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Tree species used for nesting by stingless bees (Hymenoptera: Apidae: Meliponini) in the Atlantic Rain Forest (Brazil): Availability or Selectivity

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Abstract

The stingless bees (Meliponini) are numerically dominant in tropical forests and most species depend on preexisting cavities for nesting, mainly tree hollows. However, it is still incipient the knowledge about basic characteristics of forest trees used for nesting. The basic questions addressed in this study include: would appropriate hollows be restricted to a few tree species? Would there be selectivity in the use of tree hollows in the forest? These issues are addressed from the comparison of usage patterns among forest trees in different stages of forest regeneration in the Atlantic Forest (Michelin Reserve in northeastern Brazil). Among 89 nests (from six species) found in tree hollows, in a sampled area of 32 ha of forests, 78.7% were associated with live plants and 21.3% to dead trees. This result does not support the hypothesis of selectivity for living trees, considering the high rate of living trees: dead trees (40:1). Nests were sampled from 41 tree species of 31 genera and 22 plant families. Meliponini species showed no differential association with any tree species. The absence of selectivity of tree species as nesting site is probably due to the high diversity of trees per hectare of Atlantic rainforest. The stingless bees also showed no selectivity for wood hardness, therefore the potential durability of tree hollows probably exerts weak selective pressure on bees, or at least the hardness variation range of trees used for nesting has no important influence on reproductive success of the colonies of stingless bees.

Introduction

Probably, the abundance of stingless bees (Meliponini) is constrained by floral resources (Hubbell & Johnson, 1977; Eltz et al., 2002) and/or by nesting sites (Oliveira et al., 1995; Samejima et al., 2004; Teixeira & Viana, 2005). In these eusocial bees, the choice of nesting site has profound influence on the longevity and reproductive success of colonies (Hubbell & Johnson, 1977) due to the limited flight range (Araújo et al., 2004) and progressive swarm (Nogueira-Neto, 1997).

The stingless bees use several substrates for nesting (Nogueira-Neto, 1997); however, the majority depends on preexisting cavities such as tree hollows, and few species build exposed nests (Roubik, 1989; Michener, 2000; Antonini & Martins, 2003; Batista et al., 2003; Martins et al., 2004; Silva et al., 2013). The knowledge about tree species used by stingless bees for nesting is still incipient (Moreno & Cardoso, 1997; Aguilar-Monge, 1999; Martins et al., 2004; Kleinert, 2006) and it is not known if these bees have shown some preference for nesting trees. According to Nogueira-Neto et al. (1986), some species of stingless bees may have specificity in the use of trees for nesting, which is corroborated by data sampled under low diversity of nesting trees (Batista et al., 2003; Martins et al., 2004; Teixeira & Viana, 2005; Serra et al., 2009). However, the very high diversity and very low population densities of trees in tropical forests should favor no selectivity of nesting sites: for instance in the Atlantic rainforest, the tree diversity can reach over 400 species of trees per hectare (see Guedes et al., 2005).

As an example, stingless bees would not present specificities when choosing trees for nesting in tropical dipterocarp forests and they would be opportunistic, although the tree hollows could be

selected by their characteristics, such as size (Eltz et al., 2002). In the tropical forests of Central America, Hubbell and Johnson (1977) observed low selectivity in the using of tree hollows and argued that the availability of nesting substrates would be higher than the density of stingless bee nests in the forest habitats. The hypothesis that tree hollow availability exceed the demand for nesting by stingless bees is supported by the low density of nests (2.8 nests/ha) compared to the high density of trees potentially suitable for nesting in the Atlantic rain Forest (Silva et al., 2013).

Estimates of availability and use of nesting substrates are necessary for understanding the role of choice or preferences in structuring communities of stingless bees in forests (Kleinert, 2006; Silva et al., 2013). The basic questions addressed in this study include: would availability of suitable hollows for nesting be restricted to a few tree species? Would there be selectivity in the use of tree hollows in the forest? This study compares the tree species used by the stingless bee community at different stages of forest regeneration in the Atlantic rainforest. It is assumed that the availability of tree hollows with suitable sizes varies between stages of forest regeneration, affecting choice opportunities, according to the species-specific needs of stingless bees. If the availability and selectivity of cavities are important, the communities of stingless bees must change between the stages of forest regeneration.

