazonas (Peters & Domínguez 2001), Pará (Peters & Domínguez 2001), Roraima (Gama-Neto & Hamada 2014), Bahia (Lima et al. 2016), Piauí (Campos et al. 2022), Mato Grosso (Campos et al. 2022), Espírito Santo (Campos et al. 2022) and Minas Gerais (Campos et al. 2022).

Material examined. One ♂ imago (sweep net), S8, 28.x.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ); same data as preceding except

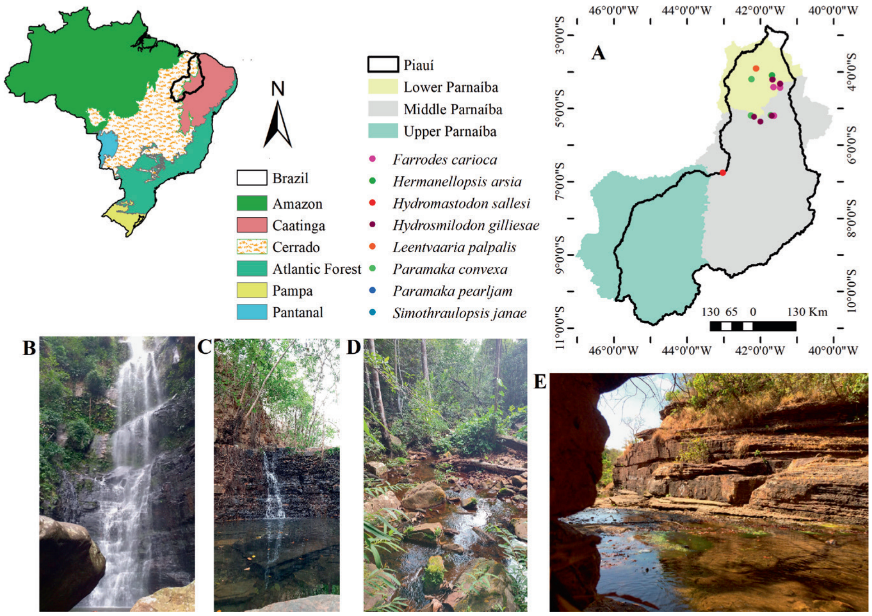

Figure 1. A, Distribution of sites with new records of Leptophlebiidae species (Ephemeroptera) for the Parnaíba river basin, Piauí, Brazil; B, Cachoeira do Urubu-Rei (site 1); C, Samambaia stream (site 14); D, Parque Nacional de Sete Cidades (site 16); E, Cachoeira da Campeira (site 27). See detailed information in Table 1.

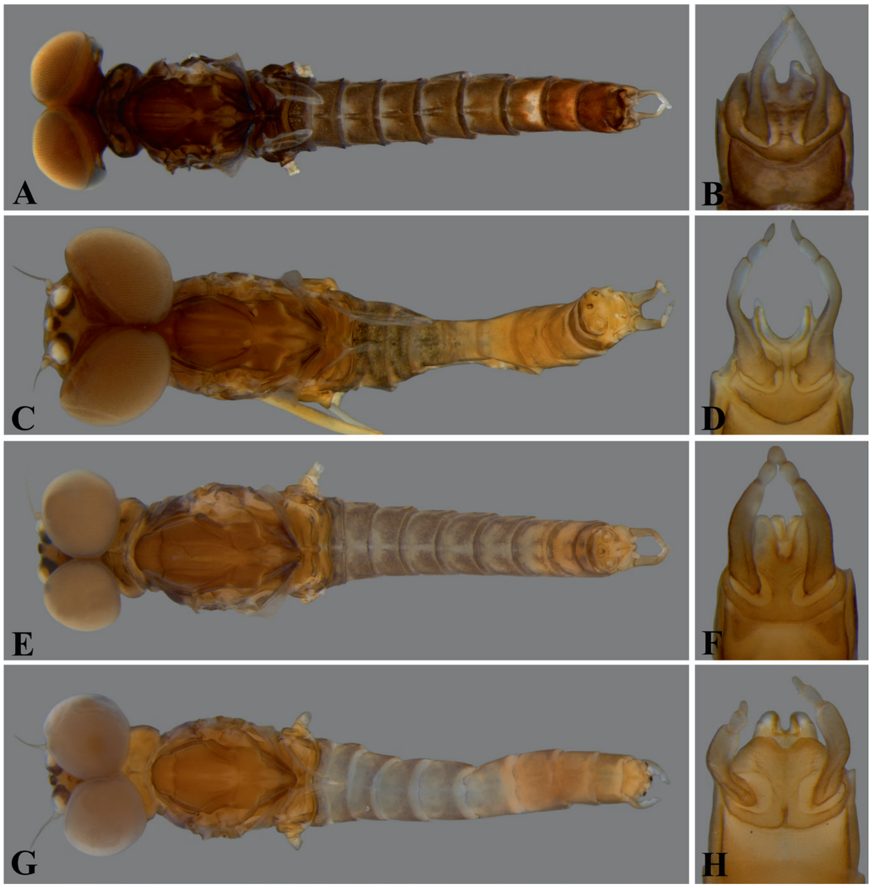

Figure 2. Male imagos of some Leptophlebiidae (Ephemeroptera) found in Piauí state, Brazil. A, Dorsal habitus of *Farrodes carioca*; B, Genitalia of *Farrodes carioca*; C, Dorsal habitus of *Hydromastodon sallesi*; D, genitalia of *Hydromastodon sallesi*; E, Dorsal habitus of *Hydrosmilodon gilliesae*; F, Genitalia of *Hydrosmilodon gilliesae*; G, Dorsal habitus of *Leentvaaria palpalis*; H, Genitalia of *Leentvaaria palpalis*.

two ♂ imagos, 10.ii.2018, L.R.C. Lima col.; same data as preceding except one ♂ imago (light trap), 15.xii.2018. One ♂ imago (light trap), S14, 18.vi.2022, L.R.C. Lima col. (CEHJ).

Hermanellopsis arsia Savage & Peters, 1983

Figure 3G–H

Previous distribution. Amazonas (Savage & Peters 1983), Roraima (Raimundi et al. 2017), Maranhão (Nascimento et al. 2020) and Pará (Oliveira et al. 2023). **New record for genus and specie for Piauí state**.

Material examined. 12 nymphs (D-shaped net), S16, 15.viii.2022, L.R.C. Lima col. (09 – CEHJ, 03 – INPA).

Hydromastodon sallesi Polegatto & Batista, 2007

Figure 2C–D

Previous distribution. Roraima (Polegatto & Batista 2007), Rondônia (Salles et al. 2016), Tocantins (Orlando et al. 2021), Maranhão (Nascimento et al. 2020), Mato Grosso (Polegatto & Batista 2007) and Mato Grosso do Sul (Silva & Salles 2017). **New record for genus and specie for Piauí state**.

Material examined. Four ♂ imagos (light trap), S8, 14.x.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ); same data as preceding except one ♂ imago, 10.ii.2018, L.R.C. Lima col. One ♂ imago (sweep net), S18, 11.iv.2023, L.R.C. Lima col. (CEHJ).

Hydrosmilodon gilliesae Thomas & Perú, 2004

Figure 2E–F

Previous distribution. Pará (Oliveira et al. 2023), Roraima (Salles et al. 2016), Tocantins (Orlando et al. 2021), Bahia (Lima et al. 2012a), Maranhão (Nascimento et al. 2020), Pernambuco (Lima et al. 2012b), Mato Grosso (Shimano et al. 2011), Espírito Santo (Salles et al. 2010) and São Paulo (Salles et al. 2016). **New record for genus and specie for Piauí state**.

Material examined. Four ♂ imagos (light trap), S1, 22.ii.2018, L.R.C. Lima col. (INPA). One nymph (D-shaped net), S2, 18.iv.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ, 03). 18 ♂ imagos (light trap), S8, 01.vi.2019, L.R.C. Lima col. (CEHJ); same data as preceding except one ♂ imago (rearing), 15.xii.2018; same data as preceding except 36 nymphs (D-shaped net), 05.ix.2022. One ♂ imago (light trap), S9, 29.iii.2018, L.R.C. Lima col. (CEHJ). One nymph (D-shaped net), S12, 27.ii.2021, L.R.C. Lima col. (CEHJ). One nymph (D-shaped net), S14, 06.ix.2022, L.R.C. Lima col. (CEHJ). 13 ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ). One ♂ imago (light trap), S20, 08.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Leentvaaria palpalis Demoulin, 1966

Figure 2G–H

Previous distribution. Roraima (Salles et al. 2016), Pernambuco (Lima et al. 2012b), Maranhão (Nascimento et al. 2020), Mato Grosso (Salles et al. 2016) and Espírito Santo (Lima et al. 2012a). **New record for genus and specie for Piauí state**.

Material examined. 10 ♂ imagos (light trap), S7, 22.ii.2018, L.R.C. Lima col. (CEHJ); same data as preceding except five nymphs (D-shaped net), 21.v.2022. 10 ♂ imagos (light trap), S8, 10.ii.2018, L.R.C. Lima col. (CEHJ); same data as preceding except one nymph (D-shaped net), 14.x.2017, L.R.C. Lima and J.A.O. Rodrigues cols. 32 ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Miroculis botafora Rodrigues, Nascimento, Raimundi & Lima, 2023

Previous distribution. Piauí (Rodrigues et al. 2023).

Miroculis fittkaui Savage & Peters, 1983

Previous distribution. Pará (Savage & Peters 1983), Roraima (Gama-Neto et al. 2018), Bahia (Campos et al. 2016), Piauí (Rodrigues et al. 2023), Pernambuco (Lima et al. 2012b) and Espírito Santo (Salles et al. 2010).

Paramaka convexa (Spieth, 1943)

Figure 3A–B

Previous distribution. Pará (Savage & Domínguez 1992), Roraima (Raimundi et al. 2017), Maranhão (Nascimento et al. 2020), Bahia (Mariano 2011) and Mato Grosso (Shimano et al. 2011). **New record for genus and specie for Piauí state**.

Material examined. One nymph (D-shaped net), S2, 18.iv.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ). One ♂ imago (light trap), S5, 12.iii.2016, L.R.C. Lima col. (CEHJ). Five ♂ imagos (light trap), S6, 04.iii.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (3 – CEHJ, 2 – INPA). 13 ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Paramaka pearljam Mariano, 2011

Figure 3C–D

Previous distribution. Mato Grosso (Mariano 2011). **New record for Piauí state**.

Material examined. 20 ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Simothraulopsis demerara (Traver, 1947)

Previous distribution. Pará (Domínguez et al. 1997), Amazonas (Domínguez et al. 1997), Roraima (Nascimento et al. 2017), Amapá (Nascimento et al. 2017), Rondônia (Nascimento et al. 2017), Tocantins (Boldrini & Krolow 2017), Ceará (Nascimento et al. 2017), Piauí (Takiya et al. 2016), Bahia (Lima et al. 2016), Pernambuco (Lima et al. 2012b), Maranhão (Nascimento et al. 2020), Mato Grosso (Nascimento et al. 2017), Goiás (Raimundi 2019), Espírito Santo (Salles et al. 2010) and Paraná (Faria & Salles 2019).

