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ABSTRACT

Pollen loads collected by *Melipona quadrifasciata anthidioides* were examined in order to detect the botanical origin of pollen and to recognise the pollination action of these bees in an urban Atlantic secondary forest. Pollen analysis followed standard methodology and without the use of acetolysis. The results revealed a great contribution of monofloral (more than 90% of a single pollen type or more than 60% if no accessory pollen was present). Quantitatively, Myrtaceae pollen samples followed Melastomataceae ones. Bifloral or heterofloral samples combine several pollen types. Besides *Myrcia* and *Eucalyptus*, the most frequent pollen types were *Solanum*, *Mimosa caesalpiniifolia* and *Alchornea*. The current study was related to the apiaries' surrounding vegetation and reflected both the resources available and the preference for native plant species by the bees. Furthermore, these results are relevant to the management of primary and secondary forests in order to preserve the environments.

KEYWORDS

Native bee; pollen loads; pollination; Atlantic forest; Rio de Janeiro

1. Introduction

The Atlantic forest biome has great plant diversity and contains many endemic species (Leão et al. 2014). This biodiversity leads to an increasing level of specialisation and interdependence between plants and animals, causing changes in a population and even extinctions. This biome in Brazil comprises an area of 1,500,000 km^2 of original forest, equivalent to approximately 12% of the national territory (Ribeiro et al. 2009), and approximately 5% is preserved.

Most tropical plant species need to be pollinated by animals. Bees (Hymenoptera, Apoidea) have a close relationship with flowers, which may be based on the exchange of rewards by the supply of nectar, pollen, oils and fragrances to the bees (Betts et al. 2015; Russel et al. 2015; Kormann et al. 2016) and both plant pollination and reproduction. Social stingless bees (Apidae, Meliponini) are important pollinators of many plant species due to their feeding habits and behaviour of foraging, which is an important factor for the maintenance of biodiversity and the dynamics of tropical communities (Pacheco Filho et al. 2015), and these bees contribute to the balance of flora and fauna populations living in natural ecosystems.

Melipona quadrifasciata Lepeletier 1836 is a stingless bee species occurring along the Brazilian east coast from southern Rio Grande do Sul to northern Paraíba State (Moure & Kerr 1950). This vigorous Brazilian bee reaches the canopy of the trees (Ramalho 2004) for foraging activities as the pollen grain productivity decreases. Traditionally, two distinct subspecies are recognised (Schwartz 1932). *Melipona quadrifasciata quadrifasciata* occurs mainly in the southern range and in the seasonal Brazilian Cerrado (savanna) vegetation, and *M. quadrifasciata anthidioides* is present in the warmer northern regions of the Atlantic rainforest; the two exhibit similar foraging behaviour (Waldschmidt et al. 2000; Batalha-Filho et al. 2009). Their occurrence in such distinct biomes reveals a successful history of survival and reproduction under diverse climatic and vegetation conditions compared to those of other species of *Melipona*. The occupation of mesic environments, covered by humid forests, can be regarded as a derived feature exhibited by this species (Silveira et al. 2002; Camargo & Pedro 2007).

Melissopalynological data from *M. quadrifasciata anthidioides* are scarce in Brazil. However, some investigations occurred in south-eastern Brazilian in an urban fragment forest located in Minas Gerais State (Antonini et al. 2006b), and in a preserved natural park in both Minas Gerais State (Antonini et al. 2006a) and São Paulo State (Oliveira-Abreu et al. 2014). However, palynological data were also observed in a 'caatinga' vegetation located in Bahia State, in the north-eastern region of Brazil (Oliveira et al. 2016).

The main goal of the present study was to analyse the pollination activity used by *M. quadrifasciata anthidioides* in a secondary urban forest, located inside the Rio de Janeiro city, through pollen grain loads analysis and to select suitable species for reforestation.

1.1. Study area

The Parque Nacional da Tijuca – PNT (Tijuca Nacional Park) is located in the municipality of Rio de Janeiro between the coordinates 22°55′–23°00′S and 43°11′–43°19′W. This area comprises 3972 ha, with a perimeter of approximately 88 km. The park extends for nearly 12,000 ha in a planimetric area that increases to approximately 14,000 ha of true surface, and elevations range