Material and Methods

The present study was conducted in the Michelin Ecological Reserve - MER (13°50′S, 39°15′W) in the Brazilian Atlantic rainforest in northeastern Brazil. The MER encompasses 3,096 ha of tropical rainforest at altitudes from 160 to 327 masl. The native forest forms a mosaic with rubber (*Hevea brasiliensis* Muell. Arg., Euphorbiaceae) plantations. The MER forest areas experienced severe anthropogenic impacts prior to 2004, generating a mosaic of forest fragments at different stages of regeneration. At the present time, the preserved nuclear areas of the largest fragments have attained a mature old growth stage of regeneration, with canopy heights of more than 20 m and many trees with circumferences >190 cm at breast height, as well as numerous old growth trees >300 cm in circumference reaching more than 30 m height. There are also extensive patches of forest at early stages of regeneration (with lower canopies and no old growth trees and with shrub and herbaceous plant cover in the understory), mainly at the edges of the largest fragments (Flesher, 2006).

Two categories of forested habitats were discriminated to verify and compare the nest density and Meliponini species richness: mature old growth forest and early stages of regeneration (IS = initial state and AS = advanced state). Four replicates of the two forest categories were sampled in each of the four largest MER forest fragments. A total of 64 25x25 m plots was established and sampled in each of the four replicates (total area of 4 ha/replicate), for an overall total of 16 ha for each of the two forest categories (Silva et al., 2013). All the trees

within each plot were visually inspected in search of nests, with special attention being paid to large trees with circumferences at breast height (CBH) >190 cm – in which stingless bee nests tend to be concentrated in forest habitats (Eltz et al., 2002; Batista et al., 2003). The botanical material was collected for preparation of exsiccates and identification of tree species.

To determine the wood density (hardness) of nesting trees (with Meliponini nests), samples of 2.0 x 2.0 cm were collected from the tree trunk at breast height (CBH = 1.30 m): one block near to the core and the other close to the bark. The mercury porosimetry technique was used to estimate the wood dry mass, according to the method of Vital (1984).

The vegetation structure was measured using the T-square method (Sutherland, 2006). Twenty random points were drawn/replica and the distance from the point to the nearest individual (x) and its distance to the nearest neighbor on orthogonal were used to estimate the density of living trees (all trees, with or without nests) in the following perimeter categories (circumference at breast height-CBH = 1.30 cm): (1) 21-50 cm; (2) 51-80 cm; (3) 81-110 cm and (4) above 110 cm. The T-square technique was not used to estimate the density of dead trees because it is not suitable for events with very low frequency (Sutherland, 2006). In this case, ten plots of 25x25 m (randomly chosen) were established for density measurement in each of the four replicates/forest stage and all the dead trees were counted (according to the perimeter categories used in the samplings of living trees). The density of living trees was calculated using the program Ecological Methodology, 2nd Ed. (Kenney & Krebs, 2000), while the density of dead trees was obtained by dividing the number of trees by the area sampled in each habitat category. In addition to the density and size of nesting trees, both stages were compared in relation to the hardness of the wood. The "Permutational Multivariate Analysis of Variance" test - PERMANOVA (Anderson, 2005) was used for data analysis because the assumptions of homoscedasticity (Levene test) and normality (Kolmogorov-Smirnov test) were not satisfied. The tests were run on Graphpad Instat and SPSS (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL, USA) software.

Three dependent variables (total abundance, richness and wood hardness of nesting trees) were tested in relation to a single factor: stage of forest regeneration in two levels (IS = initial state and AS = advanced state). The Bray-Curtis measure was used with untransformed data for comparing both stages of forest regeneration at a significance level of 0.05 (Anderson 2005).

The nonparametric correlation test (Spearman) was applied in two situations: 1) to estimate the relationship between the number of trees with nests and the number of largest trees (CBH> 80cm) per unit area of each habitat category (IS and AS); 2) to assess the relationship between the size of trees-CBH (or tree hardness) and richness and abundance of Meliponini nests. The tests were run on Graphpad Instat 3.05 software (GraphPad-Software, 1998) at a significance level of 0.05.

Selectivity was used here as synonymous for preference that would be detected when a used category of

nesting trees (size or hardness) is higher than its availability in local forest habitat.

The similarity of the nesting trees between species of stingless bees was estimated by cluster analysis (Bray-Curtis coefficient) and data were run on PAST program - (PAlaeontological STatistics, see. 1.81).