Material examined. Four ♂ imagos (light trap), S2, 18.iv.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ). 15 ♂ imagos (light trap), S7, 17.iv.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ). Nine ♂ imagos (light trap), S8, 14.x.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ); same data as preceding except five ♂ imagos, 10.ii.2018, L.R.C. Lima col.; same data as preceding except one ♂ imago (rearing), 15.xii.2018; same data as preceding except three ♂ imagos, 15.xii.2018; same data as preceding except four ♂ imagos, 01.vi.2019. 15 ♂ imagos (light trap), S11, 29.vi.2019, L.R.C. Lima col. (CEHJ). Seven ♂ imagos (light trap), S14, 18.vi.2022, L.R.C. Lima col. (CEHJ). One ♂ imago (rearing), S17, 16.viii.2022, L.R.C. Lima col. (CEHJ). Seven ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ). 38 ♂ imagos (light trap), S20, 08.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

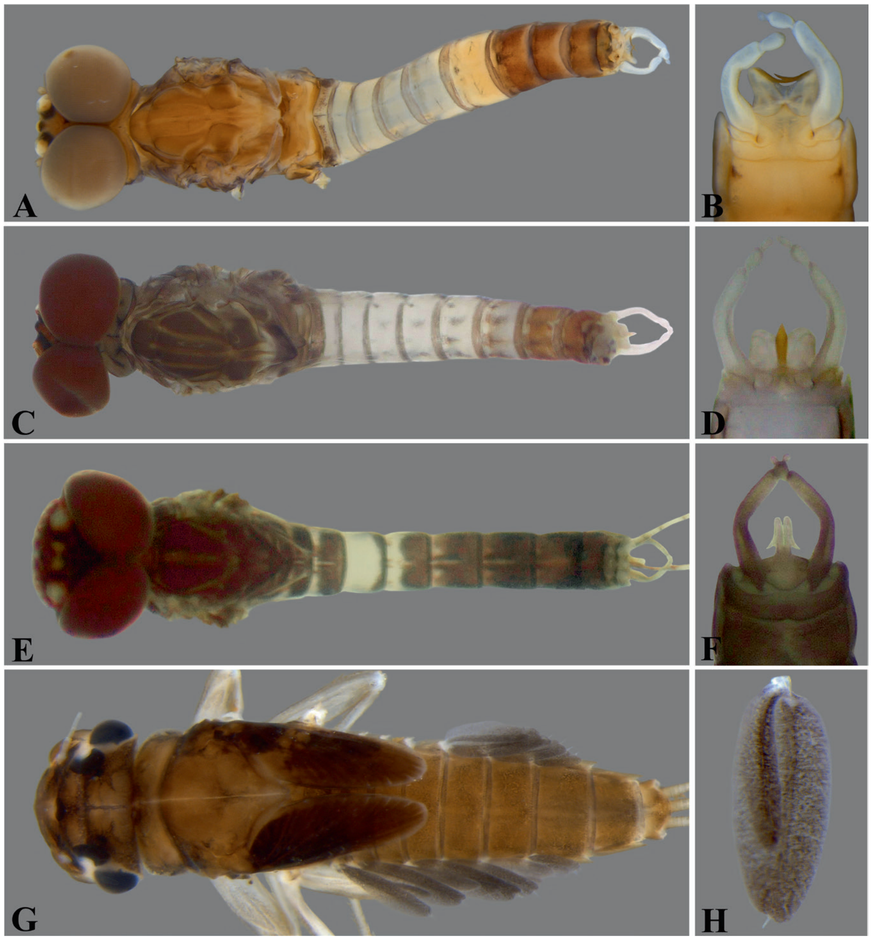

Figure 3. Male imagos and nymph of some Leptophlebiidae (Ephemeroptera) found in Piauí state, Brazil. A–F) Male imagos: A, dorsal habitus of *Paramaka convexa*; B, genitalia of *Paramaka convexa*; C, dorsal habitus of *Paramaka pearljam*; D, genitalia of *Paramaka pearljam*; E, dorsal habitus of *Simothraulopsis janae*; F, genitalia of *Simothraulopsis janae.* G–H) Nymph of *Hermanellopsis arsia*: G, dorsal habitus of *Hermanellopsis arsia*; H, gill IV of *Hermanellopsis arsia*.

Simothraulopsis janae Mariano, 2010

Figure 3E–F

Previous distribution. Bahia (Mariano 2010), Mato Grosso (Nascimento et al. 2017), Maranhão (Nascimento et al. 2020), Minas Gerais (Nascimento et al. 2017), Pará (Nascimento et al. 2017), Pernambuco (Lima et al. 2012b), Rondônia (Nascimento et al. 2017), and Roraima (Gama-Neto & Hamada 2014). **New record for Piauí state.**

Material examined. Two ♂ imagos (light trap), S19, 09.vi.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Simothraulopsis sinuosus Lima, 2018

Previous distribution. Maranhão (Nascimento et al. 2020) and Piauí (Lima 2018).

Material examined. Two ♂ imagos (light trap), S7, 18.iv.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ); same data as preceding except one ♂ imago, 07.ix.2018, L.R.C. Lima col.

Thraulodes sternimaculatus Lima, Mariano & Pinheiro, 2013

Previous distribution. Maranhão (Nascimento et al. 2020), Piauí (Boldrini et al. 2018) and Pernambuco (Lima et al. 2013).

Material examined. One ♂ imago (light trap), S7, 17.iv.2017, L.R.C. Lima and J.A.O. Rodrigues cols. (CEHJ). 11 ♂ imagos (light trap), S8, 10.ii.2018, L.R.C. Lima col. (CEHJ). 56 ♂ imagos (light trap), S19, 09.viii.2023, L.R.C. Lima, A.S. Carvalho, A.P.J. Faria cols. (CEHJ).

Ulmeritoides flavopedes (Spieth, 1943)

Previous distribution. Roraima (Lopes et al. 2003), Tocantins (Boldrini & Krolow 2017), Bahia (Lima et al. 2016), Maranhão (Nascimento et al. 2020), Piauí (Takiya et al. 2016), Pernambuco (Lima et al. 2015) and Mato Grosso (Shimano et al. 2011).

Material examined. Two ♂ imagos (rearing), S8, 15.xii.2018, L.R.C. Lima col. (CEHJ). One ♂ imago (rearing), S16, 15.vii.2022, L.R.C. Lima col. (CEHJ).

Discussion

Our study provides new information about the distribution of Leptophlebiidae species in the state of Piauí, northeastern Brazil. This represents an increase from nine to 17 species with valid occurrences in the state, and five genera reported for the first time. Although the species recorded in this study are also found in other Brazilian states and in other South American countries, this study provides a significant contribution to the knowledge of Ephemeroptera in the Northeast region of Brazil, particularly in semi-arid environments.

Leptophlebiidae inventories have been conducted in only a few areas of the Cerrado biome. The state of Piauí follows a similar pattern, with studies concentrated in the northern part of the state, specifically in the lower and middle sub-basins of the Parnaíba river (Figure 1A). This concentration is primarily due to the location of our research group, which faces resource constraints in conducting field expeditions to more distant regions. Despite notable efforts between the first record of Leptophlebiidae species distribution in Piauí (Takiya et al. 2016) and the findings presented in this study, there is still a vast unknown area in Piauí, particularly in the middle and upper sub-basins of the Parnaíba River. This knowledge gap limits our ability to understand the effects of multiple human-caused stressors on biodiversity or to utilize this information to create local conservation strategies (e.g., Baranzelli et al. 2023). This becomes even more relevant considering that to the west of the upper and middle sub-basins of the Parnaíba river lies the region known as MATOPIBA. This name refers to the area encompassing the states of Maranhão (MA), Tocantins (TO), Piauí (PI), and Bahia (BA), which is considered suitable for monoculture expansion in Brazil (Miranda et al. 2014, Polizel et al. 2021). Consequently, the transformation of this area can incur significant environmental costs, including fragmentation, water pollution, and loss of Ephemeroptera specimens unknown to science.

The new records and additional occurrence points presented in this study significantly contribute to our knowledge of Leptophlebiidae species distribution in Brazil, reducing the Wallacean shortfall of the Ephemeroptera in the Cerrado biome. We highlight that there is still a vast area in the Cerrado and Brazilian semiarid regions where the occurrence of Ephemeroptera is unknown, confirming that the diversity in this area is underestimated and that knowledge of Ephemeroptera species and their distributions can expand with increased sampling efforts in the coming years. This effort has the potential to reduce the Linnean and Wallacean shortfall about the Leptophlebiidae species. Our results also highlight the urgency of inventory efforts in the southern region of the state of Piauí, particularly in the middle and upper sub-basins of the Parnaíba river, as they are heavily influenced by MATOPIBA.

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Author Contributions

Ana Paula Justino de Faria: contributed to conceptualization; data collection; methodology and original draft.

Jackson A. O. Rodrigues: collected and identified the species; contributed to conceptualization of the manuscript.

Stênio Raniery de Sousa Nascimento: produced the photographs; reviewed and edited the manuscript.

Lucas Ramos Costa Lima: collected and identified the species; contributed to conceptualization, reviewed and edited the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data availability

The data used in our analysis is available at Biota Neotropica Dataverse https://doi.org/10.48331/scielodata.JTYTUS

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Inventory

Updated list of non-volant small mammals from the Serra da Bocaina National Park, southeastern Brazil

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Abstract: In the core of the Atlantic Forest biome, the Serra da Bocaina National Park (SBNP) is located in the Atlantic Forest Southeast area of endemism for vertebrates. Filling gaps in knowledge about the spatial distribution and occurrence of species in national parks is of fundamental importance to know how many species are protected and to guide conservation initiatives. Here we updated the non-volant small mammal species list of the SBNP, providing new data on species list and abundance, with species identified mainly by karyotype and/or molecular analysis. Twelve sampling sessions with a capture-mark-recapture approach were carried out in four sites in the SBNP from 2013 to 2016, during the paving works of the state highway RJ-165 (Estrada Parque Paraty-Cunha), municipality of Paraty, state of Rio de Janeiro, Brazil. Non-volant small mammals (Rodentia and Didelphimorphia) were sampled using Sherman® and Tomahawk® live traps (18,987 trap-nights) and pitfall traps (4,591 trap-nights). Thirty-two species (11 marsupials and 21 rodents) were recorded from 1,185 captured specimens. Species richness ranged from 18 to 28 between sites. Ten and 11 species were exclusively captured in live traps and pitfall traps, respectively. The observed richness (32 species) represented 91.4% of the estimated species richness for the study area. Sites 2 and 4 were the most similar to each other regarding species composition, and site 3 was the most dissimilar. The species with highest relative abundance were *Euryoryzomys russatus* (14%) and *Delomys dorsalis* (14%), while six species had relative abundances lower than 1%. Fourteen and 17 species were identified by karyotype and molecular analysis, respectively. The present study added 22 species to the park's non-volant small mammals list, which now has 37 species with confirmed occurrence. This species richness found in the SBNP is one of the highest ever recorded for the group of non-volant small mammals in protected areas of the Atlantic Forest in Brazil, corroborating the Serra da Bocaina region as a biodiversity hotspot.

Keywords: *Atlantic Forest; cytb; cytogenetics; faunistic inventory; molecular identification; species richness.*

Lista atualizada dos pequenos mamíferos não-voadores do Parque Nacional da Serra da Bocaina, sudeste do Brasil

Resumo: No cerne do bioma Mata Atlântica, o Parque Nacional da Serra da Bocaina (PNSB) está localizado na área Sudeste de endemismo para vertebrados na Mata Atlântica. Preencher lacunas de conhecimento sobre a distribuição espacial e ocorrência das espécies em parques nacionais é de fundamental importância para saber quantas espécies estão protegidas e orientar iniciativas de conservação. Aqui atualizamos a lista de espécies de pequenos mamíferos não-voadores do PNSB, fornecendo novos dados sobre a lista de espécies e abundância, com espécies identificadas principalmente por análises cariotípicas e/ou molecular. Doze sessões de amostragem com uma abordagem de captura-marcação-recaptura foram realizadas em quatro áreas no PNSB de 2013 a 2016, durante as obras de pavimentação da rodovia estadual RJ-165 (Estrada Parque Paraty-Cunha), município de Paraty, estado do Rio de Janeiro, Brasil. Os pequenos mamíferos não-voadores (Rodentia e Didelphimorphia) foram amostrados usando armadilhas de captura viva Sherman® e Tomahawk® (18.987 armadilhas-noite) e armadilhas de queda (4.591 armadilhas-noite). Trinta e duas espécies (11 marsupiais e 21 roedores) foram registradas em 1.185 espécimes capturados. A riqueza de espécies variou de 18 a 28 entre as áreas de amostragem. Dez e 11 espécies foram capturadas exclusivamente em armadilhas de captura viva e armadilhas de queda, respectivamente. A riqueza observada (32 espécies) representou 91,4% da riqueza de espécies estimada para a área de estudo. As áreas 2 e 4 foram as mais semelhantes entre si quanto à composição de espécies, e a área 3 foi a mais dissimilar. As espécies com maior abundância relativa foram *Euryoryzomys russatus* (14%) e *Delomys dorsalis* (14%), enquanto seis espécies tiveram abundâncias relativas inferiores a 1%. Quatorze e 17 espécies foram identificadas pelo cariótipo e por análise molecular, respectivamente. O presente estudo acrescentou 22 espécies à lista de pequenos mamíferos não-voadores do parque, que passou a contar com 37 espécies com ocorrência confirmada. Essa riqueza de espécies encontrada no PNSB é uma das maiores já registradas para o grupo dos pequenos mamíferos não-voadores em áreas protegidas da Mata Atlântica no Brasil, corroborando a região da Serra da Bocaina como um *hotspot* de biodiversidade.

Palavras-chave: *Mata Atlântica; cytb; citogenética; inventário faunístico; identificação molecular; riqueza de espécies.*

Introduction

The Atlantic Forest exhibits some of the highest rates of species diversity and endemism on the globe (Myers et al. 2000), with 8% of the world's vertebrate species occurring in this biome, 3% of those being endemic (Figueiredo et al. 2021). The species richness of vertebrates, except for reptiles and bats, increases towards the core of the Atlantic Forest (i.e., the Serra do Mar region), a pattern often attributed to the great topographic variation in this region (Figueiredo et al. 2021). Topography is also the main factor explaining the spatial pattern of species richness of Atlantic Forest marsupials, while temperature seasonality is the most critical driver of endemic species richness (Delciellos et al. 2022). Species richness of South American rodents is correlated with latitude, but also with the existing altitudinal gradient on the continent (Maestri & Patterson 2016).

