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Article

Stingless bees (Hymenoptera: Meliponini) of the Bela Vista Biological Refuge, Foz do Iguaçu, PR

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Abstract - We present an inventory based on 17 months of sampling at a strategic site within the Upper Paraná Atlantic Forest ecoregion, the largest among the regions of the Atlantic Forest. Including areas of 30-year-old forest restoration, our study qualitatively and quantitatively assesses the Meliponini (Apidae) species composition. Sampling involved periodical and discriminate sweep net collection in blooming plants. A total of 442 specimens from eight species and seven genera within Meliponini were recorded: *Scaptotrigona depilis* (31.4%), *Plebeia droryana* (26.7%), *Trigona spinipes* (25.8%), *Tetragonisca fiebrigi* (6.8%), *Tetragona clavipes* (4.8%), *P. nigriceps* (3.4%), *Schwarziana quadripunctata* (0.7%), and *Melipona quadrifasciata* (0.5%). The dominance of *S. depilis* suggests its potential for meliponiculture in the region. While *P. nigriceps* is established, its natural or introduced status remains unclear. The anthropized area supports the highest Meliponini diversity and holds promise for zootechnical strategies. Our findings, contextualized with previous distribution records, contribute to local conservation and restoration efforts and inform public policies regulating meliponiculture. Given our robust sampling effort, the Meliponini fauna composition observed provides a reliable diagnostic framework for the region.

Abelhas sem ferrão (Hymenoptera: Meliponini) do Refúgio Biológico Bela Vista, Foz do Iguaçu, PR

Resumo - Apresentamos um inventário baseado em 17 meses de amostragem em local estratégico da ecorregião da Floresta Atlântica do Alto Paraná, a maior entre as regiões desse bioma. Nosso estudo avalia qualitativa e quantitativamente a composição de espécies de Meliponini (Apidae) em áreas que incluem trechos de restauração florestal com mais de 30 anos. A amostragem envolveu coletas periódicas e dirigidas com rede entomológica em plantas floridas. Registramos um total de 442 espécimes pertencentes a oito espécies e sete gêneros de Meliponini: *Scaptotrigona depilis* (31,4%), *Plebeia droryana* (26,7%), *Trigona spinipes* (25,8%), *Tetragonisca fiebrigi* (6,8%), *Tetragona clavipes* (4,8%), *P. nigriceps* (3,4%), *Schwarziana quadripunctata* (0,7%) e *Melipona quadrifasciata* (0,5%). A dominância de *S. depilis* sugere seu potencial para a meliponicultura na região. Embora *P. nigriceps* esteja estabelecida, sua origem natural ou introduzida permanece incerta. A área antropizada apresentou a maior diversidade de Meliponini e potencial para estratégias zootécnicas. Nossos resultados, contextualizados com registros prévios de distribuição, contribuem para ações locais de conservação e restauração, além de subsidiar políticas públicas para a regulamentação da meliponicultura. Dado o esforço amostral empregado, a composição da fauna de Meliponini observada fornece um diagnóstico confiável para a região.

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Introduction

The recovery and management of pollinator fauna critically depend on a comprehensive understanding of local communities, particularly in the face of habitat degradation, climate change, and, in the case of stingless bees, the expanding practice of meliponiculture in Brazil (Freitas et al., 2009; Gonzalez et al., 2021; Galetto et al., 2022). This knowledge is essential to prevent the introduction of allochthonous species and the spread of exotic populations, mitigating potential ecological and productive impacts. Surveying local pollinator fauna provides a crucial foundation for conservation efforts and sustainable management, offering strategic data for decision-making (Pinheiro-Machado et al., 2002). Such initiatives address knowledge gaps in species distribution, commonly referred to as the Wallacean shortfall (Brito, 2010). Single-site inventories contribute to establishing baseline biodiversity estimates and identifying broader distribution patterns (Giles & Acher, 2006). A practical application of this knowledge is the selection of species with well-adapted germplasm for local phytophysiognomies, guiding the implementation of sustainable stingless bee farming (meliponaries) while promoting biodiversity conservation.