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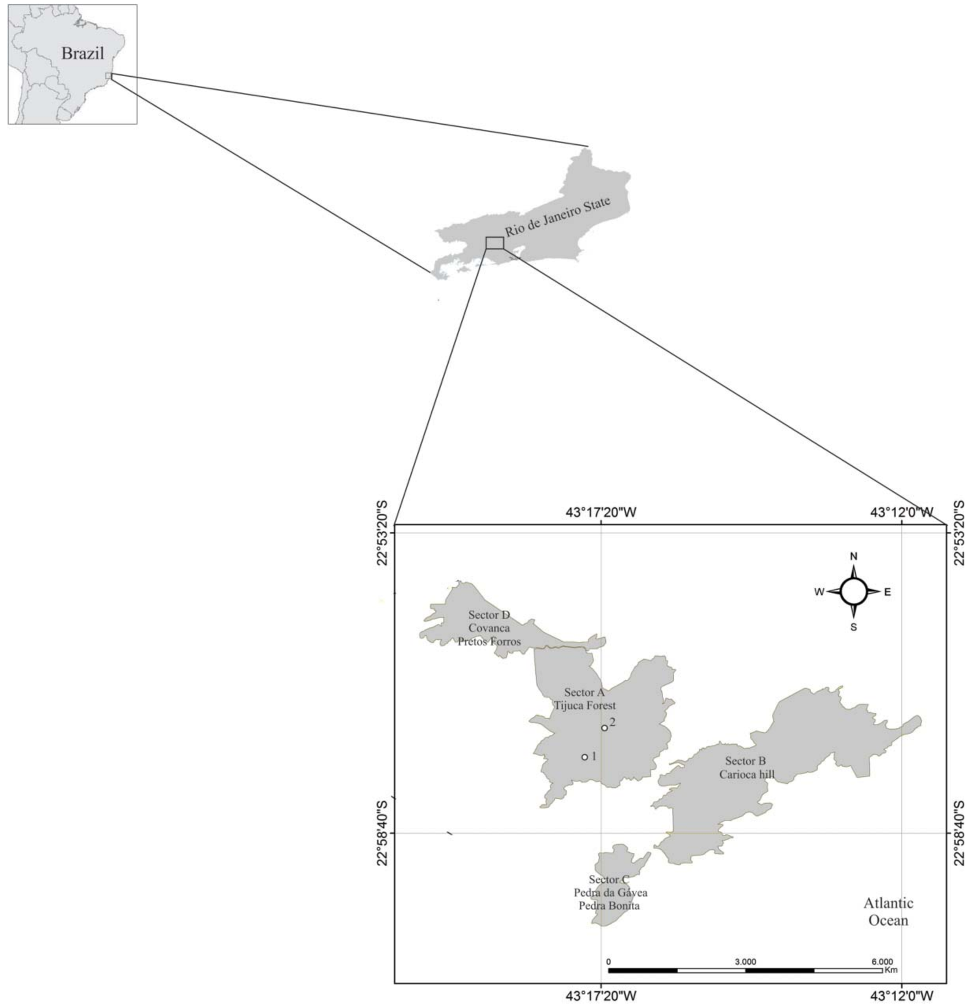

Figure 1. Sectors of the Parque Nacional da Tijuca (PNT) in Rio de Janeiro city and the two localities studied (Point 1 and Point 2).

from 40 m (quota considered for individualisation of mass) to 1021 m (Pico da Tijuca). The average annual rainfall is between 2000 and 2500 mm (Dias & Netto 2011). The PNT comprises four distinct sectors: Sector A is called the Tijuca Forest, sector B is called the Carioca Hill, sector C the Pedra da Gávea/Pedra Bonita and sector D the Covanca/Pretos Forros (Figure 1).

The predominant vegetation is tropical rainforest (ombrophilous forest). Throughout the centuries, this forest has been devastated, primarily for crop plantations such as coffee (*Coffea* spp.) and sugar cane (*Saccharum* spp.). Currently, some areas are remnants of the original forest, and others are covered with secondary vegetation under regeneration (Vieira 2001). The vegetation is extremely diverse, represented by families such as the Orchidaceae, Leguminosae, Bromeliaceae, Myrtaceae, Rubiaceae, Asteraceae, Melastomataceae, Lauraceae, Moraceae and Poaceae (MMA 2008).

2. Materials and methods

Two localities inside sector A of the PNT were chosen for sample collection. They are approximately 2210 m apart, and the altitude difference between them is nearly 10 m (Figure 2). Point 1 (22°57′22.1″S, 43°16′46.7″W) is located at an altitude of 510 m. The apiary was established inside a partial but well-preserved original ombrophilous forest vegetation of the Atlantic forest domain next to the Visitor Center. Point 2 (22°58′12.2″S, 43°17′43.4″W) is located at an altitude of 500 m. The apiary was established inside a secondary, regenerating rainforest.