Results and Discussion

Of the total of 118 nests of stingless bees found in MER, 75.4% were in trees, 9.4% amid the rocky substrate, 7.6% inside termite nests and 7.6% in soil and slopes. Among 89 nests found in tree hollows, 78.7% were found in living trees and 21.3% in dead trees. Nests of five Meliponini species were observed in hollows of living trees, of which only *Scaptotrigona xanthotricha* Moure was not found in dead trees (Table 1). Batista et al. (2003) found 5.4% of the nests of stingless bees in dead trees in a disturbed area of Atlantic rainforest.

In a recent review, Cortopassi-Laurino et al. (2009) found that living trees predominate largely over dead trees as nesting substrates to stingless bees. Roubik (1989) has also argued that the stingless bees should occupy most durable substrates, which provide good physical protection in the forest, therefore living trees. Although these data and arguments have internal coherence, they often lack a measure of availability of cavities in dead and living trees for testing preference or selectivity. In the Atlantic rainforest of MER, the density of living trees is about 40 times higher than the density of dead trees and, therefore, these data do not support the hypothesis that stingless bees would avoid dead trees (Table 2). Alternatively, we should consider that many nests found in dead trees were established while the trees were still alive.

The living trees with nests corresponded to 59 individuals and 42 species (Table 3; Fig 1). Only one nest was found in most tree individuals and species and a maximum of 3-4 nests were associated to six tree species. This overall framework suggests that availability of cavities is common

Table 2. Density of living and dead trees (trees/ha) in the initial (IS) and advanced (AS) stages of forest regeneration distributed by circumference at breast height (CBH): CBH 1: 21-50 cm; CBH 2: 51-80 cm; CBH 3: 81-110 cm and CBH 4: above 110 cm).

CBH	Living trees		Dead trees	
	AS	IS	AS	IS
1	536.5	599.7	8.50	5.00
2	257.1	165.1	7.25	9.25
3	92.9	61.2	8.25	9.75
4	96.3	41.1	4.25	2.50

in many tree species and trees selectivity by stingless bees is very low or nonexistent in this forest habitat.

Among the 194 tree species recorded in MER (Rocha-Santos & Talora, 2012) 41 (21%) showed Meliponini nests, therefore apparently the appropriate tree hollows for these bees are well spread in the flora. If we consider the relatively high values of alpha and beta tree diversity in the Atlantic rainforest (e.g, Guedes et al., 2005), the data on MER refute the argument of Hubbell and Johnson (1977) that most of the nests of stingless bees would be found in relatively few species of trees out of the total available in any vegetation.

In a disturbed area of Atlantic rainforest, with depressed richness of trees, Batista et al. (2003) found only 18 tree species with nests of stingless bees; while Eltz et al. (2003) recorded nests in 38 species of trees in lowland dipterocarp forests, Malaysia. In areas of Caatinga, Castro (2001), Martins et al. (2004), Teixeira and Viana (2005) and Souza et al. (2008) found most nests of stingless bees in only five tree species (*Caesalpinia pyramidalis* Tul.; *Commiphora leptophloeos* Mart. J.B. Gillett.; *Schinopsis brasiliensis* Engl.; *Copaifera coriacea* Mart. and *Amburana cearensis* Schwacke & Taub.). In savannah-Cerrado areas, Kerr (1971), Aquino et al. (2007), Antonini and Martins (2003) and Serra et al. (2009) found a predominance of nests in the species *Caryocar brasiliense* Cambess., *Qualea parviflora* Mart. and *Salvertia convallariaeodora* A. St.-Hil.. In mixed forest of Araucaria, Witter and colleagues (2010) reported certain

Table 1. Characteristics of stingless bees' nests found in tree hollows in Atlantic Rainforest (Michelin Ecological Reserve): Advanced Stage (or old growth mature; AS) and Initial Stage (IS) of forest regeneration; Circumference at breath height (CBH)= 1.30m.

	Number of nests AS IS		Average height	Height Range	CBH Mean	CBH Range	Nesting Trees
Melipona scutellaris Latreille	6	5	8.4m (±4.2m)	2.5-13m	125cm (±39.7cm)	59-197cm	Living and Dead
Plebeia droryana (Friese)	-	2	85.5cm (±62.9cm)	41-130cm	55.5cm (±14.8cm)	45-66cm	Living and Dead
Scaptotrigona bipunctata (Lepeletier)	9	5	5.1m (±5.1m)	171cm-15m	120.1cm (±34.1cm)	70-180cm	Living and Dead
Scaptotrigona xanthotricha (Lepeletier)	13	8	3.2m (±4.2m)	25cm-13m	127.2cm (±34.8cm)	76-180cm	Living
Tetragonisca angustula (Latreille)	14	20	3.1m (±4.7m)	1cm-11m	119.3cm (±85.3cm)	41-232cm	Living and Dead
Trigona fuscipennis Friese	-	1	4.5m	-	119.3cm	-	Living