Despite Paraná's long-standing tradition in bee research, systematic surveys of bee fauna remain scarce in the state's western region (Pereira et al., 2021). This aligns with the pattern identified by Pinheiro-Machado et al. (2002), where most fauna surveys in Brazil result from undergraduate and master's research, explaining the limited documentation of bee fauna in the Foz do Iguaçu region.

The Upper Paraná Atlantic Forest ecoregion, the largest among the 15 ecoregions of the Atlantic Forest biome, is originally dominated by Seasonal Semideciduous Forest (Di Bitetti et al., 2003). Known for the Iguaçu National Park, the largest conservation unit for this ecoregion, it also includes the Itaipu Binacional Hydroelectric Power Plant on the Paraná River, at the Brazil-Paraguay border. Since 1979, Itaipu has conducted the world's largest reforestation program conducted by an hydroelectric company, successfully restoring over 99% of its Permanent preservation areas (Itaipu, 2015; Nickele et al., 2023).

Seizing the opportunity to address this knowledge gap, Embrapa Forestry and UNILA, in collaboration with the Itaipu Parquetec - Núcleo de Inteligência Territorial, launched a project to document the Meliponini fauna in the Itaipu dam region. This study presents a species survey, contextualizing the data from historical and geographical perspectives while correlating them with phytophysiognomies. In addition to portraying the stingless bee taxocoenosis diversity in an area of the Upper Paraná Atlantic Forest ecoregion, we also aim to provide a basis for public policies that support meliponiculture. This survey may provide useful insights for Itaipu company in order to design stingless bee conservation strategies for the Bela Vista Biological Refuge (RBV).

Material and methods

Bela Vista Biological Refuge (RBV) is located in the Foz do Iguaçu municipality, Paraná State, Southern Brazil (25°44'90″S, 54°55'42″W). An overview of the RBV boundaries with a map of the sampling areas were presented in Vicente-Ferreira et al. (2024). The region is part of an area connecting Argentina, Brazil and Paraguay. RBV is one of the ten environmental conservation areas of Itaipu Binacional, among biological reserves and refuges, in Brazil and Paraguay. It encompasses a relevant Transboundary Green Corridor where the Iguazú and Paraná rivers converge: the Upper Paraná Atlantic Forest ecoregion. Established in 1984 the RBV has an area of 1,920 ha distributed near Itaipu's Hydroelectric Power Plant Dam and Lake. The area encompasses anthropized areas, recomposition areas (some with the exotic species *Leucaena leucocephala* (Lam.) de Wit.) and areas of secondary forest (alluvial secondary forest and pioneer alluvial formations). Based on the proportionality of each phytophysiognomies in RBV we assumed 23 transects to access stingless bees biodiversity. These were distributed in the secondary forest and recomposition sectors (nine in each) and in pioneer alluvial formations (five).

The RBV has a tradition of conducting flora and fauna inventories. Consequently, it maintains permanent plots to facilitate surveys across its various phytophysiognomies. The plots are georeferenced and have been previously screened during extensive surveys, providing a wealth of data on local fauna and flora. Utilizing these areas would be advantageous for enabling further comparisons and generating reports. To capitalize on the existing permanent plots in RBV, we established transects for our survey. Nonetheless whenever possible we

complemented the search sampling also outside the transects. The searches were based on periodical and discriminate sweep net sampling in blooming plants. Transects were selected based on vegetation distribution, prioritizing areas with minimal anthropic interference and higher levels of conservation. To support this selection, we also analyzed images of natural vegetation remnants prior to the formation of Lake Itaipu and the reforestation program. The transects were distributed to homogeneously represent the following phytophysiognomies within the RBV area: a) Alluvial secondary forest; b) Pioneer alluvial formation; c) Forest recomposition and d) Secondary forest (Figure 1). Within the recomposition areas there are parcels that include forestry experiments (largely with *Leucaena*) that were not surveyed. We also didn't sample the *Leucaena* regeneration areas.