In the Serra do Mar region, the Serra da Bocaina National Park (SBNP) is located in one important center of endemism for small mammals in the Atlantic Forest (Dalapicolla et al. 2021). The Atlantic Forest Southeast area of endemism, as established by Dalapicolla et al. (2021), is the largest of the seven areas of endemism identified by these authors and the one with the largest number of protected areas that cover 55% of its extension. Despite this relative high level of protection, the southeastern Atlantic Forest, as well as the rest of the biome, is still severely threatened due to intense historical and current deforestation pressure (Rezende et al. 2018). Additionally, existing protected areas do not always overlap with diversity and endemism hotspots, as observed for marsupials (Delciellos et al. 2022) and small mammals (Dalapicolla et al. 2021). Thus, filling knowledge gaps on species occurrence and spatial distribution is of fundamental importance to obtain a more complete overview of how much of the Atlantic Forest species are protected and to guide conservation initiatives such as management and creation of protected areas in identified biodiversity hotspots.

The SBNP was created 52 years ago by the Federal Decree 68,172 of March 4th, 1971 (IBAMA 2001), but a comprehensive survey of mammals had not been carried out until recently. From 2010 to 2016, we conducted a survey of non-volant small mammals in the southern portion of the park, in the state of Rio de Janeiro, as part of the program to evaluate environmental impacts of the paving works of the state highway RJ-165 (Estrada Parque Paraty-Cunha). Initially, from 2010 to 2011, three sampling sessions were carried out to inventory mammal species in the region, culminating in the publication of Delciellos et al. (2012). Here, we updated the SBNP non-volant small mammal species list with the data obtained from 2013 to 2016, providing new data on species occurrence and abundance.

Material and Methods

1. *Study area*

The study was conducted in four sites along the RJ-165 state highway (Estrada-Parque Paraty-Cunha), traversing the SBNP in the municipality of Paraty, Rio de Janeiro state, Brazil (Figure 1). The sites encompass an altitudinal range from 731 to 1,193 m a.s.l. (Delciellos et al. 2018). The climate of Paraty is type Af (Tropical Rainforest) following Köppen-Geiger classification, the mean annual temperature is 23.3 °C and the mean annual precipitation is 2,284 mm (https://pt.climate-data.org/). The super-humid season occurs from October to April and the humid season from May to September (https://pt.climate-data.org/). There is no water deficit in the region (https://pt.climate-data.org/). The vegetation is classified as ombrophilous dense montane forest (IBGE 2012). This study was part of the Mammal Monitoring Program

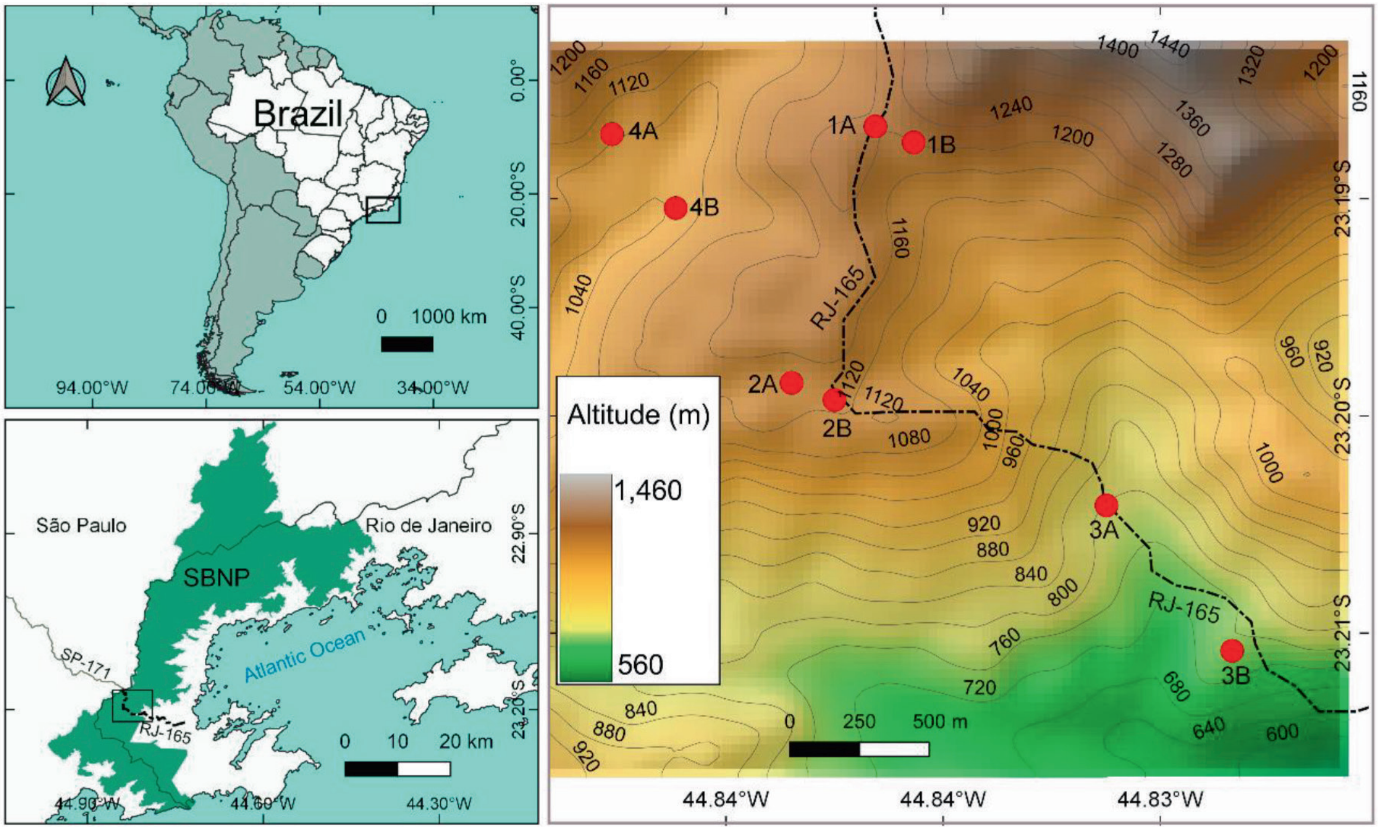

Figure 1. Study area in the Serra da Bocaina National Park (SBNP), municipality of Paraty, Rio de Janeiro state, Brazil. The Digital Elevation Model (MDE) database from the Instituto Brasileiro de Geografia e Estatística (IBGE 2023) was used to generate the contour lines and the topographic map. Topography is represented by shading. The study was carried out in four sites (1–4) distributed along the RJ-165 state highway, each site with two transects (A and B; red dots) for sampling non-volant small mammals.

of the roadworks project of the RJ-165 highway (IBAMA/MMA process no. 02001.003937/2008-18, authorizations no. 248/2013 and 610/2015).

2. *Sampling of non-volant small mammals*

Non-volant small mammals from the orders Didelphimorphia and Rodentia were sampled in twelve sampling sessions with a capture-mark-recapture approach carried out between June 2013 and December 2016 (Sampling sessions: 1 = June 2013; 2 = September 2013; 3 = December 2013; 4 = April 2014; 5 = June 2014; 6 = October 2014; 7 = January 2015; 8 = October 2015; 9 = December 2015; 10 = February 2016; 11 = July 2016; 12 = December 2016) using live traps (Tomahawk® and Sherman®) and pitfall traps. Two 290 m transects (A and B) with 30 trap stations were established at each sampling site. Trap stations consisted of either a Tomahawk trap (45 × 15 × 17 cm), placed on the ground and baited with a mixture of banana, bacon, grinded peanut and oat, or a Sherman trap (31 × 8 × 9 cm) set in the understory at a height of 1.5–2.0 m above the ground and baited with slices of banana. An additional 10 Sherman traps were set in the canopy (> 3.5 m above ground) along each transect during the first two sampling sessions, and then in the understory from the third session onwards, because the ropes that suspended the traps in the canopy were continually stolen. All traps were activated over five consecutive nights during each sampling session, and checked in the morning. The total sampling effort using live traps was 18,987 trap-nights.

Twenty pitfall traps (60 L plastic buckets) were set at each sampling site, totaling 80 buckets in the study area. In each site, buckets were buried in the ground, 10 m apart. A drift fence of plastic sheeting was set 0.5 m high, the base buried up to 0.1 m, and extended along the ground connecting the buckets to orientate the capture of wandering animals. Ideally, at each site these 20 buckets were installed in two transects, each with 10 buckets. Eventually, the 10 buckets sequence could not be done because of rough terrain, thus we deviate from the obstacle (e.g., rocks) and, in this case, the distance between buckets has become larger than 10 m. Pitfall traps were activated five consecutive nights during each sampling session. They were checked in the morning during the first seven sampling sessions, and then three times a day from the eighth session onwards to reduce mortality in the buckets by hypothermia due to the combination of low temperatures and high rainfall in the study area. Increasing the frequency of bucket checks was an imposition by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Before this imposition, animal mortality in the buckets was high despite all possible measures to avoid eventual deaths, such as holes in the buckets, styrofoam plates to avoid drowning, and hydrophobic cotton pieces for the animals to warm up. The total sampling effort using pitfall traps was 4,591 trap-nights.

The mammal specimens captured were identified in the field to species or genus, sexed, weighed using a spring balance, measured (heady-body and tail lengths), and marked with a numbered ear-tag (at first capture). Unidentified specimens were collected, taxidermized, and deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil (Table 1). Some specimens were karyotyped (Table 2) and/or had the mitochondrial DNA sequenced and analyzed (Table 3). The threatened status of species followed the classifications at regional (states of Rio de Janeiro and São Paulo; Bergallo et al. 2000, São Paulo 2018), national (MMA 2022) and global (IUCN 2022) levels (Table 1).

3. *Karyotypic and molecular analysis*

Karyotypes were prepared in the field and chromosomes in metaphases from 26 specimens were obtained by *in vitro* culture (culture of bone marrow grown in Dulbecco´s MEM with 10% fetal bovine serum and colchicine) plus ethidium bromide, following Geise (2014) with modifications – culture kept at 36.5 °C for 1h40 min. Conventional staining with Giemsa 5% was used to observe diploid number (2n), fundamental number of autosomes (FNa), and chromosome morphology. Microscopic analyzes were performed on the optic photomicroscope (Nikon Eclipse 50i), using an increase of 1,000 with an immersion objective of 100 plus 10 ocular lenses. Karyotypes were mounted, compared with those available in the literature. Chromosomes in metaphases were deposited at the Laboratório de Mastozoologia, Universidade do Estado do Rio de Janeiro (Geise & Aguieiras 2021). Chromosome morphology follows Levan et al. (1964).

Genomic DNA of 87 specimens belonging to 11 rodent and two marsupial genera was extracted from the liver and/or epithelial tissue using the salt protocol and proteinase K (Bruford et al. 1992). In the Polymerase Chain Reaction (PCR), the primers MVZ05 and MVZ16 (Smith & Patton 1993) were used to amplify the first 801 base pairs (bps) of the mitochondrial Cytochrome b (cytb) gene. Cytb is widely used in mammals as a species identification tool (Bradley & Baker 2001, Agrizzi et al. 2012). PCR was performed using 2.5 µl of 10× buffer, 1.0 µl of $MgCl_2$ at 50 mM, 0.5 µl of deoxynucleotide triphosphate mix (10 mM of each nucleotide), 0.3 µl of each primer at 10 µM, 3 units of Taq Platinum (Invitrogen Corporation, Carlsbad, California) and 1.0 µl of template DNA, totalizing 25 µl of final volume of PCR reaction. The PCR was carried out with an initial denaturation temperature of 94 °C for 5 min followed by 39 cycles (30 s of denaturation at 94 °C, 45 s at 48 °C for primer annealing and 45 s at 72 °C for extension of the molecule) and a final extension at 72 °C for 10 min. The PCR products were purified using ExoSAP enzymes (GE Healthcare Life Sciences). The sequencing reactions were run using BigDye Terminator 3.1 (Applied Biosystems, Inc.) and the same primers used for the PCR. The sequences were read in an ABI 3500 automated capillary sequencer (Applied Biosystems, Inc.) and aligned using Geneious Prime software (Biomatters Ltd, Auckland, New Zealand). After that the sequences were submitted to the BLAST tool incorporated in Geneious for the certification of the correct sequencing process.