Thirteen pollen samples were collected at Point 1 and 18 at Point 2 from July 2014 to December 2015. The samples were collected manually by capturing *M. quadrifasciata anthidioides* bees at the hive entrance. The flight activity of the bees was observed monthly, and approximately five bees were collected with an entomological net at approximately 10:00 a.m. at the beginning of each month. The collected materials from the bee baskets were kept in Eppendorf vials and processed by the standard methodology proposed by Barth et al. (2010) without the use of acetolysis, as described below. Each sample, consisting of a variable number of pollen loads, was homogenised by stirring in 70% ethanol. Centrifuge tubes were filled to 13 mL and allowed to stand for 30 minutes or overnight. Each sample was then sonicated for 5 minutes to dissociate pollen grain agglomerates. If samples contained a large amount of oil after centrifugation, they were subjected to a second ethanol extraction.

The resulting sediment was kept in a 1:1 mixture of water: glycerin for 30 minutes. One drop of this well-homogenised pollen grain suspension was applied to a microscope slide that was then covered with a 22 × 22-mm coverglass and sealed with nail varnish. The pollen stock suspension was kept for a long time in glycerin at room temperature in Eppendorf vials.

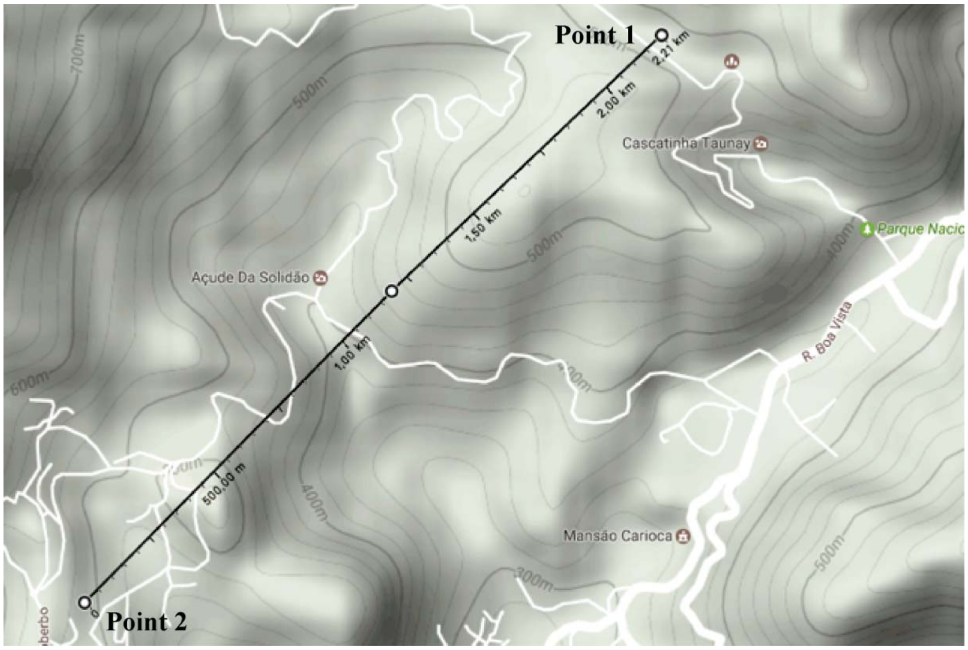

Figure 2. Distance between Point 1 and Point 2, roads (light lines) inside the Parque Nacional da Tijuca (PNT) and level contours.

Two microscope slides were prepared, and more than 500 pollen grains of each sample were counted and identified. Light and polarised light microscopy was used. Attention was also given to other structures detected, as yeast, dark and colourless fungal spores, vegetal hairs, and organic and inorganic material were also preserved in the final sediment obtained.

Samples were classified as monofloral, bifloral or heterofloral batches according to the pollen classes previously established by Barth et al. (2010). Pollen grain illustrations presented by Barth (1989), Roubik & Moreno (1991) and Moreti et al. (2002), and a palynological reference collection with tropical species deposited at Fiocruz (Fundação Oswaldo Cruz) were used for pollen identification. The pollen grains of the Myrtaceae genera (except *Eucalyptus*) have very similar morphology (Barth & Barbosa 1972). Slight variability in pollen morphology composes groups of genera.

Principal component analysis (PCA) was performed using the software PAST (Paleontological Statistics version 3.09) (Hammer et al. 2001). The matrix included all pollen types present in each sample, with their absolute count values. Variability between the samples was expressed using the first two axes. The Euclidean distance (dissimilarity measure) in conjunction with the unweighted pair group method with arithmetic mean (UPGMA) clustering method was used to evaluate the formation groups among the samples studied based on the abundance of pollen types identified.

3. Results

A total of 31 pollen load samples comprised points 1 and 2. Considering monofloral and bifloral samples, Point 1 presented 9/13 monofloral samples and 3/13 bifloral samples, and Point 2 showed 12/18 monofloral samples and 6/18 bifloral samples (Table 1). The two collection points presented frequencies described below.