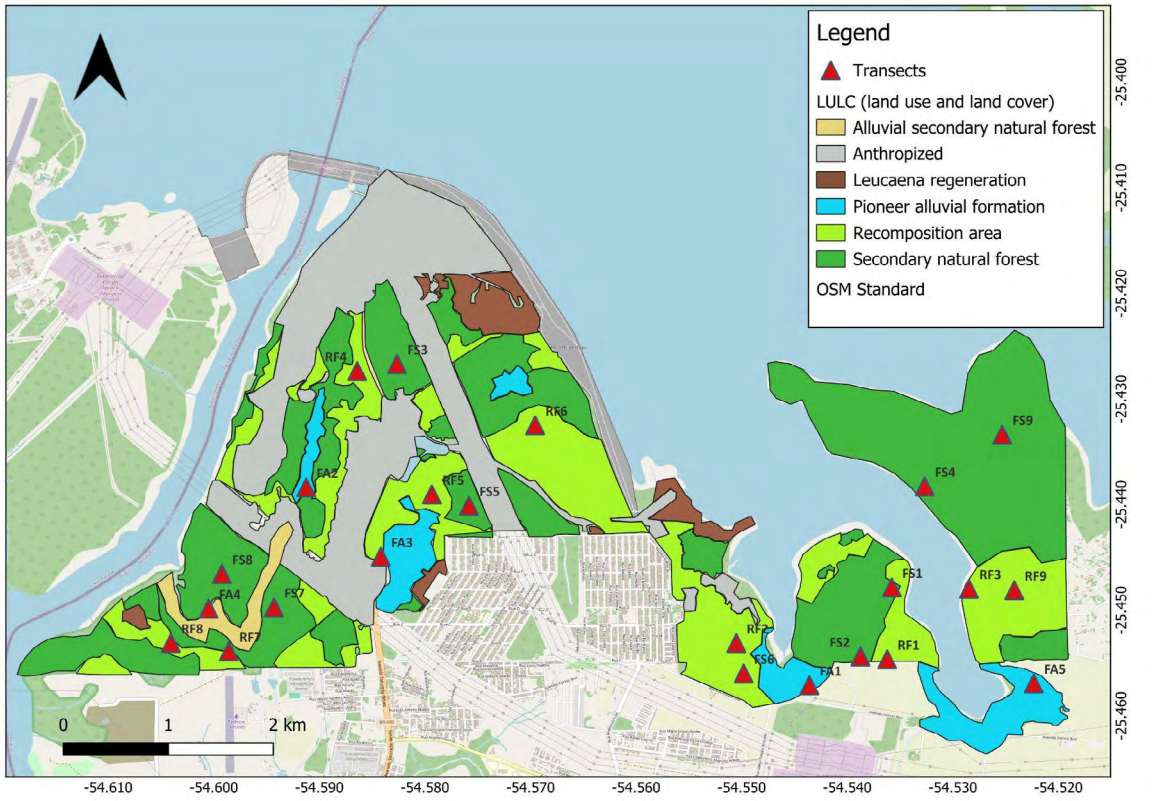

Figure 1. Bela Vista Biological Refuge land use and land cover (LULC), with the position of our transects to illustrate our sampling range over the phytophysiognomies. Transects (red triangles) are numbered followed by a code of the correspondent phytophysiognomy. (FA: Alluvial secondary forest; FS: Secondary forest; RF: Forest recomposition).
Source: Open Street Maps (2015).

Each transect comprised a non-linear length of 100 m with a sampling area 15 m wide (7.5 m on each side) within the length of the transects. We did not set a reference catch-effort time leaving the collector free to decide the convenience. Collections were conducted by at least two researchers using sweep nets on plants whenever inflorescences were present.

Sampling was performed using long sweep-net (Bioquip pole: aluminum, 5 sections of 61 cm each; hoop: 45 cm diameter; net: 1 mm white nylon mesh allowing sampling of floral resources up to 3 m in height), and a plastic lethal flask (ethyl acetate or directly in ethanol 70 °GL) to collect and kill the specimens. An entomological aspirator was required in some cases to sort specimens from the net. We used different conical centrifuge plastic tubes (50 mL) for each site identifying it with paper labels (date; coordinates; collector id; type of sample). We conducted surveys representing each season through multiple expeditions per season, starting in the summer of 2021 (December 2021) and concluding in the fall of 2023 (April) totalizing 17 months of survey.