For the molecular identification by phylogenetic reconstruction, additional sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/), together with sequences of closely related taxa, included as outgroups (See Data Availability). The sequences obtained during

Table 1. Updated list of non-volant small mammals from the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Abundance is the number of individuals per species during the study period, from June 2013 to December 2016. * = Previous records in the SBNP (Delciellos et al. 2012), not recorded from June 2013 to December 2016. Legend for type of record: CA = Capture; LT = Live trap; PIT = Pitfall trap; RO = Roadkill; VO = Visual observation. Legend for methods of species identification: K = Karyotype; M = Morphology; MA = Molecular analysis. Legend for status of conservation: DD = Data deficient; NT = Near threatened; VU = Vulnerable; EW = Extinct in the wild; IUCN = International Union for Conservation of Nature (IUCN 2022); RJ = state of Rio de Janeiro (Bergallo et al. 2000); SP = state of São Paulo (São Paulo 2018). Legend for voucher specimens: MN = Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

Species	Type of record	Method of species identification	Abundance	Status of conservation	Voucher specimens
Order Didelphimorphia					
Family Didelphidae					
Caluromys philander (Linnaeus, 1758)	CA(LT), RO	M	2		MN81492
**Chironectes minimus* (Zimmermann, 1780)	VO	M	–	NT (RJ)	Not collected
Didelphis aurita Wied Neuwied, 1826	CA(LT, PIT), RO, VO	M	72		MN83220, MN83787
Gracilinanus microtarsus (Wagner, 1842)	CA(LT, PIT)	M	63	NT (RJ)	MN81007, MN81045, MN83207, MN83210
Marmosa paraguayana Tate, 1931	CA(LT)	M	33		MN81032
Marmosops incanus (Lund, 1840)	CA(LT, PIT)	M	6		MN80996, MN81020, MN81044, MN81048, MN81049, MN81060

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Species	**Type of record**	**Method of species identification**	**Abundance**	**Status of conservation**	**Voucher specimens**
Marmosops paulensis (Tate, 1931)	CA(LT)	MA	1	NT (RJ), VU (SP)	MN83200
Marmosops spp.	CA(LT, PIT)	M	138		
Metachirus myosuros (Temminck, 1824)	CA(LT)	M	15		MN81834, MN83232
**Monodelphis americana* (Müller, 1776)	CA(PIT)	M	–		MN77794
Monodelphis iheringi (Thomas, 1888)	CA(PIT)	MA	9	VU (SP), DD (IUCN)	MN81006, MN81017, MN81046, MN81844
Monodelphis pinocchio Pavan, 2015	CA(PIT)	M, MA	2		MN83182
Monodelphis scalops (Thomas, 1888)	CA(PIT)	MA	7	NT (RJ)	MN81015, MN81040, MN81041, MN81481, MN81498, MN81840, MN83024
Monodelphis spp.	CA(PIT)	M	57		
Philander quica (Temminck, 1824)	CA(LT, PIT), RO	M	39		MN83784
Order Rodentia					
Family Sciuridae					
Guerlinguetus brasiliensis (Gmelin, 1788)	CA(LT)	M, MA	28		MN80992, MN81003, MN81009, MN81013, MN81026, MN81029, MN81057, MN81469, MN81471, MN81473, MN81486, MN83223, MN83783, MN83786
Family Cricetidae					
Abrawayaomys ruschii Cunha & Cruz, 1979	CA(PIT)	K, M, MA	5		MN81816, MN83192, MN83194
**Akodon cursor* (Winge, 1887)	CA(PIT)	K	–		MN77792
Akodon montensis Thomas, 1913	CA(PIT)	MA	2		Not collected
Akodon spp. or *Castoria angustidens*	CA(LT, PIT)	M	54		
Blarinomys breviceps (Winge, 1887)	CA(PIT)	K, M	6	EW (RJ)	MN77786, MN81027, MN81483
Brucepattersonius nebulosus E. F. Abreu-Júnior, J. F. Vilela, A. U. Christoff, V. H. Valiati & A. R. Percequillo, 2019	CA(PIT)	K, MA	2		MN83197
Brucepattersonius soricinus Hershkovitz, 1998	CA(LT, PIT)	M, MA	11	DD (IUCN)	MN81004, MN81809, MN81810, MN83198
Brucepattersonius spp.	CA(PIT)	M	4		
Castoria angustidens (Winge, 1887)	CA(LT)	K, M, MA	1		MN77791, MN83198

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Species	**Type of record**	**Method of species identification**	**Abundance**	**Status of conservation**	**Voucher specimens**
Delomys dorsalis (Hensel, 1872)	CA(LT, PIT)	K, M	166		MN77787, MN77788
Drymoreomys albimaculatus Percequillo, Weksler, and Costa, 2011	CA(PIT)	K, M, MA	4	NT (IUCN)	MN81462, MN83791
Euryoryzomys russatus (Wagner, 1848)	CA(LT, PIT)	K, M, MA	168		MN80997, MN81008, MN81050, MN81811, MN81818, MN83235
Juliomys ossitenuis Costa, Pavan, Leite & Fagundes, 2007	CA(LT, PIT)	K, MA	6		MMN81807, MN81847, MN81852
Juliomys pictipes (Osgood, 1933)	CA(PIT)	K, MA	16		MN77793, MN80998, MN81005, MN81841, MN81843, MN81863, MN83190, MN83217
Juliomys spp.	CA(LT, PIT)	M	3		
Nectomys squamipes Brants, 1827	CA(LT)	K, M	10		MN81059, MN81828, MN83789
**Oecomys catherinae* Thomas, 1909	CA(LT)	M	–		Not collected
**Oligoryzomys flavescens* (Water house, 1837)	CA(LT, PIT)	M	–		Not collected
Oligoryzomys nigripes (Olfers, 1818)	CA(PIT)	K	1		MN81014
Oligoryzomys spp.	CA(LT, PIT)	M	86		
Oxymycterus dasytrichus (Schinz, 1821)	CA(PIT)	M, MA	2		MN81850, MN83191
Oxymycterus spp.	CA(LT, PIT)	M	10		
Rhipidomys itoan B. M. A. Costa, Geise, Pereira & L. P. Costa, 2011	CA(LT, PIT)	K, M, MA	35		MN81016, MN83201, MN83221
Sooretamys angouya (G. Fischer, 1814)	CA(LT), RO	K, M, MA	23		MN80994, MN81025, MN81028, MN81061, MN81812, MN81826, MN81851, MN83193 MN83212, MN83218
Thaptomys nigrita (Lichtenstein, 1829)	CA(LT, PIT)	K, M	57	VU (RJ)	MN81812, MN81851, MN83193
Family Echimyidae					
Phyllomys nigrispinus (Wagner, 1842)	CA(LT, PIT)	K, MA	4		MN83181, MN83183, MN83187, MN83196
Phyllomys sulinus Leite, Christoff & Fagundes, 2008	CA(LT)	MA	1	DD (IUCN)	MN81062
Phyllomys spp.	CA(PIT)	M	2		
Trinomys dimidiatus (Günther, 1876)	CA(LT)	K, MA	34	VU (SP)	MN81018, MN81813

Table 2. Updated list of karyotyped rodent specimens captured in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Legend: F = Female; M = Male.

Species	Voucher number	Sex	Diploid number	Fundamental number of autosomes	Reference
Abrawayaomys ruschii	MN83194	F	58	58	Present study
Akodon cursor	MN77792	F	14	20	Delciellos et al. (2012)
Blarinomys breviceps	MN77786	F	28	Not determined	Delciellos et al. (2012)
Blarinomys breviceps	MN81027	M	28	50	Present study
Brucepattersonius nebulosus	MN83197	F	52	52	Present study
Castoria angustidens	MN77791	F	46	46	Delciellos et al. (2012)
Delomys dorsalis	MN77787, MN77788	F	82	80	Delciellos et al. (2012)
Drymoreomys albimaculatus	MN81462	F	62	62	Present study (Delciellos et al. 2015)
Euryoryzomys russatus	MN80997, MN81827	F	80	88	Present study
Juliomys ossitenuis	MN81807, MN81847, MN81852	M/M/F	20	36	Present study (Delciellos et al. 2020)
Juliomys pictipes	MN81841, MN81843, MN83190	F	36	34	Present study (Delciellos et al. 2020)
Nectomys squamipes	MN81828	M	56	56	Present study
Oligoryzomys nigripes	MN81014	F	62	79	Present study
Phyllomys nigrispinus	MN83183, MN83187, MN83196	F	85/84/85	–	Present study (Delciellos et al. 2018)
Rhipidomys itoan	MN81016	M	44	50	Present study
Sooretamys angouya	MN83184, MN83185, MN83197	F/M/M	58	60	Present study
Thaptomys nigrita	MN81812, MN81851, MN83193	M	52	52	Present study
Trinomys dimidiatus	MN81018, MN81813	M	60	116/114	Present study

Table 3. List of specimens from which cytochrome b sequence data was identified with BLAST analysis and used for phylogenetic analyses. Legend: B ID% = Blast Percent Identity; BOCA = Field number from collected specimens in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil; BQC = Blast Query Cover; MN = Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Species	Specimens number	BQC% / B ID%	Outgroups	Model
Abrawayaomys ruschii	BOCA666/MN83194	99 / 99	*Delomys*	GTR + I+ G
Akodon montensis	BOCA1509	94 / 99	*Blarinomys*	HKY + I + G
	BOCA1513	94 / 99		
Brucepattersonius soricinus	BOCA1143	96 / 99	*Oxymycterus*	GTR + I + G
	BOCA1163	100 / 99		
	BOCA1155	99 / 99		
	BOCA1494	97 / 99		
Brucepattersonius nebulosus	BOCA667/MN83197	98 / 99	*Oxymycterus*	GTR + I + G
	BOCA795	100 / 100		
Castoria angustidens	BOCA221	92 / 97	*Blarinomys*	HKY + I + G
Drymoreomys albimaculatus	BOCA336/MN81462	100 / 100	*Cerradomys*	GTR + I + G

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Species	Specimens number	BQC% / B ID%	Outgroups	Model
Euryoryzomys russatus	BOCA1332/MN83235	100 / 99	*Cerradomys*	GTR + I + G
	BOCA1333	100 / 100		
	BOCA265	50 / 86		
	BOCA852	94 / 96		
	BOCA889	100 / 97		
Juliomys ossitenuis	BOCA357/MN81807	98 / 99	*Oxymycterus*	GTR + I + G
	BOCA365/MN81852	98 / 99		
	BOCA792/MN81847	98 / 98		
	BOCA 814	99 / 93		
	BOCA1324	98 / 99		
	BOCA1353	98 / 98		
Juliomys pictipes	BOCA18/MN80998	100 / 98	*Oxymycterus*	GTR + I + G
	BOCA47/MN81005	100 / 98		
	BOCA201	100 / 99		
	BOCA759/MN81841	100 / 98		
	BOCA766/MN81843	100 / 98		
	BOCA798	100 / 98		
	BOCA818	100 / 98		
	BOCA820	100 / 98		
	BOCA904	100 / 98		
	BOCA995	100 / 98		
	BOCA1026	100 / 95		
	BOCA1154/MN83217	100 / 98		
	BOCA1157	100 / 97		
	BOCA1231	100 / 97		
	BOCA1233	100 / 97		
	BOCA1355	98 / 98		
Marmosops paulensis	BOCA826/MN83200	100 / 99	*Didelphis*	GTR + I + G
Monodelphis iheringi	BOCA49/MN81006	100 / 99	*Didelphis*	GTR + I + G
	BOCA92/MN81017	100 / 99		
	BOCA241/MN81046	100 / 99		
	BOCA769/MN81844	100 / 99		
	BOCA1401	96 / 98		
	BOCA1403	99 / 99		
	BOCA1479	90 / 99		
	BOCA1504	99 / 99		
	BOCA1520	99 / 98		
Monodelphis pinocchio	BOCA328/MN83182	99 / 99	*Didelphis*	GTR + I + G
Monodelphis scalops	BOCA86/MN81015	100 / 99	*Didelphis*	GTR + I + G
	BOCA234/N81040	100 / 99		
	BOCA235/MN81041	100 / 99		
	BOCA404/MN81481	100 / 99		
	BOCA587/MN81498	100 / 99		
	BOCA756/MN81840	100 / 99		
	BOCA1034	100 / 99		
Oxymycterus dasytrichus	BOCA1510	100 / 99	*Delomys*	GTR + I + G
	BOCA1500	100 / 96		
Rhipidomys itoan	BOCA832	100 / 98	*Oxymycterus*	GTR + I + G
	BOCA1171/MN83221	100 / 99		

Continue...