Table 3. Tree species most used as nesting substrate by stingless bees in initial and advanced stages of forest regeneration. Ecological Group: T = tolerant to shade; I = Intolerant to shade; NC = Not Classified (Lorenzi, 2002a; 2002b; 2009; Rocha-Santos & Talora, 2012). *S.b* (*Scaptotrigona bipunctata*); *S.x* (*Scaptotrigona xanthotricha*); *T.a* (*Tetragonisca angustula*); *M.s* (*Melipona scutellaris*) and *T.f* (*Trigona fuscipennis*). (*) = sample not identified.

| Family	Tree species	Ecological group and hardness	Number of nests		Meliponini species
			IS	AS	
Anacardiaceae	*Thyrsodium spruceanum* Salzm. Ex Benth.	T (0.41 g/cm)	2	1	*T.a; S.b*
Apocynaceae	*Symphonia globulifera* L.f.	T (0.14 g/cm)	1	0	*T.a*
Araliaceae	*Dendropanax bahiensis* Fiaschi.	NC (0.35 g/cm)	0	1	*S.x*
Burseraceae	*Protium icicariba* (DC.) Marchand.	T (0.53 g/cm)	0	3	*S.x; M.s*
Burseraceae	*Protium* sp.	NC(0.64 g/cm)	2	0	*M.s; T.a*
Chrysobalanaceae	*Licania hypoleuca* Benth.	T (*)	1	0	*T.f*
Clusiaceae	*Aspidosperma c.f. spruceanum* Benth. ex Müll.Arg.	T (0.38 g/cm)	1	0	*T.a*
Clusiaceae	*Clusiacea* sp.2	NC (0.69 g/cm)	1	0	*S.b*
Clusiaceae	*Clusiacea* sp.1	NC(0.60 g/cm)	0	1	*S.x*
Clusiaceae	*Garcinia macrophylla* Mart.	T (0.17 g/cm)	1	0	*S.x*
Clusiaceae	*Tabernaemontana flavicans* Willd. Ex Roem. & Schult.	T (*)	0	1	*M.s*
Cunoniaceae	*Lamanonia ternata* Vell.	T (0.66 g/cm)	1	0	*S.x*
Elaeocarpaceae	*Sloanea guianensis* (Aubl.) Benth.	T (0.83 g/cm)	2	1	*P.d*
Elaeocarpaceae	*Sloanea obtusifolia* (Moric.) K. Schum.	I (0.15 g/cm)	0	1	*T.a*
Euphorbiaceae	*Pera glabrata* (Schott) Poepp. ex Baill.	I (0.23 g/cm)	0	1	*T.a*
Fabaceae	*Albizia pedicellaris* (DC.) L.Rico.	I (0.091 g/cm)	0	1	*S.b*
Fabaceae	*Arapatiella psilophylla* (Harms) R.S.Cowan.	T (0.13 g/cm)	1	0	*S.x*
Fabaceae	*Inga capitata* Desv.	T (0.63 g/cm)	1	0	*T.a*
Fabaceae	*Inga edulis* Mart.	T (0.70 g/cm)	1	0	*T.a*
Fabaceae	*Inga thibaudiana* DC.	I (0.86 g/cm)	1	0	*M.s*
Fabaceae	*Inga* sp.1	NC(0.78 g/cm)	1	0	*S.b*
Fabaceae	*Peltogyne confertiflora* (Mart. Ex Hayne) Benth.	T (0.74 g/cm)	0	1	*M.s*
Fabaceae	*Stryphnodendron pulcherrimum* Willd. Hochr.	I (0.69 g/cm)	4	0	*S.x*
Fabaceae	*Swartzia polita* (R.S.Cowan) Torke	T (0.79 g/cm)	0	1	*S.b*
Fabaceae	*Swartzia* sp.1	NC(0.89 g/cm)	0	1	*S.x*
Hypericaceae	*Vismia guianensis* (Aubl.) Choisy	I (0.90 g/cm)	1	0	*T.a*
Hypericaceae	*Vismia guianensis* (Aubl.) Choisy	I (0.90 g/cm)	1	0	*T.a*
Icacinaceae	*Emmotum nitens* Miers.	T (0.83 g/cm)	0	1	*T.a*
Lauraceae	*Ocotea* cf. *canaliculata* (Rich.) Mez	I (0.64 g/cm)	1	0	*S.b*
Lauraceae	*Ocotea longifolia* Kunth.	T (0.43 g/cm)	1	0	*M.s*
Melastomataceae	*Henriettea succosa* (Aubl.) DC.	T (0.75 g/cm)	1	1	*T.a*
Melastomataceae	*Tibouchina* sp.	NC(*)	1	0	*S.x*
Meliaceae	*Trichilia lepidota* Mart.	T (0.31 g/cm)	0	3	*T.a; S.b*
Moraceae	*Artocarpus heterophyllus* Lamk.	T (0.92 g/cm)	1	0	*T.a*
Myrsinaceae	*Myrsine* sp.1	T (0.62 g/cm)	0	1	*T.a*
Myristicaceae	*Virola gardneri* (A.DC.) Warb	T (*)	0	1	*S.x*
Peraceae	*Pogonophora schomburgkiana* Miers ex Benth.	I (*)	1	0	*T.a*
Phyllanthaceae	*Amanoa guianensis* Aubl.	I (0.79 g/cm)	0	1	*T.a*
Rubiaceae	*Psycotria carthagenensis* Jacq.	T (0.86 g/cm)	0	2	*T.a; S.x*
Salicaceae	*Casearia* sp.1	NC (0.84 g/cm)	0	1	*S.x*
Sapotaceae	*Pouteria venosa* (Mart.) Baehni.	T (0.93 g/cm)	0	1	*S.x*
Urticaceae	*Pourouma velutina* Mart. Ex Miq.	I (0.16 g/cm)	2	0	*T.a; S.x*
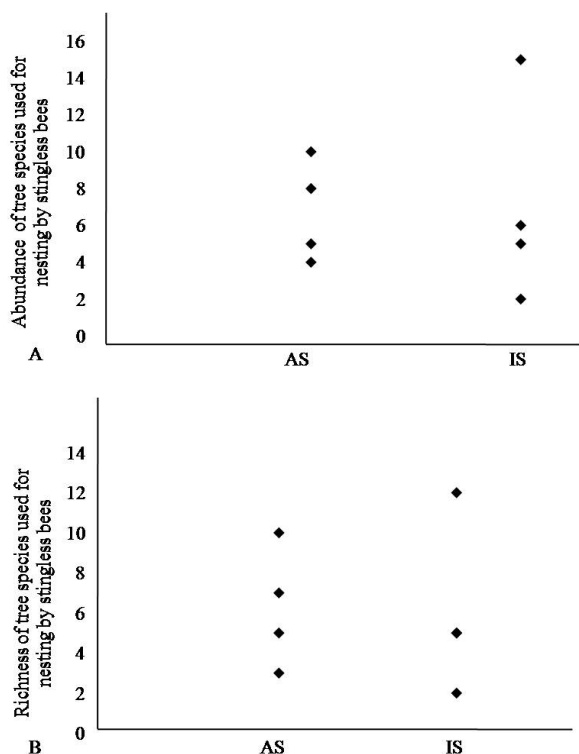
Fig 1. Abundance (A) and richness (B) of tree species used for nesting by stingless bees in each replica forest habitat in advanced stage (AS) and initial stage (IS) of regeneration in the Michelin Ecological Reserve (MER).