Specimens were pin mounted and labeled to identification under stereoscope. The specimens were deposited in the Danúncia Urban Entomological collection (UNILA, Foz do Iguaçu, Brazil).

The Meliponini species distribution in Paraná State was based on Camargo et al. (2023), complemented with data from the Global Biodiversity Information Facility (GBIF) database (GBIF, 2021) and Species Link (Species Link, 2021; Canhos et al., 2022).

Besides the active sampling strategy, we also recorded any natural nest found during our inventory. For each natural nest we collected a sample of 10 specimens as ID vouchers using the same preservation methods as the ones for active sampling. The specimens that originated from nest sampling were not computed in this survey in order to allow species richness and abundance estimation under active sampling. Nonetheless we address these captures here as a way to enrich our discussion. Diversity indexes were calculated as described by Magurran (2013).

Results

We collected 442 specimens belonging to eight species distributed in seven genera within the Meliponini tribe. The representativeness of each species in total was, in decrescent order: *Scaptotrigona depilis* (Moure, 1942), *Plebeia droryana* (Friese, 1900), *Trigona spinipes* (Fabricius, 1793), *Tetragonisca fiebrigi* (Schwarz, 1938), *Tetragona clavipes* (Fabricius, 1804), *Plebeia nigriceps* (Friese, 1901) *sensu* Camargo & Moure (1988), *Schwarziana quadripunctata* (Lepeletier, 1836) and *Melipona quadrifasciata* Lepeletier, 1836.

All eight species recorded in the Bela Vista Biological Refuge (RBV) were present in the anthropized area, including three exclusive. This area also had the highest diversity indexes, that also includes dominance, besides the species richness. The Shannon evenness index was higher in the two phytophysiognomies with higher species richness, but very low in those with low species richness. Anthropized area was followed by the secondary natural forest (Table 1), while in the pioneer alluvial formations was less diverse and uniform regarding species abundance followed by the recomposition area. The species most widely distributed across the different vegetation types were the three with the highest number of individuals, each present in three distinct vegetation types (Figures 1 and 2).

Table 1. Vegetal formation and species diversity considering sweeping net sampling and respective diversity and evenness indexes.

	Anthropized	Secondary forest	Alluvial secondary forest	Forest recomposition	Specimens	Relative abundance (%)
Melipona quadrifasciata	2	0	0	0	2	0.5
Plebeia droryana	74	25	0	19	118	26.7
Plebeia nigriceps	15	0	0	0	15	3.4
Scaptotrigona depilis	120	11	8	0	139	31.4
Schwarziana quadripunctata	1	2	0	0	3	0.7
Tetragona clavipes	17	0	1	3	21	4.8
Tetragonisca fiebrigi	30	0	0	0	30	6.8
Trigona spinipes	108	5	1	0	114	25.8
Specimens number	367	43	10	22	442	
Simpson index (1-D)	0.76	0.59	0.38	0.25	0.76	
Shannon index (H)	1.57	1.06	0.64	0.40	1.57	
Evenness (J)	0.76	0.76	0.58	0.58	0.75	

The planted forest, pioneer alluvial formations and *Leucaena* regeneration areas had no captures and were removed from the table and calculations.

Besides the active sampling by the sweeping net strategy it is convenient to mention other observations based on nests located during our inventory. We found 34 natural nests and also 34 colonies were captured in trap-nests. Most of the nests observed or captured belonged to species that had already been sampled through active collection (*P. droryana*, *P. nigriceps*; *S. depilis*; *Tetragonisca fiebrigi*; *Tetragona clavipes* and *Trigona spinipes*). Exceptions that were noted as natural nests but not captured by sweeping net was *Lestrimelitta chacoana* Roig-Alsina, 2010 (Colombelli et al., 2024). The nests of this species were also mainly observed in anthropized area, near the buildings of RBV administration.