3.1. Collection point 1

The most common pollen type was of *Myrcia* (53.8%, in seven samples), followed by pollen types of exotic *Eucalyptus*, Melastomataceae and *Mimosa caesalpiniifolia* (15.4%, each type in two of the samples). Monofloral pollen loads were obtained from *Myrcia* (30.8%, four samples), *Eucalyptus* (15.4%, two samples), Melastomataceae (15.4%, two samples) and *Solanum* (7.7%, one sample). Bifloral pollen loads consisted of *Mimosa caesalpiniifolia* (in two samples) and *Alchornea* (in one sample). Only one heterofloral sample was obtained.

3.2. Collection point 2

The *Myrcia* pollen type at Point 2 was also the most common (61.1%, in 11 samples), followed by Melastomataceae (27.9%, in five samples), *Eucalyptus* (16.6%, in three samples) and *Anadenanthera* (5.6%, in one sample) (Plate 1).

Monofloral pollen loads were obtained from *Myrcia* (38.9%, in seven samples), *Eucalyptus* (16.6%, in three samples), Melastomataceae (5.6%, in one sample) and *Anadenanthera* (5.6%, in one sample). Bifloral pollen loads consisted of *Mimosa caesalpiniifolia* (in two samples). No heterofloral samples were collected. In addition to pollen types recognised in the monofloral and bifloral pollen loads, pollen grains of several plant species were collected but were of low frequency. Considering a 3 to 15% inclusion percentage, important pollen grains of *Vernonia* (5.0%) constituted a portion of a monofloral *Eucalyptus* sample in August 2014 at Point 1 together with *Myrcia* (14.5%) pollen. The bifloral sample in December 2014 at Point 2 presented, in addition to Melastomataceae and *Myrcia*, important pollen grains of Loranthaceae (10.2%) and *Anadenanthera* (3.8%). The unique heterofloral sample obtained in January 2015 was composed mainly of *Anadenanthera* (42.4%), *Solanum* (38.4%) and *Eucalyptus* (18.2%) pollen types.

Table 1. Pollen loads collected by *Melipona quadrifasciata anthidioides* at two points located in the Parque Nacional da Tijuca (PNT) from July 2014 to December 2015.

		Pollen types and pollen load evaluation	
Season/year	Month	Collection point 1	Collection point 2
Winter/2014	July	*Myrcia* (100%) **Monofloral**	*Eucalyptus* (80.5%) **Monofloral**
	August	*Eucalyptus* (80.5%) **Monofloral**	*Myrcia* (97.9%) **Monofloral**
Spring/2014	September	*	*Myrcia* (100%) **Monofloral**
	October	Solanum (100%) **Monofloral**	*Myrcia* (100%) **Monofloral**
	November	Melastomataceae (100%) **Monofloral**	*Myrcia* (100%) **Monofloral**
Summer/2014–2015	December	*Myrcia* (100%) **Monofloral**	Melastomataceae (62.1%) *Myrcia* (23.6%) **Bifloral**
	January	Heterofloral	*Anadenanthera* (100%) **Monofloral**
	February	*Mimosa caesalpiniifolia* (71.8%) *Myrcia* (22.7%) **Bifloral**	*Mimosa caesalpiniifolia* (69.6%) Melastomataceae (18.5%) **Bifloral**
Autumn/2015	March	*Myrcia* (100%) **Monofloral**	*Mimosa caesalpiniaefolia* (51.7%) *Myrcia* (36.1%) **Bifloral**
	April	*Mimosa caesalpiniifolia* (80.5%) *Myrcia* (19.5%) **Bifloral**	*Myrcia* (96.7%) **Monofloral**
	May	*Alchornea* (65.2%) *Myrcia* (34.8%) **Bifloral**	*Myrcia* (63.4%) Melastomataceae (36.6%) **Bifloral**
Winter/2015	June	*	*Eucalyptus* (80.1%) Melastomataceae (19.9%) **Bifloral**
	July	*	*Eucalyptus* (100%) **Monofloral**
	August	*Eucalyptus* (100%) **Monofloral**	*Eucalyptus* (100%) **Monofloral**
Spring/2015	September	*	*Myrcia* (100%) **Monofloral**
	October	*Myrcia* (100%) **Monofloral**	*Myrcia* (98.9%) **Monofloral**
	November	*	Melastomataceae (78.8%) *Myrcia* (21.2%) **Bifloral**
Summer/2015	December	Melastomataceae(100%) **Monofloral**	Melastomataceae (99.0%) **Monofloral**

*Not collected.

3.3. Additional structured elements

Other structured elements were detected in the pollen sediments of four samples only in 2014, which consisted mostly of brown amorphous organic material, silica crystals, and fungal spores and hyphae. Fern spores were absent.