nesting specificity by *Melipona bicolor schencki* Gribodo in a few species of Lauraceae (59.52%). In urban area in the center of Curitiba city (Paraná State, Brazil), Taura and Laroca (1991) also found a greater number of nests of stingless bees in only two tree species, *Jacaranda mimosaefolia* D. Don. and *Platanus* sp.

A common denominator to these studies with stingless bees in non-forested habitats is the dominance of some tree species and/ or the availability of tree hollows suitable for nesting in a small proportion of arboreal flora. By analogy, the plant family with highest frequency of stingless bee nests in MER was Fabaceae (Table 3) that was also highlighted in the review of nesting trees by Cortopassi-Laurino et al. (2009). In MER, Fabaceae surpasses the others in richness and abundance in all stages of forest regeneration (Rocha-Santos & Talora, 2012), and this predisposes its use as nesting trees by stingless bees: this usage reflects availability and not preference or selectivity. Often, the authors who have studied stingless bee communities in forest habitats just point out the use of "suitable tree hollows for nesting" (mainly with apparent suitable size), regardless of tree species (Hubbell & Johnson, 1977; Oliveira et al., 1995; Eltz et al., 2003). When the availability of tree hollows is widely distributed in the tree flora, as in the MER rainforest, the apparent tree selectivity by Meliponini disappears.