...Continuation

Species	Specimens number	BQC% / B ID%	Outgroups	Model
Sooretamys angouya	BOCA356/MN83184	100 / 99	*Cerradomys*	GTR + I + G
	BOCA362/MN83185	100 / 99		
	BOCA677/MN83197	96 / 99		
	BOCA841	89 / 97		
Trinomys dimidiatus	BOCA59	99 / 99.87	*Clyomys*	HKY + I + G
	BOCA94/MN81018	99 / 99.87		
	BOCA168	99 / 99.87		
	BOCA225	99 / 99.87		
	BOCA283	99 / 99.87		
	BOCA383	99 / 99.87		
	BOCA465	99 / 99.75		
	BOCA528	99 / 99.87		
	BOCA551	99 / 99.75		
	BOCA576	99 / 99.87		
	BOCA597/MN81813	99 / 99.87		
	BOCA692	99 / 99.87		
	BOCA702	99 / 99.87		
	BOCA868	99 / 99.87		
	BOCA924	99 / 99.87		
	BOCA1061	99 / 99.75		
	BOCA1069	99 / 99.87		
	BOCA1134	99 / 99.87		
	BOCA1177	99 / 99.87		
	BOCA1190	99 / 99.75		
	BOCA1211	99 / 99.87		
	BOCA1220	99 / 99.87		
	BOCA1446	99 / 99.87		
	BOCA1472B	99 / 99.87		

this study were deposited in GenBank (See Data Availability). Bayesian Inference was run in MrBayes v3.2 (Ronquist et al. 2012) over 10^6 generations, with one tree being sampled every 10^3 generations, resulting in a total of 10^3 trees. We discarded the first 10% of the samples as burn-in and obtained a consensus from the remaining trees. Only the nodes with a Posterior Probability (PP) higher than 95% were considered robust. The jModelTest 2.1.7 program (Darriba et al. 2012) was used to establish the best evolutionary model for the data, using the Bayesian Information Criterion (BIC).

4. *Statistical analysis*

Sample sufficiency was evaluated by the calculation of the expected number of species (Sest) and species richness estimated using Chao 2, an incidence-based non-parametric estimator (Colwell & Coddington 1994), using EstimateS 9.1 software (Colwell 2013). The similarity among sites in presence/absence data for non-volant small mammal species was assessed using the Sorensen index (Bray-Curtis, single link method) in a cluster analysis (Mingoti 2007). The "% Information remaining" (i.e., a rescaling of Wishart's objective function; Bakker 2023) was used for dendrogram graphical representation, in the software PCOrd 4.14 (McCune and Meford 1999).

Results

From 2013 to 2016, 32 species of non-volant small mammals (11 marsupials and 21 rodents) were recorded from 1,185 captured specimens (Table 1). Species richness ranged from 18 to 28 between sites (Site 1 = 26; Site 2 = 28; Site 3 = 18; Site 4 = 25). Ten species were exclusively captured with live traps (Sherman and Tomahawk) and 11 exclusively with pitfall traps (Table 1). The observed richness (32 species) was lower than the species richness estimated using Chao 2 (Mean ± SD = 35.06 ± 3.82) and represented 91.4% of the estimated species richness for the study area (Figure 2).

The genera with the highest relative abundance were *Euryoryzomys* (14%), *Delomys* (14%), and *Marmosops* (12%) (Figure 3). The genera with lower relative abundance (< 1%) were *Abrawayaomys*, *Blarinomys*, *Caluromys*, *Drymoreomys*, *Nectomys*, and *Phyllomys* (Figure 3). Four species are Near threatened, four are Vulnerable, and one is Extinct in the wild at regional level; and three are Data deficient and one is Near threatened at global level (Table 1). No species is threatened at national level (Table 1). In the cluster analysis comparing all sampling sites, sites 2 and 4 were the most similar to each other regarding species composition, and site 3 was the most dissimilar (Figure 4).

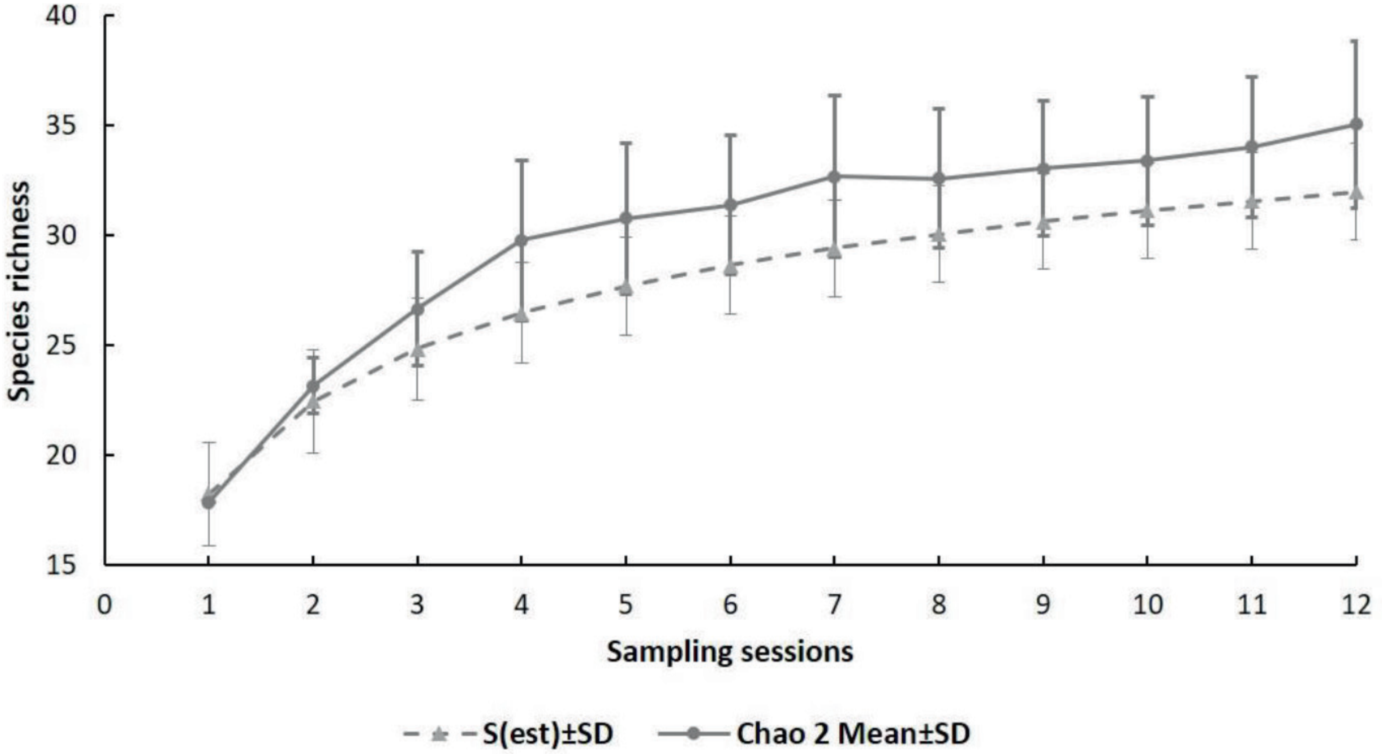

Figure 2. Expected number of species (Sest) and species richness estimated using Chao 2 (Chao 2 Mean) for non-volant small mammals (Didelphimorphia and Rodentia) recorded during twelve sampling sessions in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Bars are the standard deviation.

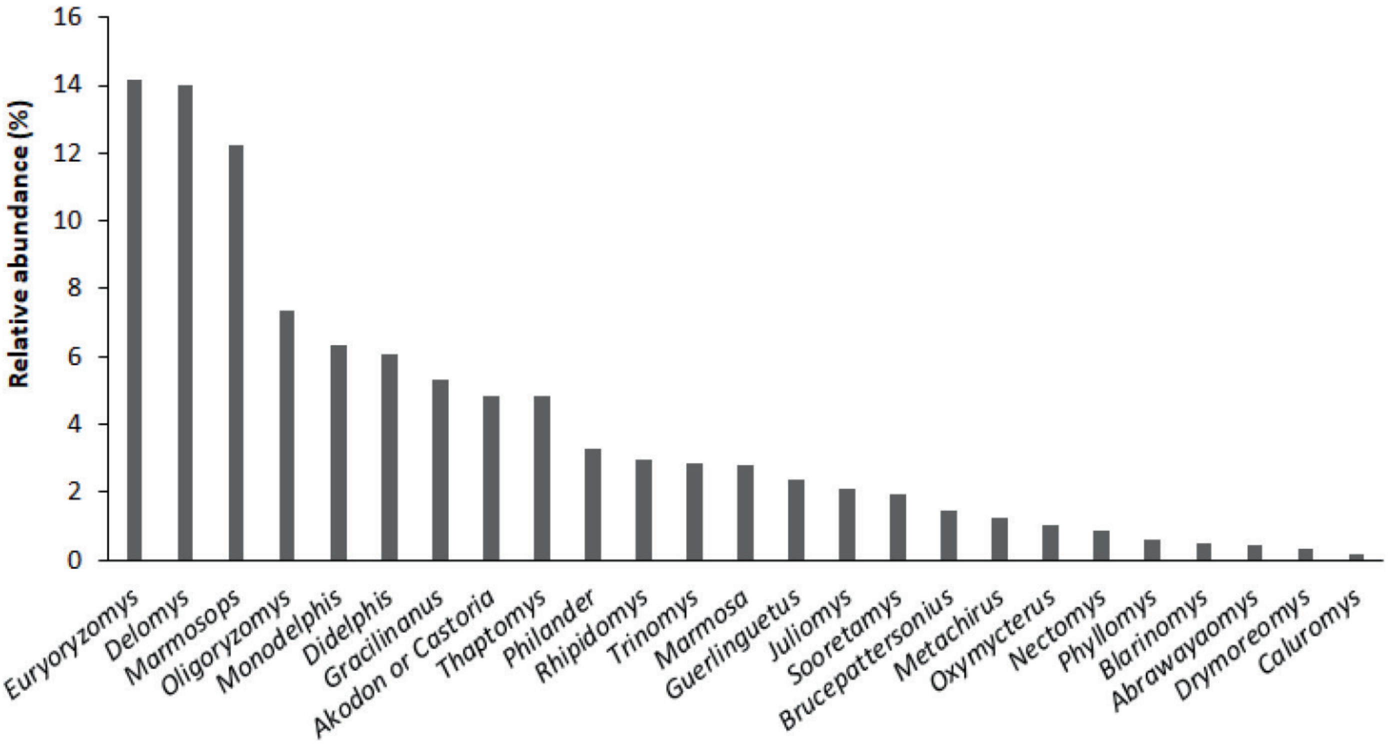

Figure 3. Relative abundance (%) for non-volant small mammals' genera (Didelphimorphia and Rodentia) recorded during twelve sampling sessions in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. See Table 1 for the list of recorded species by genus.

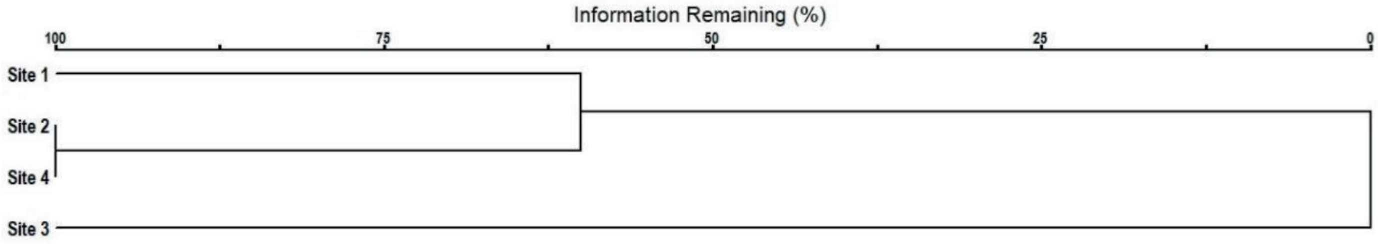

Figure 4. Cluster analysis of sampling sites (Sites 1 to 4) based on species composition of non-volant small mammals (Didelphimorphia and Rodentia) recorded during twelve sampling sessions in the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. The % information remaining is a rescaling of Wishart's objective function (Bakker 2023).