3.4. Statistical evaluation

The monofloral pollen types of collected samples at the two points (1 and 2) of Parque Nacional da Tijuca were ordered according to the similarities in their occurrences, which resulted in a great similarity among samples (Figure 3). At Point 1, a variance of 85.7% of the dataset represented by the *Myrcia*, *Eucalyptus*, Melastomataceae and *Solanum* pollen types was observed. However, at Point 2, a variance of 83.2% was observed, characterised by the presence of *Anadenanthera*, *Eucalyptus*, *Myrcia* and Melastomataceae pollen types.

4. Discussion

Melipona are native bees that prefer native vegetation for foraging (Ramalho 2004; Antonini et al. 2006b); however, these bees do forage exotic plants when few native food resources are available. With a high availability of specific resources, these bees remain faithful to a source and collect pollen and/or nectar for the maintenance of the hives (Law et al. 2000). According to Roubik (1989), bee foraging behaviour can be affected when access to resources is influenced by environmental conditions, dispersion and competition. Our samples indicated a strong preference for native plants.

Considering the annual seasons (Table 1), during winter, from June to August, in 2014 and 2015, only eight samples were obtained. The predominance of exotic *Eucalyptus* pollen in five samples proves the lack of available native plant species. It is known that native bees have a higher preference for native vegetation (Wilms et al. 1996; Morandin & Kremen 2013) when