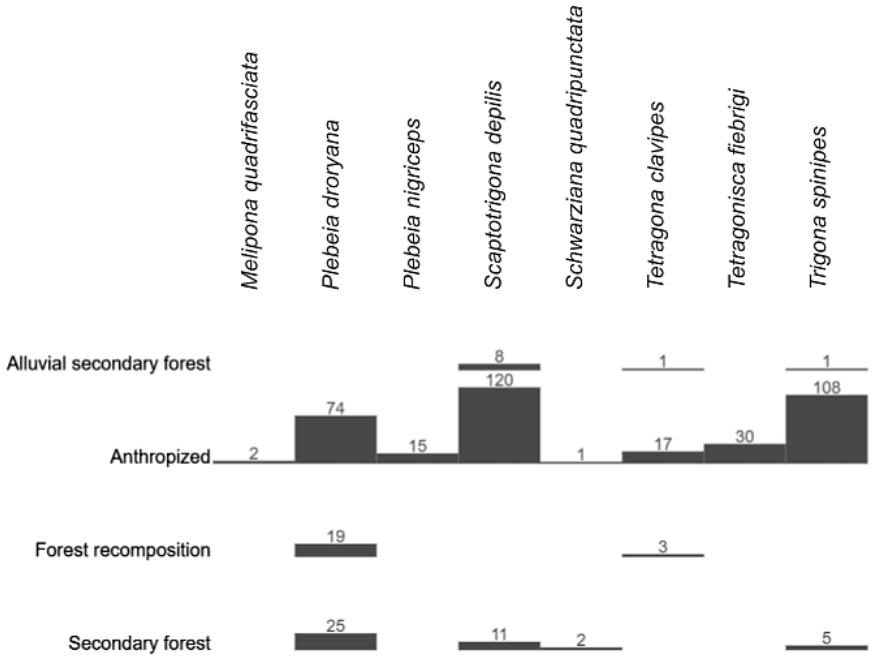

Figure 2. Diversity of Meliponini species by vegetation types. The species are listed in columns by the number of individuals captured (represented in bars), while the sampled vegetation types are displayed in rows. Pioneer alluvial formations were removed since there was no capture.

Discussion

Of the 40 stingless bee species recorded for the state of Paraná, 39 in the Moure catalogue (Camargo et al., 2023), plus *Lestrimelitta chacoana* in Melo (2023) and Colombelli et al. (2024), only nine were recorded in the Bela Vista Biological Refuge (RBV). One of these species is likely a reintroduction, as two colonies of *Melipona quadrifasciata* were present in the RBV's meliponary, having been brought from other regions, and the specimens collected visiting flowers were found in the anthropized area near the meliponary.

Several species recorded in Paraná are not expected to occur in the semideciduous Atlantic forests of western Paraná, where the RBV is located, as their distribution is likely restricted to other regions. For instance, *Melipona mondury* Smith, 1863 and *Scaptotrigona xanthotricha* Moure, 1950 are known only from the Ombrophilous Dense Atlantic Forest in eastern Paraná, while *Paratrigona subnuda* Moure, 1947 and *Plebeia saiqui* (Friese, 1900) have been recorded in Curitiba, the nearest known locality to Foz do Iguaçu (Species Link, 2021; Camargo et al., 2023;).

On the other hand, some species were likely native to the Foz do Iguaçu region but are now absent from the natural areas of the RBV. Besides *M. quadrifasciata*, *Cephalotrigona capitata* (Smith, 1854), *Melipona torrida* Friese, 1916, and

Oxytrigona tataira (Smith, 1863) can be assumed to have originally occurred in the RBV area, based on geographical records from Camargo et al. (2023) and Species Link (Species Link, 2021).

We consider it beneficial to evaluate the results through comparisons with other surveys conducted at a similar taxonomic level, focusing on specimen and species numbers. While such comparisons are not entirely comparable due to inherent differences in sampling variables, they can serve as indicators of sampling effort and provide a baseline for addressing potential discrepancies.

The first comparison examines species richness and diversity relative to the surveyed area. Felix & Freitas (2021), despite operating on a much larger sampling scale, the Ceará state, but within a similar time frame, recorded 34 species across 14 genera. Differences in species richness may be attributed to the broader sampling range and the proximity to the equator.