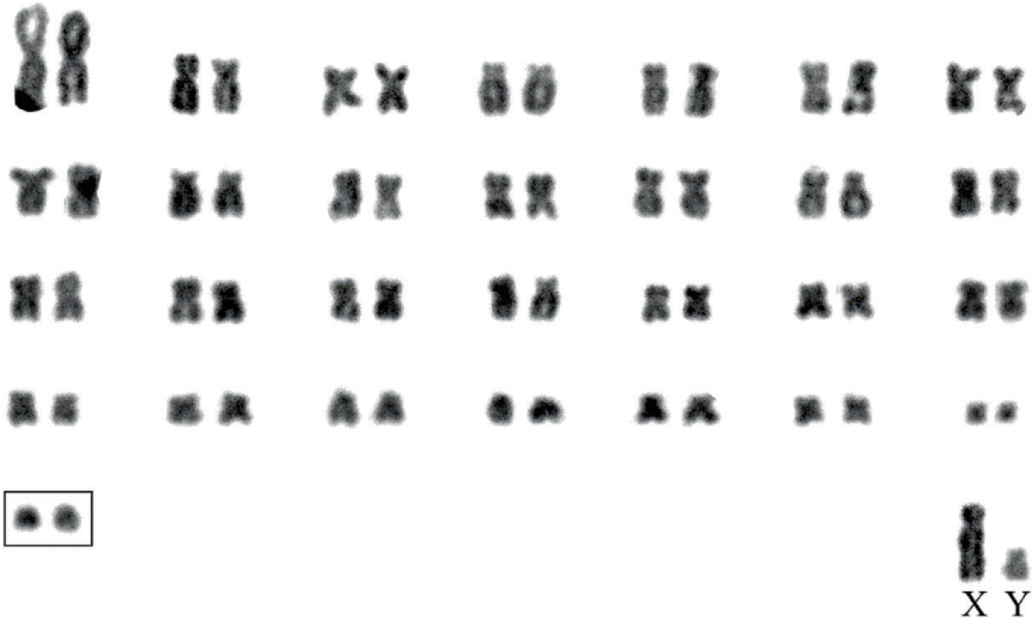

Figure 5. Karyotype (stained with conventional Giemsa) of a male of *Trinomys dimidiatus* (MN 81813) from the Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro state, Brazil. Chromosome complement with diploid number (2n) = 60 and Fundamental number of autosomes (FNa) = 114. The pair of acrocentric chromosomes is highlighted in the box.

Twenty-six specimens belonging to 14 rodent species were karyotyped (Table 2; See Data Availability). Undescribed chromosomal variation was found in *Trinomys dimidiatus* (MN 81813), that presented a distinct fundamental number of 114, composed by 28 pairs of biarmed and one pair of acrocentric chromosomes (Figure 5). The sexual pair is composed by X large submetacentric, and the Y is a small metacentric chromosome. The karyotypes of the other 13 species do not differ from the literature (Table 2).

Seventeen species were identified by molecular analysis, belonging to three families (Cricetidae, Echimiydae and Didelphidae) and 13 genera (Table 3; See Data Availability). Three genera were recorded with more than one species occurring in sympatry (*Brucepattersonius soricinus* and *B. nebulosus*; *Juliomys ossitenius* and *J. pictipes*; *Monodelphis iheringi*, *M. pinocchio* and *M. scalops*). The most common evolutionary model was General Time-Reversible (GTR + I + G) followed by Hasegawa-Kishino-Yano (HKY + I + G) (Table 3). The cytb gene was efficient in recovering the monophyly of the species and all species are formally described in the literature. Also, the blast tool of NCBI platform showed a great potential for the first screening of the analyzed specimens (Table 3).

Discussion

Thirty-two species were recorded in the present study, adding 22 species to the park's non-volant small mammals list. Most of these new records probably are due to the large sample effort carried out during a long period of time in the study area; species identification using a variety of methods, such as karyotypic and/or molecular analyses (e.g., *Juliomys* (Delciellos et al. 2020), *Phyllomys* (Delciellos et al. 2018)); or to species recently described, such as *B. nebulosus* (Abreu-Júnior & Percequillo 2019), *D. albimaculatus* (Percequillo et al. 2011), and *M. pinocchio* (Pavan 2015). Adding five species (*Akodon cursor*, *Chironectes minimus*, *Monodelphis americana*, *Oecomys catherinae*, and *Oligoryzomys flavescens*) exclusively recorded in a previous study (Delciellos et al. 2012), we obtain a list with 37 species of non-volant small mammals with confirmed occurrence in the SBNP (Table 1).

The species richness found in the SBNP (37 species) is one of the highest ever recorded for the group of non-volant small mammals in protected areas of the Atlantic Forest in Brazil, corroborating the region as a biodiversity hotspot (Dalapicolla et al. 2021, Delciellos et al. 2022). Similar species richness (37 species) was found for the Serra dos Órgãos National Park (Cronemberger et al. 2019), but in this park several areas were sampled and a higher sampling effort was carried out, including the longest small mammal monitoring study in Brazil (Gentile et al. 2023). The Bananal Ecological Station (BES) is located about 60 km from the study area in the SBNP, both protected areas being part of the same large remnant of Atlantic Forest in the Serra do Mar (Abreu-Júnior & Percequillo 2019). Thirty-two species were recorded in the BES, including rare endemic rodent species, such as *Phaenomys ferrugineus*, *Phyllomys kerri* and *Rhagomys rufescens* (Abreu-Júnior & Percequillo 2019), which were not registered within the SBNP. In other protected areas, species richness was frequently lower than that found in the SBNP, as in the Tinguá Biological Reserve (21 species; Travassos et al. 2018), Desengano State Park (21 species; Modesto et al. 2008), Morro Grande Forest Reserve (23 species; Pardini & Umetsu 2006), and Foz do Iguaçu National Park (24 species; Brocardo et al. 2019), but it is important to highlight that differences among methods used and sampling effort were not taken into account in this comparison among studies.

The use of pitfall traps in the Atlantic Forest is challenging, because of both the rough terrain with many rocks that make it difficult to install large buckets and the difficulty of keeping the animals alive once trapped in the buckets. The last situation is usually associated with a combination of low temperatures, high rainfall, and predators (Barros et al. 2015). However, the use of pitfall traps is highly recommended, as in the present study 13 out of 37 species were recorded exclusively using this method. Furthermore, in addition to capturing the most common species in the non-volant small mammals community, this sampling method was also helpful in capturing arboreal (e.g., *J. pictipes* and *J. ossitenuis*, Delciellos et al. 2020; *Phyllomys nigrispinus* and *P. sulinus*, Delciellos et al. 2018), rare (e.g., *D. albimaculatus*, Delciellos et al. 2015; *M. pinocchio*), and threatened species (e.g., *B. breviceps*, Delciellos et al. 2012).

Species richness and composition differed among the four sampling sites in the SBNP. Site 3 had the lowest species richness and it was the most dissimilar regarding species composition. The biotic and abiotic factors that cause this variation among sites were not evaluated in the present study. One of the possible explanations for the pattern we found is the large altitudinal gradient that exists in the SBNP. A large altitudinal gradient can be associated with a great variability in topography (Eisenlohr et al. 2013), which in turn can promote habitat heterogeneity (Rodrigues et al. 2020) and species diversity (Rodrigues et al. 2019). Topography (i.e., surface roughness) can also promote a higher species richness by providing a higher area availability and favoring speciation by restricting dispersal of individuals (Janzen 1967, Johnson et al. 2003, Delciellos et al. 2022). In the Atlantic Forest, a positive relationship between topography and species richness was found for tetrapods (Figueiredo et al. 2021) and marsupials (Delciellos et al. 2022).

Our study carried out at the SBNP revealed one of the highest diversities of non-volant small mammals ever recorded in the Atlantic Forest. If we add to the SBNP species list the species recorded exclusively in the BES, we obtain a surprisingly even higher species richness (42 species) for Serra da Bocaina region. However, despite being a center of endemism and a diversity hotspot for non-volant small mammals (Dalapicolla et al. 2021, Delciellos et al. 2022), the area of the SBNP located in the municipality of Paraty has been suffering with at least two main anthropic pressures in the last decade that are clearly identifiable. The first is an irregular and diffuse anthropic expansion in the park's surroundings in the municipality of Paraty, quantified by loss of forest cover and an increase in built-up areas or pasturelands (Welerson et al. 2021). The second is the paving of the RJ-165 road that crosses the SBNP, which provided increased traffic and vehicle speed, as well as easier access for humans and domestic animals to the park, and an increase in the number of wild animals being run over (Rodrigues 2020, Aguieiras 2021). The impact of these factors specifically on non-volant small mammals remains to be evaluated in future studies.

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Author Contributions

Ana Cláudia Delciellos: Substantial contribution in the concept and design of the study. Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Marcia Aguieiras: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Roger Rodrigues Guimarães: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Ana Carolina Loss: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

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Bruno Henrique de Castro Evaldt: Contribution to data collection, and data analysis and interpretation.

Marcelo de Assis Passos Oliveira: Contribution to data collection, data analysis and interpretation, and manuscript preparation.

Lena Geise: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision, adding intellectual content.

Oscar Rocha-Barbosa: Substantial contribution in the concept and design of the study.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

Specimens were handled following protocols approved by the American Society of Mammalogists (Sikes & Animal Care and Use Committee of the American Society of Mammalogists 2016); and captured and collected following the permissions of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA/MMA process 02001.003937/2008-18, authorizations 248/2013 and 610/2015).

Data Availability

Delciellos, Ana Cláudia, 2023, “Replication Data for: Non-volant small mammals of the Serra da Bocaina National Park, southeastern Brazil: an updated species list with new data on karyotype description and phylogeny”, https://doi.org/10.48331/scielodata.NPBXGK, SciELO Data, DRAFT VERSION, UNF:6:vFcDoxWDHKr71+QStMU7jQ== [fileUNF]

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Inventory

Medium to large-sized mammals of an urban protected area and its ecological corridor in Rio de Janeiro City, Brazil

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Abstract: Protected areas play a fundamental role in the preservation of species, environmental education, and ecosystem services maintenance. Most of the world population live in urban areas, which highlight the importance of preserved areas in urban centers. However, many urban protected areas in Brazil lack species surveys, which limits biodiversity conservation and implementation of management plans. In this study, we surveyed the species of medium and large mammals of Chico Mendes Municipal Natural Park (MNP) and Canal das Taxas corridor through camera traps, thus providing the first comprehensive *in situ* survey of terrestrial mammals for the area. We also verified whether there was mammalian fauna exchange between the Chico Mendes and Marapendi MNPs through the vegetation corridor formed by the Canal das Taxas. Between November 2020 and July 2021, with a sampling effort of 1,334 trap-days, we recorded five native and five exotic species in the studied areas, including one Vulnerable species in the Municipality and State of Rio de Janeiro (*Cuniculus paca*). We also confirmed that Canal das Taxas works as a wildlife corridor for native species. Finally, we highlight that species checklists based on secondary information for the region are of limited usefulness and recommend *in situ* surveys even in small, urban protected areas of Rio de Janeiro city.

Keywords: *Camera trap; conservation unit; ecological corridor; terrestrial mammals; urban areas.*

Mamíferos de médio a grande porte de uma Unidade de Conservação urbana e seu corredor ecológico na cidade do Rio de Janeiro, Brasil

Resumo: As áreas protegidas têm papel fundamental na preservação das espécies, na educação ambiental e na manutenção dos serviços ecossistêmicos. A maior parte da população mundial vive em áreas urbanas, o que evidencia a importância das áreas preservadas nos centros urbanos. No entanto, muitas áreas protegidas urbanas no Brasil carecem de levantamentos de espécies, o que limita a conservação da biodiversidade e a implementação de planos de manejo. Neste estudo, inventariamos as espécies de mamíferos de médio e grande porte do Parque Natural Municipal Chico Mendes e do corredor do Canal das Taxas por meio de armadilhas fotográficas, fornecendo assim o primeiro levantamento *in situ* abrangente de mamíferos terrestres para a área. Também verificamos se houve intercâmbio de fauna de mamíferos entre os parques Chico Mendes e Marapendi através do Canal das Taxas. Entre novembro de 2020 e julho de 2021, com um esforço amostral de 1.334 armadilhas-dia, registramos cinco espécies nativas e cinco espécies exóticas nas áreas de estudo, incluindo uma espécie Vulnerável no município e estado do Rio de Janeiro (*Cuniculus paca*). Confirmamos que o Canal das Taxas funciona como um corredor de fauna para espécies nativas. Por fim, ressaltamos que listas de espécies baseadas em informações secundárias para a região são de utilidade limitada e recomendamos levantamentos *in situ*, mesmo em pequenas Unidades de Conservação urbanas da cidade do Rio de Janeiro.

Palavras-chave: *Armadilha fotográfica; Unidade de Conservação; corredor ecológico; mamíferos terrestres; áreas urbanas.*

Introduction

Protected areas play a fundamental role in the preservation of species, since they promote the protection of endangered species, the preservation and restoration of biodiversity, environmental education, and ecosystem services maintenance (Henry-Silva 2005, Hummel et al. 2019, Kabisch et al. 2017). However, in Brazil, basic information on the biodiversity of these parks, such as from fauna species surveys, is often lacking, which limits the proper formulation and implementation of management strategies. In fact, many protected areas do not have management plans proposed or implemented in the country (Barros & Leuzinger 2019). For example, in the state of Rio de Janeiro, 83.4% of protected areas – called conservation units – still did not have published management plans in 2019 (CNUC 2023), and in the city of Rio de Janeiro, only 27% of conservation units have these plans (De Mattos Bezerra & Lira 2020). Moreover, several of these protected areas have species checklists based on secondary information, without adequate *in situ* surveys.