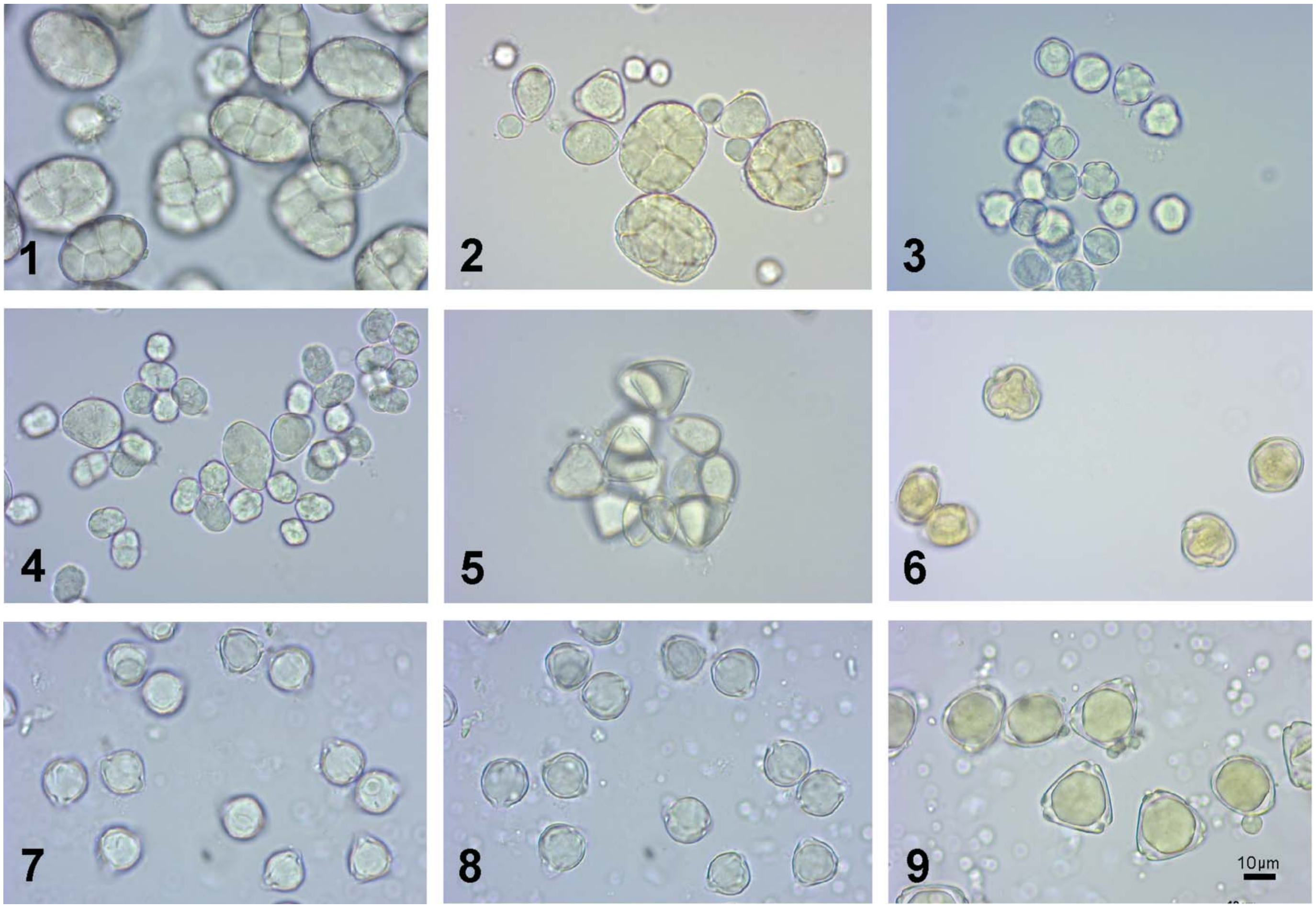

Plate 1. Pollen grains of bee load samples obtained in two collecting points of a tropical Atlantic forest, Parque Nacional da Tijuca (PNT), Rio de Janeiro, Brazil. 1. Pollen load of 100% *Anadenanthera* polyads. 2. Mixed pollen load of *Anadenanthera*, *Eucalyptus* and *Mimosa*. 3. Melastomataceae. 4. *Mimosa caesalpiniifolia* pollen grains, two of the *Mimosa invisa* and one of the *Myrcia* pollen type. 5. A 100% *Myrcia* pollen load. 6. *Alchornea*. 7. *Solanum*, pollen grain surface view. 8. *Solanum*, optical section. 9. *Eucalyptus* pollen grains. Scale bar = 10 μm for all images.

it is available than do introduced bees, which would be the case for *Apis mellifera* L. On the other hand, the presence of *Eucalyptus* species can be essential to healthy maintenance of the hives when there are few resources available. *Myrcia* pollen obtained from a unique monofloral sample at Point 1 as well as pollen of the Melastomataceae from a bifloral sample showed a prolonged activity of summer flowering. No pollen entrance occurred during June–July 2015 at Point 1, which was devoid of any favourable environmental conditions, with higher precipitation in June than in July.

In the spring, monofloral samples of *Myrcia* pollen were largely recorded at Point 2 during each month. Point 1 samples showed a lack of pollen entrance in the hive for 3–6 months, and no collection could be performed. In addition to a unique monofloral sample of *Myrcia* pollen in October, sustainability was covered by *Solanum* and Melastomataceae. The summer offered a great variety of pollen resources. Mostly Melastomataceae pollen was collected at both sampling points. The 100% entrance of *Anadenanthera* pollen in the hive at Point 2 showed the fidelity and preference of *M. quadrifasciata anthidioides* for this plant, with *A. colubrina* ('angico-branco') being the most common species in the area. The unique heterofloral pollen sample obtained (January 2015 at Point 1) showed that environmental conditions with abundant precipitation were not conducive. Therefore, this bee species is a generalist, and this species was also observed in small proportions in the bifloral samples. The short flowering of *Mimosa caesalpiniifolia* ('sabiá') in February has been proven using the bifloral samples at the two points of collection.

One season of autumn was included in the present study, in which six samples were collected. *Myrcia* pollen was the most abundant in monofloral and bifloral samples. In March at Point 2 and in April at Point 1, the pollen grains of the *Mimosa caesalpiniifolia* consisted of bifloral samples, as did *Alchornea* in May. Considering the botanical families, Myrtaceae, which is represented by *Myrcia* and *Eucalyptus* pollen types, was the most representative at the two points of our collection. This fact was also observed by Antonini et al. (2006b) for *M. quadrifasciata* in Minas Gerais State, and by Oliveira-Abreu et al. (2014) for *M. quadrifasciata anthidioides* regarding pollen samples collected from São Paulo State. The *Melipona* subspecies bee is considered a pollen-feeding generalist (Absy et al. 1984), but when favourable situations occur, these bees become specialised and obtain pollen exclusively from certain families (Antonini et al. 2006a). This behaviour was clearly observed in the PNT Atlantic forest in the present study.

Pollen of *Myrcia* was collected mainly during the spring and autumn seasons. Strong rainfall in the summer (December and January) may be partly responsible for this. Peak flowering of