Trees used as nesting sites by Meliponini have wood density (or hardness) ranging from 0.13 to 0.93 g/cm³ and about 70% of these trees have wood hardness above 0.6 g/cm³. The hardness had no significant relationship with nest abundance (p=0.4210) and richness (p=0.2779) of stingless bees (Fig 2). There is no significant variation in the distribution of total nests related to trees' hardness,

however, *Plebeia droryana* (Friese) and *Melipona scutellaris* Latreille often used trees with higher densities (0.86 g/cm³ and 0.75 g/cm³, respectively), while *Tetragonisca angustula* (Latreille) often occupies cavities in trees with low hardness (below 0.55 g/cm³). In these cases, we can rule out the effect of trees size used for nesting, as a confounding variable, mainly in the case of *T. angustula*, a generalist species able to use small hollows (Silva et al., 2013, 2014).

It was detected a difference in hardness of nesting trees between the two stages of forest regeneration (p=0.0002), in a first analysis of the four replicates. However, one of the replicas was detected as an outlier, with very low values of hardness in 'IS' (0.13g/cm³) in comparison with the others replicas of 'IS' stage of forest regeneration (around or above 0.5 g/cm³) and, a posteriori, it was excluded from the analysis. With this procedure, the differences in wood hardness disappeared between the two stages of
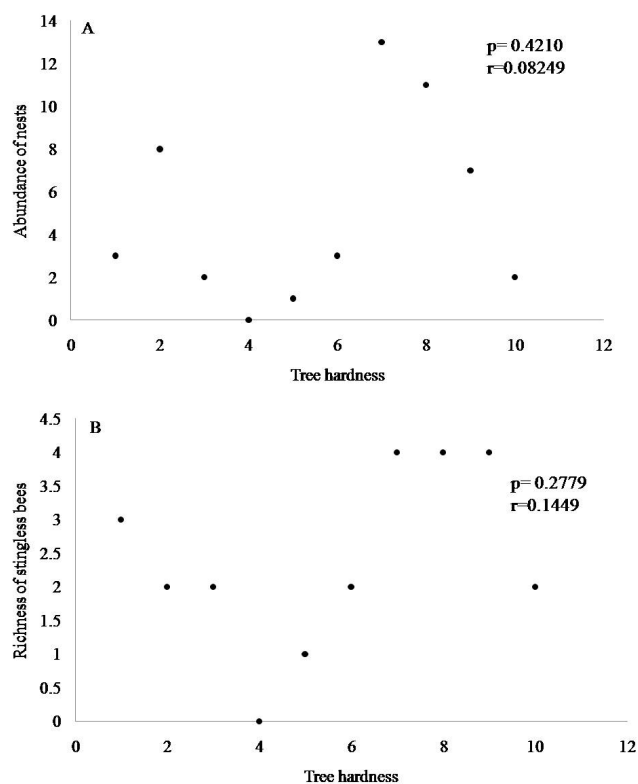

Fig 2. Correlation between the abundance of nests (A), species richness of stingless bees (B) regarding the hardness of trees that were found with nest of stingless bees.

forest (p >0.05). This result support the previous argument of similar hollow availability in trees regardless of the wood hardness or ecological group (e.g., tolerant or intolerant to shade; Table 3). Moreover, one nest per tree/family with varying wood hardness was the predominant pattern in both forest stages of regeneration (Table 3), indicating that the hardness *per se* would not be relevant in the process of hollow formation and availability for Meliponini.

In summary, probably the hardness and durability of hollows in the trees have not exerted significant selective pressure on stingless bees in the rainforest, or at least the hardness variation range has had no influence on colonies reproductive success and longevity.

The average CBH of nesting trees in MER was 132.3cm (±66.1cm). The CBH had negative significant relationship (p=0.0224, r=-0.4953) with nest abundance and no significant relationship with stingless bee richness (p=0.1072) (Fig 3). The lower occurrence of nests in larger trees should be a sampling artifact, however, reflecting the pattern of availability of CBHs categories, i.e. the great reduction in abundance of larger trees (Table 2). In fact, the variation in the abundance of nests of stingless bees is not significant (p=0.944) between the largest categories 3 and 4 of CBH.