Currently, 55% of the world's population lives in urban areas, and this proportion is expected to increase to 70% by 2050 (United Nations 2019). Protected areas located near or within large urban centers may be more susceptible to certain anthropic impacts (Mcdonald et al. 2008, Filho et al. 2017), such as high number of visitors, hunting and fishing, pollution, and introduction of exotic species (SMAC 2016, Gibaldi 2019, Gibaldi et al. 2020, Pacheco et al. 2020). On the other side, they may offer a good opportunity to implement environmental education projects due to the large number of visitors. The city of Rio de Janeiro has 59 protected areas (CNUC 2023). Among them is the Chico Mendes Municipal Natural Park (Chico Mendes MNP), a small protected area inserted in the urbanized landscape of the Recreio dos Bandeirantes district that was created with the goal of preserving, protecting, and restoring the area's landscape heritage, the coastal sand dune (restinga) ecosystem, and the Lagoinha lagoon, as well as providing green spaces for leisure (FUNBIO et al. 2014). However, this park faces problems such as water pollution by sewage and solid waste, presence of exotic species, loss of the restinga ecosystem, impact on the trails generated by excessive visitors, among others (FUNBIO et al. 2014).

Knowledge about the mammals that inhabit the area of Chico Mendes MNP is incipient (FUNBIO et al. 2014). The management plan of Chico Mendes MNP was published nine years ago and was based on a three-day primary data survey, being mainly complemented by potential species occurrences based the scientific literature (FUNBIO et al. 2014). Therefore, *in situ* sampling is necessary to adequately characterize the biodiversity of the park, including their mammalian fauna. Furthermore, there may be fauna exchange between the Chico Mendes and Marapendi MNPs through a vegetation corridor that connects both parks, so sampling such corridor (Canal das Taxas) can help define whether it functions as an ecological corridor between the two parks. Ecological corridors are one of the main processes for forest defragmentation, thus being important to preserve biodiversity (Seoane et al. 2010). In small protected areas, the connection promoted by ecological corridors with other areas of natural habitat can be essential to the long-term viability of some populations, especially for species of medium to large size.

The survey of the mammal fauna of Chico Mendes MNP and Canal das Taxas corridor is also important to identify the presence of exotic species that may become or are invasive alien species (IAS), i.e., species that have been introduced – accidentally or intentionally – outside their natural range and that pose a risk to biodiversity conservation (Zalba & Ziller 2007); IAS can cause several types of impacts on native species and ecosystems, such as predation and herbivory of native fauna and flora; competition with and exclusion of native species; changes in the original habitats; physical environment and ecosystem processes; and hybridization with native species, among other impacts (Sampaio and Schmidt 2013).

In this context, the objective of this study was to survey the species of medium and large mammals of Chico Mendes MNP and Canal das Taxas corridor and identify the potential fauna exchange between the Chico Mendes and Marapendi MNPs through Canal das Taxas, thus contributing to the update of the management plan of the protected areas.

Material and Methods

1. Study area

Chico Mendes Municipal Natural Park (23°01'23.3"S; 43°28'15.2"W) has 40.65 hectares and was created in 1989 by Municipal Decree 8.452. It has areas of restinga vegetation, shrubs and closed tree formations and flooded forests, serving as habitat for several endemic and endangered species (FUNBIO et al. 2014). A water body (Lagoinha) occupies much of its area (Figure 1) and, in the rainy season, its volume increases via flooding additional land. Chico Mendes MNP faces problems such as pollution of the water body, presence of exotic species, human invasions, and impacts on the trails generated by visitors (FUNBIO et al. 2014).

Chico Mendes MNP is connected to Marapendi MNP (23°01'1.58" S; 43°26'34.59" W) by Canal das Taxas, which connects Lagoinha and Marapendi lagoons in Marapendi MNP (Figure 1). The Canal is approximately 1.5 kilometers long and is bordered by vegetation that extends no more than 50 meters from the edges. Canal das Taxas is included in the Green Corridor project, an initiative of the municipal, state and federal governments that involves actions to replant native species, improve basic sanitation, and provide environmental education (SMAC 2015). However, the importance of Canal das Taxas as an ecological corridor has not been properly evaluated yet in relation to the mammal fauna.

The Chico Mendes MNP, Canal das Taxas, and the Marapendi MNP, along with the Barra da Tijuca MNP, Nelson Mandela MNP and Marapendi Environmental Protection Area form a complex of 359 hectares of protected areas inserted in the urban area. Marapendi MNP (23°01'01.8 "S 43°26'58.6 "W) covers 152 hectares, with predominantly restinga forest and mangrove vegetation bordering Marapendi Lagoon (SMAC 2016). It is negatively impacted by solid waste, the presence of exotic species, and human occupation. Marapendi Lagoon is also impacted by raw sewage discharge and by unregulated fishing and water transport (Gibaldi 2019). The Marapendi MNP headquartes is nearby Canal das Taxas and this area is also fenced, like the Chico Mendes MNP. However, other areas of the park are relatively less protected and apparently are more impacted by anthropic activities (Gibaldi 2019).

2. Data collection

Between November 2020 and July 2021, nine Scoutguard ® SG560C White LED camera traps were arranged to cover most of Chico Mendes

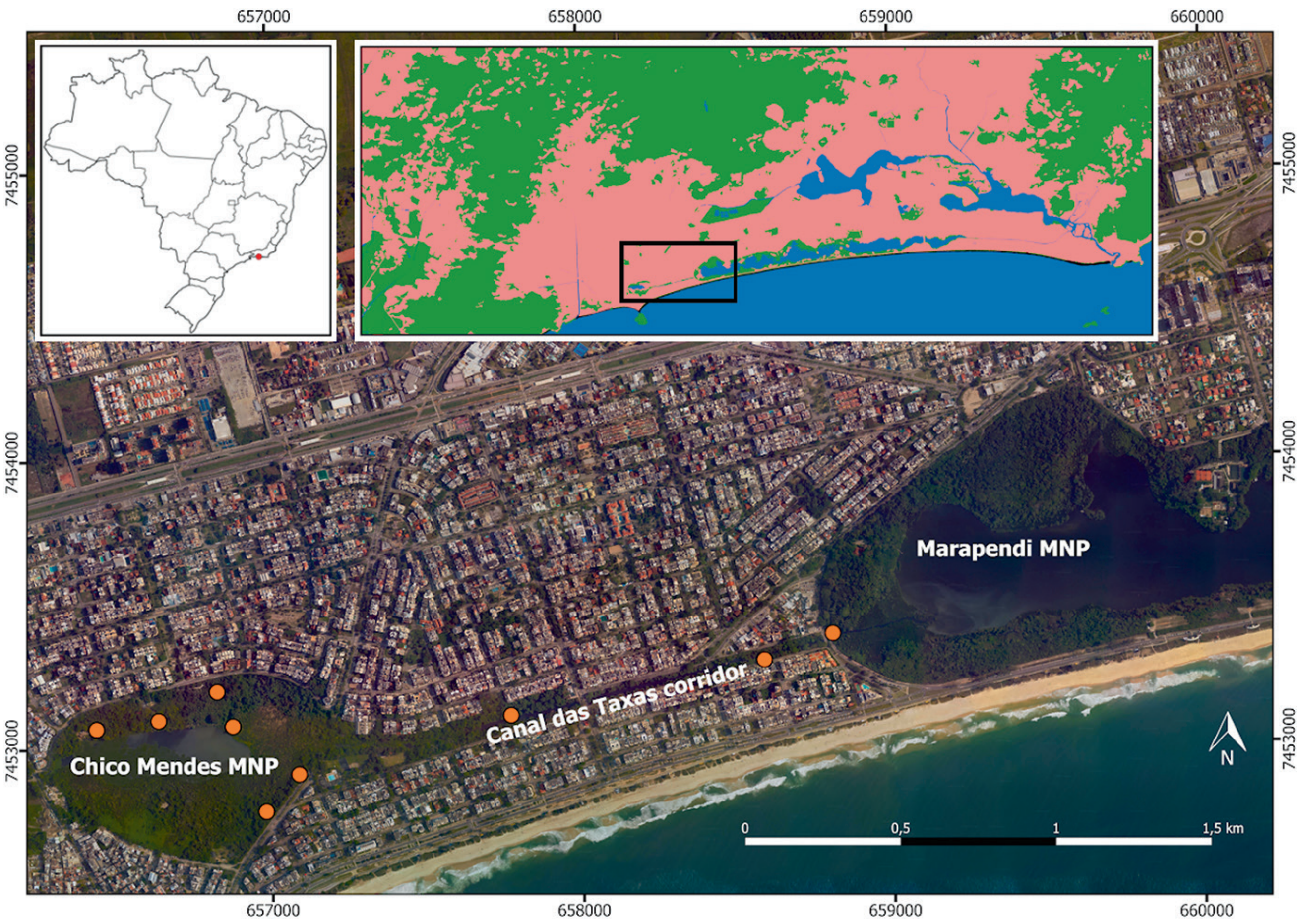

Figure 1. Map showing camera traps (yellow marks) in Chico Mendes Municipal Natural Park (Chico Mendes MNP), Canal das Taxas corridor, and Marapendi Municipal Natural Park (Marapendi MNP). The map at the upper, left side of the figure shows the location of the study area in Brazil. The map at the upper, central part of the figure shows the overall location of the study area on a regional scale; green color is vegetated areas, pink is urbanized areas and blue is water bodies.

MNP, as well as the Canal das Taxas and the border of Marapendi MNP (Figure 1). The minimum spacing between traps was approximately 150 meters. Two of the traps were set along Canal das Taxas and one trap was set at the border of Marapendi MNP and next to Canal das Taxas, to verify the potential interchange of mammals between Chico Mendes and Marapendi MNPs (Figure 1).

The cameras were inspected every month to change batteries and memory cards. The traps were baited with a small quantity of banana, bacon, and peanut meal on the first day of trapping only. Baits were renewed whenever the traps' batteries were checked (roughly once a month). The procedures were carried out according to authorization from the Municipal Environmental Secretariat, permit number 10/2020, process number 14/000.632/2020.

3. *Data analysis*

To verify the sampling sufficiency of the survey for Canal das Taxas and Chico Mendes MNP, the species accumulation curve for medium to large-sized mammals was calculated using the first-order jackknife richness estimator and its standard deviation ("Jack 1 Mean" and "Jack 1 SD") and 1000 randomizations, using the EstimateS program version 9.1 (Colwell 2016). The curve was generated based on the frequency of species' photographic records, considering each field excursion (month) as a sampling unit. Records of medium to large-sized exotic species (*Felis catus*, the domestic cat; see Table 1) and the black-eared opossum (*Didelphis aurita*) were included.

The relative frequency of species occurrence was based on independent photographic records of species: consecutive pictures of the same species within a one-hour interval were disregarded to minimize pseudo-replication in the analyses (Hurlbert 1984). Because distance between cameras was small and individuals might have been photographed in more than a camera within one hour, we considered photographic records as if they had been obtained by a single camera, thereby not discriminating between cameras in distinct places. For the relative frequency of occurrence, records of non-volant, smaller species (rodents, marsupials and primates) were also considered, although the methodology employed was not the most appropriate for small mammals or arboreal species. Finally, the camera-trap on Marapendi MNPs was not considered in these analyses, since data of this camera-trap was used only to verify potential interchange between parks through the Canal das Taxas.

Results and Discussion

With a total sampling effort of 1,334 trap-days, ten species of mammals were recorded in Chico Mendes MNP, Canal das Taxas and/or Marapendi MNP, distributed in nine families and five orders

(Table 1). The species accumulation curve for Chico Mendes MNP and Canal das Taxas stabilized on the seventh fieldwork (Figure 2), showing that sampling effort was adequate for terrestrial medium to large-sized mammals.

Five exotic species were recorded: *Callithrix jacchus*, *Rattus rattus*, *Felis catus, R. norvergicus*, and *Canis lupus familiaris*. The first three occurred in all the areas sampled, whereas *R. norvergicus* was recorded in Canal das Taxas only and *C. familiaris* in the Marapendi MNP camera trap, next to the Canal das Taxas (Table 1; Figure 1). In addition, human presence was also detected at one point in Chico Mendes MNP where access by people was temporarily prohibited. Except for *Cuniculus paca*, all species recorded were mentioned in the Chico Mendes MNP management plan as species with "probable occurrence" in the area, since that previous survey was largely based on potential records instead of *in situ* observations (FUNBIO et al. 2014). *Cuniculus paca* is classified as Vulnerable both in the state of Rio de Janeiro and city of Rio de Janeiro (Tanizaki-Fonseca et al. 2000, SMAC 2022), although all the species are of Least Concern according to the IUCN RedList (IUCN, 2022).