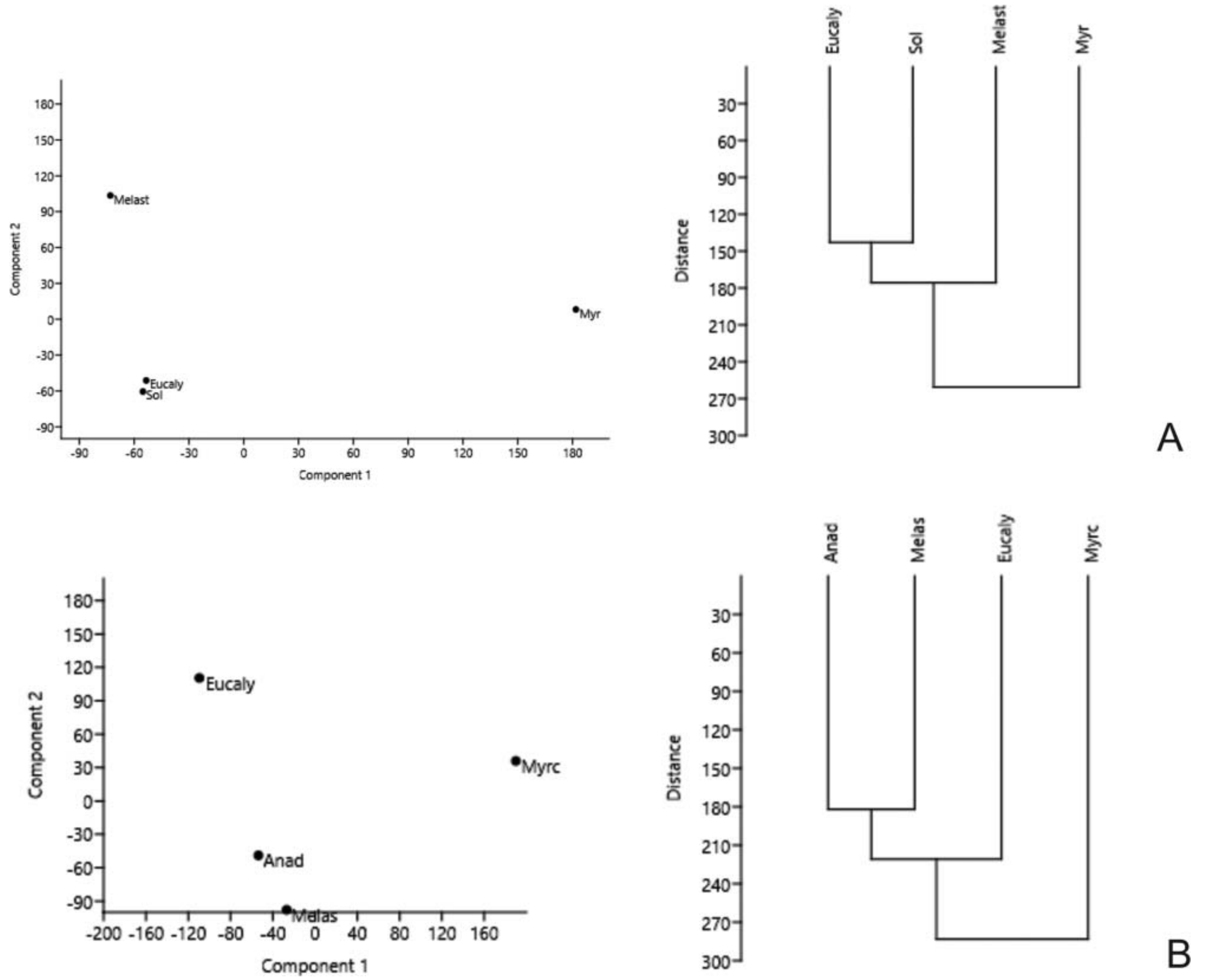

Figure 3. Biplot of the principal component analysis (PCA) and cluster analysis based on the Euclidean distance for pollen types in monofloral samples collected by *Melipona quadrifasciata anthidioides* in Parque Nacional da Tijuca (PNT), Rio de Janeiro city, using absolute value variables per sample. A = Point 1; B = Point 2; Anad = *Anadenanthera*; Eucaly = *Eucalyptus*; Melast = Melastomataceae; Myrc = *Myrcia*; Sol = *Solanum*.

some *Myrcia* species was observed in August by Proença & Gibbs (1994) in a natural reserve area located in the central part of Brazil, and in September by Amorim et al. (2013) in an Atlantic forest in southern Bahia. Also, in the PNT Atlantic forest, *Myrcia* species were frequently visited by *M. quadrifasciata* in an urban forest fragment at Belo Horizonte city (Antonini et al. 2006b) and by *M. quadrifasciata anthidioides* in a natural park of São Paulo State (Oliveira-Abreu et al. 2014). Lorenzi (2002) highlighted that this genus is characterised as containing heliophilic perennials as well as hygrophytes and xerophytes.

Eucalyptus pollen was predominant in our samples during the winter. *Eucalyptus* species were introduced to Brazil at the end of the nineteenth century (Pinto Júnior & Goulart 2014), being widely pollinated by *A. mellifera* bees (Pacheco et al. 1986). Some flowering *Eucalyptus* species were also observed during autumn–winter by Law et al. (2000) on the eastern coast of Australia, as occurred in the present study. However, Ashton (1975) highlighted that *Eucalyptus* species can have abundant flowering in any season depending on the weather conditions.