Samejima et al. (2004) indicated that the low density of stingless bee nests in disturbed areas of forest would be related primarily to the absence of largest trees for nesting (CBH above 150cm or diameter above 24cm). Hubbell and Johnson (1977) argued that the colonization of secondary forests by stingless bees would depend on the tree size, and the initial stage of forest regeneration should be colonized primarily by small bees and later by larger ones. The small nesting trees should be less
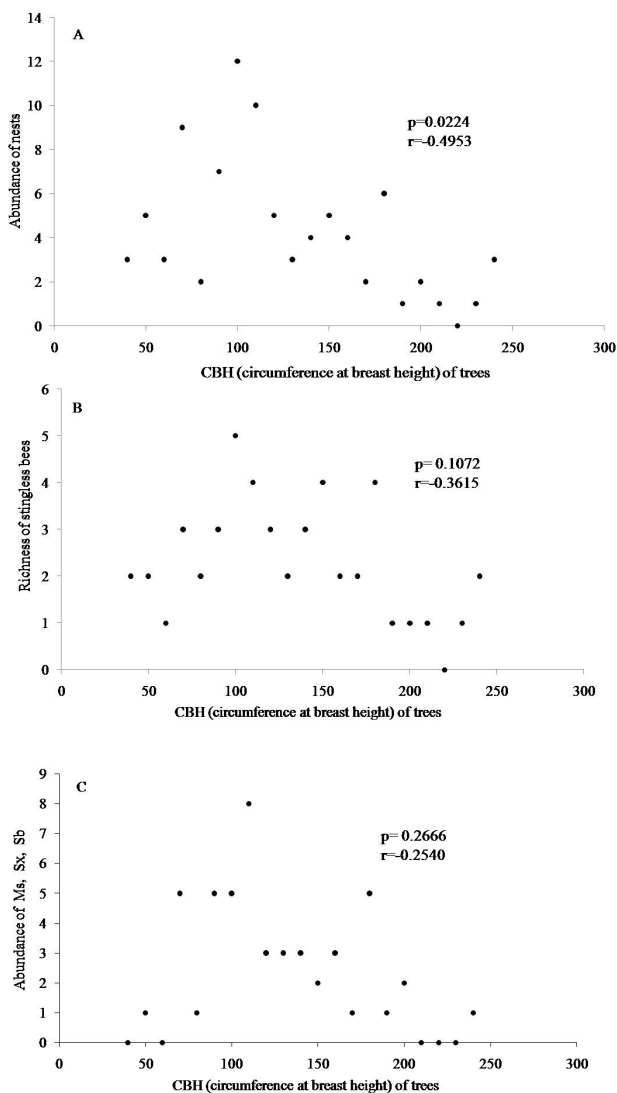
accessible to Meliponini species with large colonial biomass (Hubbell & Johnson, 1977; Roubik, 1983; Samejima et al., 2004). Sampling with trap nests also supports the argument that different species might respond to different thresholds of minimum size of cavities in the forest (Silva et al., 2014). A positive relationship between stingless bees body size and minimum diameter of nesting trees was also detected (Kleinert 2006), probably because the minimum biomass of colonies would also be lower in smaller species of stingless bees.

However, in MER rainforest, the stingless bees with very different body sizes that would fit the profile of "large colonial biomass" (*S. xanthotricha*, *S. bipunctata* and *M. scutellaris*) show no significant variation (p=0.2666, r=-02540) in the occupancy of trees with different CBHs (Fig 3C). Likewise, the use of tree hollow sizes does not group the stingless bee taxa or sizes; on the contrary, affinities are random (Fig 4). For instance, stingless bee species with more sampled nests also used a higher number of tree hollows and tend to have greater nesting overlap (e.g. *T. angustula* and *S. xanthotricha*). These results support the argument of weak selectivity for different hollow sizes among different species of stingless bees.
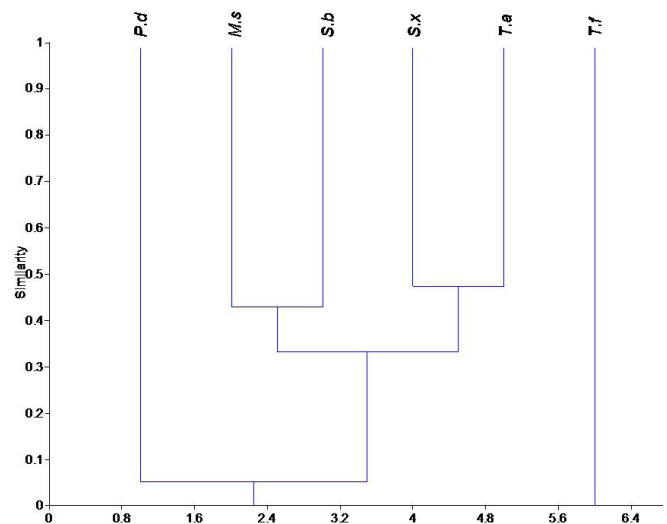

Fig 3. Correlation between the abundance of nests (A), species richness of stingless bees (B) and abundance of nests of *Melipona scutellaris* (Ms), *Scaptotrigona xanthotricha* (Sx) and *S. bipunctata* (Sb) (C) regarding the CBH (circumference at breast height) of trees that were found with nest of stingless bees.