Three native species of medium-sized mammals that were listed in the management plan as "likely to occur" were not recorded in the present study: the spiny tree porcupine (*Coendou spinosus*), brown-throated sloth (*Bradypus variegatus*), and agouti (*Dasyprocta leporina*) (FUNBIO et al. 2014). The two first species are arboreal and commonly found in parks within the urban area of Rio de Janeiro and were seen at Chico Mendes by park employees in the last ten years (Fernanda G.M.P Lima, Pers. Comm). They may occur in the park even though we did not record them in the camera traps because the method used was not adequate to sample arboreal species. The agouti has also been seen by park employees (Fernanda G.M.P Lima, Pers. Comm) although we did not record this species in camera traps, even having applied a large sampling effort. Since rescued agoutis have been translocated and released in the Marapendi MNP (Fernanda G.M.P Lima, Pers. Comm), such visual record by employees might be of one of the translocated individuals that reached MNP Chico Mendes through the Canal das Taxas. Under this scenario, an established population of agoutis might not occur in the area. In addition, according to park employees, a crab-eating fox (*Cerdocyon thous*) has also been

Table 1. Mammals detected through camera traps in protected areas in the city of Rio de Janeiro. Site: CM = Chico Mendes Natural Municipal Park; CT = Canal das Taxas corridor; MA = Marapendi Natural Municipal Park. Data for MA came from one camera trap placed at the limits of this park with Canal das Taxas (see Figure 1).

Order	Family	Species	Common name	Site
Didelphimorphia	Didelphidae	*Didelphis aurita* Wied-Neuwied, 1826	Black-eared opossum	CM, CT, MA
Cingulata	Dasypodidae	*Dasypus novemcinctus* Linnaeus, 1758	Nine-banded armadillo	CM, CT, MA
Rodentia	Cuniculidae	*Cuniculus paca* (Linnaeus, 1766)	Paca	CM, CT, MA
	Caviidae	*Hydrochoerus hydrochaeris* (Linnaeus, 1766)	Capybara	CM, CT, MA
	Muridae	*Rattus rattus* (Linnaeus, 1758)	Black rat	CM, CT, MA
	Muridae	*Rattus norvergicus* Berkenhout, 1769	Norway rat	CT
Primates	Callitrichidae	*Callithrix jacchus* (Linnaeus, 1758)	White-tufted marmoset	CM, CT, MA
	Procyonidae	*Procyon cancrivorus* (Cuvier, 1798)	Crab-eating raccoon	MA
Carnivora	Canidae	*Canis lupus familiaris* Linnaeus, 1758	Domestic dog	MA
	Felidae	*Felis catus* Linnaeus, 1758	Domestic cat	CM, CT, MA

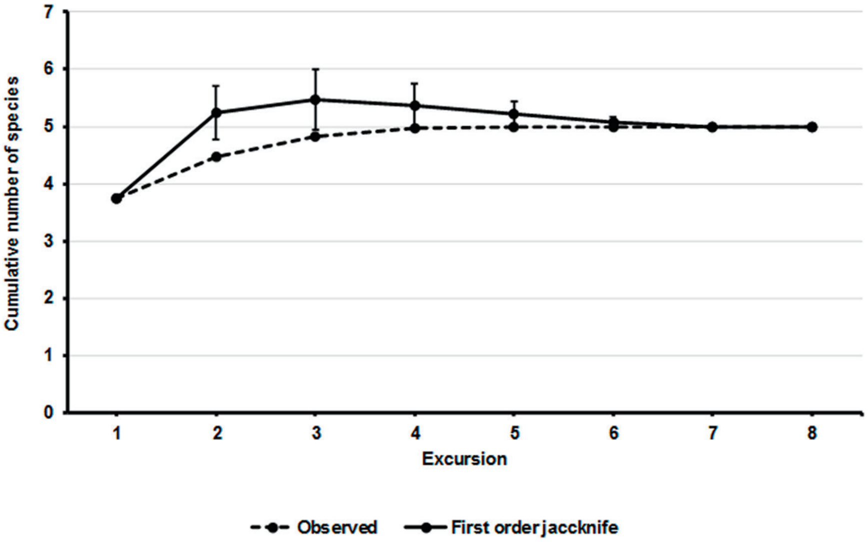

Figure 2. Species accumulation curve of terrestrial medium to large-sized mammals of Chico Mendes Municipal Natural Park and Canal das Taxas corridor, with data from camera traps set between 2020 and 2021. The richness estimator used was the first-order Jackknife. The "Observed" curve corresponds to the accumulated number of species observed in the study area. The bars correspond to the standard deviation.

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seen in the park in 2021. This is certainly the single (male) individual that was released in Marapendi MNP (Fernanda G.M.P Lima, Pers. Comm) and is using Chico Mendes MNP area as well.

The species with the highest frequency of occurrence were *D. aurita* and *H. hydrochaeris*, which occurred in both Chico Mendes and Marapendi MNPs, as well as in Canal das Taxas (Figure 3). *Didelphis aurita* is a synanthropic species that can reach high population densities even in urban areas (Gentile et al. 2018), whereas capybaras adapt well to secondary or disturbed forests, reproduce throughout the year, with litters that can reach up to eight pups (De Oliveira & Bonvicino 2006), besides being closely associated with the water bodies in both parks and triggering the camera traps more easily. The capybara and black-eared opossum are considered generalist species that are tolerant to disturbances and favored by forest fragmentation (Fonseca & Robinson 1990, Olifiers et al. 2005).

The three species of rarest occurrence were exotic species: the house cat (*Felis catus*), the white-tufted marmoset (*Callithrix jacchus*) and the Norway rat (*Rattus norvergicus*). The presence of domestic cats, stray or feral, inside a protected area can cause several negative impacts on native fauna, such as competition with other species, disease transmission, and predation (Loss & Marra 2017). Animals preyed upon by cats include birds, invertebrates, small mammals, amphibians, and reptiles (Baker et al. 2005, Mella-Méndez et al. 2022). The presence of exotic marmosets poses a risk mainly to the region's avifauna, due to the predation of bird eggs and chicks by marmosets and potential competition for resources with other species (Lyra-Neves et al. 2007). *Callithrix jacchus* and *C. penicillata* adapt easily and exhibit great ability to occupy new habitats; they are generalists, possess behavioral flexibility and high reproductive rate (Reis et al. 2008), which make them potential invaders (Traad & Weckerlin 2012). *Callithrix penicillata* (black-tufted marmoset) was also recorded as probably occurring in Chico Mendes MNP, according to the management plan (FUNBIO et al. 2014), although we could not detect it in this study. This species, as well as hybrid forms, are likely to occur in the area. The presence of *Rattus* species also deserves attention, since they are reservoirs of important diseases such as leptospirosis and are potential vectors of bubonic plague (Carter & Cordes 1980).

The management plan of Chico Mendes MNP indicates two native species that transit between Chico Mendes and Marapendi MNPs through Canal das Taxas: capybaras and brown-throated sloths (*B. variegatus*) (FUNBIO et al. 2014). With the exception of the crab-eating raccoon (*P. cancrivorus*) and the domestic dog (*C. l. familiaris*), which were recorded only in Marapendi MNP border, all other species occurred in both parks and/or in Canal das Taxas, indicating potential exchange of these species between the parks. Indeed, when we analyzed the pelage pattern of pacas recorded in the camera traps, we found the presence of the same individual in Chico Mendes MNP, Canal das Taxas and Marapendi MNP, confirming the interchange of individuals of native

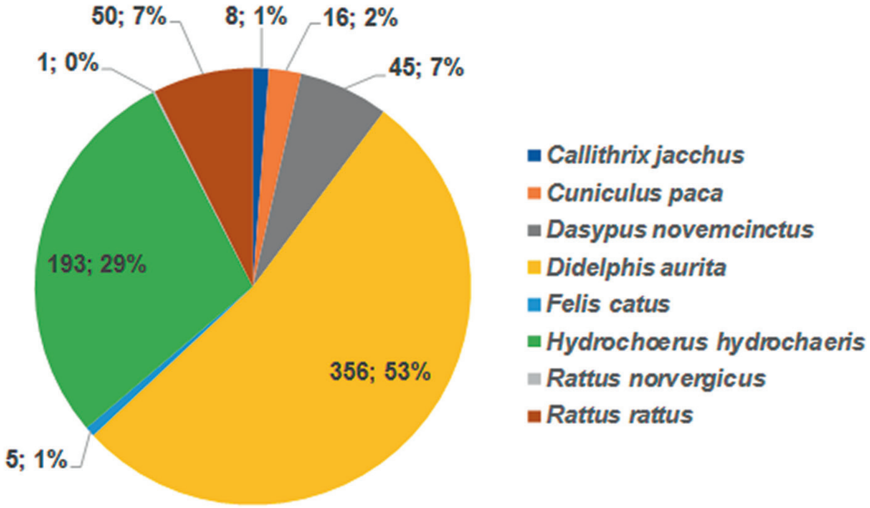

Figure 3. Number of records and relative frequency of occurrence of mammal species in Chico Mendes Natural Municipal Park and Canal das Taxas corridor using camera traps between 2020 and 2021. Records of non-volant, smaller species (rodents, marsupials and primates) were also shown. Data obtained in the single camera trap placed within the Marapendi Natural Municipal Park was not included.

Figure 4. Photographic records of the same individual of Cuniculus paca in Chico Mendes Municipal Natural Park (Chico Mendes MNP), Canal das Taxas corridor, and Marapendi Municipal Natural Park (Marapendi MNP).

mammal fauna between the parks (Figure 4). However, the presence of white-tufted marmosets, cats, and rats in Canal das Taxas is also an indication that these exotic species are using the corridor, although they also occur around the parks in unforested areas. Although the presence of the ecological corridor is important for the transit of native species between the parks, it may also facilitate the movement of exotic species. Therefore, the management of this corridor and of Chico Mendes MNP should take exotic species into account.

Although domestic dogs were not recorded in Chico Mendes MNP in this study, they were identified in the park during the survey for the management plan (FUNBIO et al. 2014). The fencing of Chico Mendes MNP is relatively intact, which may explain the absence of domestic dog records in the current study. Several researches have reported that small and medium-sized mammals are most preyed upon by domestic dogs, especially the opossum *D. aurita* (Galetti & Sazima 2006, Campos et al. 2007, Rangel & Neiva 2013, Lessa et al. 2016). Both domestic dogs and cats tend to hunt either by instinct and/or by lack of care – such as proper feeding – negatively influencing the behavior, feeding, and reproductive success of native fauna (Young et al. 2011, Silva-Rodríguez & Sieving 2011). Fencing maintenance in Chico Mendes MNP is therefore an important management action which were highlighted in the management plan (FUNBIO et al. 2014).

Finally, several municipal parks in Rio de Janeiro do not have *in situ* surveys. This study showed that a checklist based on secondary sources for the region (that is, potential occurrence of species) is misleading, at least for mammals, because it has missed important species while listed others that do not seem to occur in the park (except perhaps for occasional translocated individuals). Municipal parks that have management plans but no *in situ* mammals surveys include: Catacumba MNP (26.5 ha), which has two native species of medium to large-sized non-volant mammals that are likely to occur (SMAC 2008); Serra do Mendanha MNP (1,444.86 ha), with 21 species of probable occurrence (FUNBIO & SEA RJ 2012a); Grumari MNP (804.73 ha) and Prainha MNP (146.93 ha), which potentially have 17 species each (FUNBIO & SEA RJ 2012b); Bosque da Barra MNP (53.65 ha), with 10 species (SMAC 2014); and Paisagem Carioca MNP (159.82 ha), which has five species of probable occurrence (SMAC 2013); for all these parks, *D. aurita* was included in the species richness counting. We believe that *in situ* surveys should always be performed for the management plan of protected areas, preferable employing an array of available techniques for sampling terrestrial as well as arboreous species.

Conclusion

Chico Mendes MNP and Canal das Taxas have a mammalian fauna that is probable a subset of the species in Marapendi MNP, including with respect to exotic species. Although small, Chico Mendes MNP is mostly fenced, with public access controlled. Thus, although it does not have a very distinct mammalian fauna, Chico Mendes MNP may be helping to compose an additional and relatively more protected area – like a refuge – for native species that use the region's park complex. The finding that Canal das Taxas, in the stretch between Chico Mendes and Marapendi MNPs, might be effectively contributing to the exchange of native mammals species corroborates its importance for the long-term maintenance of these populations and underscores the need to effectively implement the Recreio Green Corridor Plan in the region. Finally, we believe that adequate, in situ surveys are a requirement for the management plan of protected areas.

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Associate Editor

Diego Astúa

Author Contributions

Beatriz Elvas: Contribution to data collection; resources; conceptualization; data analysis; writing – original draft and manuscript preparation.

Cecília Bueno: Contribution to conceptualization; resources; critical revision.

Natalie Olifiers: Contribution to conceptualization; data analysis; resources; critical revision, adding intellectual content.

Conflicts of Interest

The author(s) declare(s) that they have no conflict of interest related to the publication of this manuscript

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

Data Availability

Supporting data are available at <https://doi.org/10.48331/scielodata.OMHLOH>.

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