In Brazil, exotic *Eucalyptus* pollen type was observed in samples obtained by *M. quadrifasciata* on the university campus of São Paulo (USP), an urban area (Pirani & Cortopassi-Laurino 1993). However, Antonini et al. (2006a) did not observe this pollen collected by *M. quadrifasciata* in the two studied urban areas that comprise large *Eucalyptus* spp. plantations. The authors highlighted that the high richness of the native vegetation observed in these areas could favour their exploration. Oliveira-Abreu et al. (2014) found a *Eucalyptus* pollen type in analysed samples from *M. quadrifasciata anthidioides* from December to September in São Paulo State. This fact highlights that the strong collection of this pollen plays an important role in the diet of these bees when the frequency of *Myrcia* pollen decreases or when the pollen is absent.

Monofloral samples of the Melastomataceae pollen type were obtained during the summer at both collection points. Together with Melastomataceae, the *Myrcia* pollen type seems to be of great importance in the maintenance of *M. quadrifasciata anthidioides* colonies. This fact was also observed by other authors in different areas of the Atlantic rainforest throughout Brazil (Ramalho 2004; Antonini et al. 2006b; Oliveira-Abreu et al. 2014). The high precipitation during summer could have favoured the flowering of *Anadenanthera* spp. and *Mimosa* *caesalpiniifolia*, since the majority of pollen types collected by *M. quadrifasciata anthidioides* have a great adaptation to humid soils (Lorenzi 2002). Pirani & Cortopassi-Laurino (1993) reported intense Melastomataceae pollen collection in an urban area of São Paulo State.

Solanum pollen collected in October at Point 1 was an isolated occurrence. Several *Solanum* species (*S. granulosoleprosum*, *S. tabacifolium*, *S. paniculatum* and *S. didymium*) were observed by Antonini et al. (2006b) being visited by *M. quadrifasciata* in an urban fragment forest in Minas Gerais State. Members of the Solanaceae family have poricide anthers, and the stingless bees are specialised in extracting pollen by 'buzz pollination' (Laroca 1970; Bezerra & Machado 2003). Species of *Melipona* visit Solanaceae plants also in Amazonian forest areas of Brazil (Absy & Kerr 1977; Absy et al. 1980) and these plants are of great importance to the diet and maintenance of the hives.

Alchornea comprises plant species that commonly occur in the rainforest and are considered pioneers (Webster 2004). *Alchornea* pollen was found as isolated pollen in several samples collected by different stingless bees from southern Brazil (Freitas et al. 2012). Less-frequent pollen types collected by *M. quadrifasciata anthidioides* represent an opportunistic behaviour of the bees to supply their protein needs. As such, they become important pollinators, even during short plant flowering times. The bees take advantage of these offerings and supplement their preferred botanical species with these other sources. Structured elements sometimes occurred in pollen load samples when *Myrcia* pollen grains were predominant. This means that these elements are not necessarily related to the volume of pollen grains collected by the bees and that they are coming from a natural environment.

PCA showed that the two points in relation to the mean and standard error showed a certain homogeneity (i.e. of Point 1 and Point 2). Significant statistical variation between the two points was not observed, and the difference was due only to the occurrence of Melastomataceae at Point 1 and *Solanum* at Point 2. In Atlantic forest areas, Melastomataceae can be found from riparian forests of high altitudes to slope regions. In Rio de Janeiro State, different species occur, with emphasis on the *Miconia* and *Tibouchina* genera (Barberena et al. 2008). The flowers of these genera are widely visited and pollinated by different species of stingless bees (Baungratz & Silva 1988; Ramalho 2004). However, *Solanum* (Solanaceae) occurs in semi-deciduous forests, in secondary formations and in sloping areas with well-drained soils (Lorenzi 2002), which corroborates the fact that collection point 2 is located in a well-preserved area that contains secondary vegetation (Figure 3). The results of the PCA of the studied samples in the present paper are in agreement with the results of the pollen analysis.

5. General considerations

(1) *Melipona quadrifasciata anthidioides* showed a preference for native plant species, mainly of the Myrtaceae family, except *Eucalyptus*, which is an exotic and introduced plant genus. The identification of pollen grains in the loads showed the importance of *Eucalyptus* to bee maintenance when harvesting from short-flowering taxa, such as *Alchornea*, *Anadenanthera*, Melastomataceae and *Solanum*.
(2) The monthly follow-up of pollen load collections for 1.5 years proved the fidelity of *M. quadrifasciata anthidioides* to a few plant taxa (monofloral pollen samples) compared to the high botanical diversity of the Atlantic rainforest.
(3) There is reciprocity between the actions of stingless bees (native bees) and forest maintenance through pollination activity. Monthly or weekly studies could provide more detailed information on the relationship between pollen grains and native plant species resources for the native bees in the south-eastern Brazilian tropical Atlantic forest, considering environmental changes such as temperature, rainfall and anthropogenic activities.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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