In the Itaipu survey, we sampled a base representing 14.9% of the total specimens collected in Ceará by Felix & Freitas (2021). Notably, our sampling area accounts for only 0.3% of the total area surveyed in Ceará, suggesting that our effort achieved a comparable level of representativeness. It is important to highlight that in the Ceará survey, only 35 samples (8.7%) out of 401 were explicitly identified as collected using sweep nets. However, the study does not specify the exact number of specimens exclusively obtained through this method. If we project adjustments based solely on sweep net sampling, our results align more closely in numerical terms, indicating a robust sampling effort for a relatively small area.

Among the eight species sampled at RBV, only two were not initially listed as likely to occur: *Plebeia nigriceps* and *M. quadripunctata*. For the remaining species, our expectations were supported by their order of abundance. Regarding the most captured species (*Scaptotrigona depilis*), it was reasonable to point it as an expected and abundant Meliponini. It was the most frequently sampled species in our study. Although this species has not been specifically reported in the region of Foz do Iguaçu and its surroundings (GBIF, 2021), its presence is not surprising, as it was previously documented in northern Misiones, Argentina, by Alvarez et al. (2018) Zamudio & Hilgert (2015) genus solely; and Alvarez (2016). The known efficient use of floral resources from Myrtaceae species (Ramalho, 1990) may indicate a well-structured availability of Myrtaceae resources in the environments where the species was most frequently sampled (anthropized area, secondary forest and alluvial secondary forest).

Although the presence of *Plebeia droryana* in the region was already known (Alvarez et al., 2018), our record is significant as it reinforces the species' distribution to the western region of Paraná State. This is particularly relevant given that Global biodiversity information facility data are predominantly concentrated in the eastern portion of Brazil (GBIF, 2021). *P. droryana* and *Tetragona clavipes* appear to have a good capacity for establishment in areas with limited resource availability, as they were the only two species collected in the forest restoration area.

Trigona spinipes is widely distributed across the Neotropical region and is considered the most geographically widespread species among Meliponini. It occurs in nearly all regions of Brazil, often in high abundance (Almeida & Laroca, 1988; Cortopassi-Laurino & Ramalho, 1988; Kleinert & Giannini, 2012). This species is generalist or polylectic, utilizing a diverse range of botanical families as food sources (Kleinert & Giannini, 2012; Moura et al., 2017). Its broader ecological niche, uniform exploitation of floral resources (Lorenzon et al., 2003), and flexibility in nesting (without dependence on tree hollows or mature forest structures) enhance its adaptability and widespread presence.

Given its foraging radius of approximately 630 m from the nest (Kerr, 1959) and the presence of two nests confirmed within the anthropized area, we expected high numbers of *T. spinipes* across all surveyed vegetation types, with significant representativity. This expectation was met in the anthropized area, where the species was abundant. However, *T. spinipes* was absent from the planted forest, pioneer alluvial formations, and forest recomposition.

This pattern suggests the absence of nests in the areas where the species was less frequently or not captured. Such findings highlight that, despite its ecological flexibility, *T. spinipes* may still depend on specific environmental conditions to establish nests, limiting its occurrence in certain vegetation types.

Tetragonisca fiebrigi was anticipated as a probable species in our dataset, as its natural occurrence has been documented in the Atlantic Forest of the Upper Paraná region (Alvarez et al., 2018). Being a species commonly found even in altered areas, its absence outside the anthropized area may indicate limitations that warrant further investigation.

Considering not only the records obtained through sweeping net captures but also those from bait nests and observations of natural nests, the sampling of *Tetragona clavipes* can be interpreted as evidence of well-established populations in the study area.

It is worth noting that *Plebeia nigriceps* (*sensu* Camargo & Moure, 1988) was already present in the surveyed area, initially observed as a single colony established in a rational hive within an incipient meliponary at the beginning of the inventory. Subsequently, four additional natural nests were identified.

Based on previous records, *Plebeia nigriceps* is considered a naturally occurring species in the Atlantic Forest of the Upper Paraná region, particularly in the western part of the state. Specimens collected in Rio Grande do Sul, as documented in GBIF (2021), support the hypothesis of a natural distribution extending to our study area and further south to neighboring states. However, an alternative interpretation exists regarding the identification of these specimens from Rio Grande do Sul. Roig-Alsina & Alvarez (2017) identified them as *Plebeia merinoides*, reporting its widespread occurrence in Missiones, Argentina, including Iguazú National Park. Whether representing a natural occurrence or a possible introduction, our record is significant as it reinforces the taxonomic identity of *Plebeia nigriceps*.