Fig 4. Similarity among Meliponini species in use of plant families. Bray-Curtis coefficient. Caption: *Plebeia droryana* (P.d); *Trigona fuscipennis* (T.f); *Scaptotrigona bipunctata* (S.b); *Melipona scutellaris* (M.s); *Scaptotrigona xanthotricha* (S.x); *Tetragonisca angustula* (T.a).

The lack of variation in stingless bee richness with CBH trees (Fig 3) and the lack of differences (species and abundance) between stages of forest regeneration support the argument that trees above a CBH´s threshold (or hollow size threshold) are used and shared by most stingless bee species in local communities. For example, trees above 80cm of CBH (Table 2) or trap nests above 3L (Silva et al., 2014) were used by stingless bees with wide range of body sizes and colonial biomasses.

If there is any influence of the diameter of the trees on the stingless bees' community in MER, it is unlikely that this factor is sufficient to explain the absence of spatial dynamics

in the community between stages of forest regeneration. Even if the longevity of trees and tendency to develop hollows were influenced by the type of wood (e.g. hardness, chemicals, fiber, etc.) or by characteristics of tree growth (tolerant or intolerant to shade; Table 3), there are not evidences that these attributes have effective expression on nesting site availability and usage by stingless bees of Atlantic rainforest in MER, contrary to suppositions made by several authors.

Of the total number of nesting trees in MER, 51.6% were found in the early forest stage of regeneration and 49.4% in the old growth stage of forest regeneration. The differences were not significant between the two stages regarding the abundance of living trees with nests (p=0.9170) and richness of nesting trees (p=0.8326). According to Silva and colleagues (2013) the abundance of nests and the species richness of stingless bees were also similar in both stage of forest regeneration. Probably, there is no variation in the availability of suitable tree hollows per unit area between the two different stages of forest regeneration.

There are contrasting approaches and mainly conflicting interpretations about the relative importance of pioneer or slow growth trees as nesting substrate for Meliponini in tropical forests (Hubbell & Johnson, 1977; Batista 2003; Eltz et al., 2003; Samejima et al., 2004). Often, a higher wood hardness of slow growth trees (e.g. tolerant to shade) corresponds to a higher mechanical strength and natural wood durability (Florsheim, 1992), which would mean less exposure to fungi and insects and weathering action (Burger & Richter, 1991). However, the similar availability of hollows on pioneer and slow growth trees is likely a key determinant of abundance and spatial distribution of stingless bees nests in forests at different stages of regeneration, and not the physical characteristics of these trees *per se*.

Alternatively, the range of variation in tree hollow availability between the stages of forest is well above the demand by the bees' community. For example, Hubbell and Johnson (1977) estimated that the stingless bees should occupy 34% of tree hollows available in a tropical dry forest in Costa Rica. The low proportion of nests per trees with suitable sizes (1 nest per 100 trees with CBH >60cm), in Atlantic rainforest of MER (Silva et al., 2013) also supports that availability of tree hollows overcomes the stingless bees demands in both stage of forest regeneration.

The MER data support the general argument of oversupply of arboreal substrates for nesting in the forest for stingless bees, suggesting access control mechanisms operating at the community level that would also explain the low nest density (2.8 nests/ha; Silva et al., 2013). On the other hand, selectivity for tree species does not exist or at least is not relevant in spatial structuring (or temporal) of communities of stingless bees in this rainforest.

The extreme disruption of forest habitats, accompanied by extensive deforestation and savannization of landscapes, however, tends to reduce the diversity of trees and change considerably the pattern of tree hollows supply, exposing stingless bees to greater convergence in the use of nesting substrates. In this process, generalist species in using cavities with higher swarm rates should become dominant in the communities, as probably is happening with *T. angustula* (Silva et al., 2013, 2014). In such a scenario, the management of stingless bees in the forest should be closely associated with the management of diversity of trees for regeneration of tropical rainforests.

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