Schwarziana quadripunctata had no previous records in Foz do Iguaçu or nearby areas in GBIF (2021), although it had been found in northern Misiones, Argentina (Alvarez et al., 2018), including an occurrence in Iguazú National Park.

Melipona quadrifasciata, a species naturally occurring in the tri-border region, was recorded with only two specimens, both captured within the anthropized area, close to the RBV headquarters. Near this area there is a meliponary that had 13 nests of this species, acquired from Prudentopolis (25°12′46″ S, 50°58′40″ W) and Medianeira (25°17′42″ S, 54°05′38″ W) and introduced in the RBV area. Notably, the species was not found in areas of natural vegetation. Historical records for this species in the Misiones region, reported by Alvarez (2016), are particularly insightful. These records date back to the early 20th century, with a few extending until the 1960s. More recently, Alvarez et al. (2018) reported the species in only one location in Misiones, where individuals were collected from managed hives, while it was absent from samples collected in Iguazú National Park. Given that the species is commonly found in meliponaries within the region, the scenario presented by those authors aligns with our findings and supports the hypothesis of a significant population decline followed by potential reintroductions, likely driven by the increasing popularity of this species in meliponiculture practices. Considering discussions regarding the impact of meliponiculture on natural populations, these data highlight contributions of beekeeping activities that extend beyond their productive aspect. They provide direct benefits to producers, enhance environmental services, and offer an often-overlooked outcome: the conservation of species in natural areas affected by human intervention.

The presence of *Lestrimelitta chacoana* aligns with the diversity of other species that provide substantial resources for this cleptobiotic species (Collombeli et al., 2024). It is natural that the species was not represented on inflorescences under the sweeping net strategy. This highlights the importance of complementary sampling methods (detailed in Collombeli et al., 2024), such as natural nest observations, to ensure more comprehensive species detection.

Several studies have suggested that urbanization exerts a positive effect on abundance-based diversity indices while simultaneously reducing the richness of threatened species (e.g., Fortel et al., 2014; Fauviau et al., 2024). In this context, our findings reinforce the observation of higher species richness in the anthropized area, characterized by a higher Shannon index and a lower Simpson index. This pattern is likely driven by factors previously identified as critical for bee diversity, such as gardens, which typically provide abundant floral resources year-round, and the high diversity of land-cover types. Conversely, the recomposition area exhibited the lowest diversity indices, suggesting that the ongoing restoration process has not yet mitigated the environmental impacts resulting from prior disturbances.

Conclusions

Since we achieved a sampling base that is at least equivalent to, if not superior to, other surveys, the results regarding the Meliponini fauna composition (both in terms of species found and those not found) can be considered a reliable diagnosis.

The high abundance of *Scaptotrigona depilis* in the surveyed area aligns with its increasing prominence in meliponiculture practices. This suggests that the

species holds significant potential as one of the most suitable options for meliponiculture development in the Foz do Iguaçu region.

The resource limitations in the pioneer alluvial formations and forest recomposition areas are evident based on the distribution patterns of various species with well-documented niche plasticity and biological adaptability, particularly *Trigona spinipes* and *S. depilis*.

Although it is evident that *Plebeia nigriceps* is established in the area, it remains uncertain whether it occurs naturally or was reintroduced. For *Melipona quadrifasciata* the few specimens captured are suggestive of reintroduction.

The anthropized area harbors the highest diversity of Meliponini and shows promise for supporting zootechnical strategies, such as breeding, to aid in the restoration and conservation of the most representative local bee diversity.

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Conflict of interest

All authors declare that they have no conflict of interest.

Authors' Contributions

Guilherme Schnell e Schühli: conceptualization, formal analysis, investigation, methodology, supervision, writing original draft, writing review & editing. **Julia Colombelli Agostini**: investigation and methodology. **Yoan Delky Ibanez Ojeda**: investigation. **Fernando César Vieira Zanella**: conceptualization, formal analysis, investigation, methodology, supervision, writing review & editing.

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