#### **ORIGINAL ARTICLE**

# Efficiency of trap nests in attracting stingless bees in the central Brazilian Amazon

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### ABSTRACT

Obtaining colonies of stingless bees in the wild for the formation or expansion of meliponaries and other purposes is permitted by law in Brazil using bait containers or trap nests, and other non-destructive methods. We tested the efficiency of trap nests made from plastic bottles for attraction and nesting of stingless bees in the central Brazilian Amazon. We used 2-L and 5-L bottles and three types of attractants (cerumen and geopropolis from *Melipona seminigra*, *M. interrupta* and a mix of the two). We used 216 trap nests distributed in three experimental areas during 13 months. Visitation by six species of stingless bees in 58 (26.9%) trap nests, and nesting by three species in 12 (5.6%) trap nests in two areas near meliponaries was recorded. There was no significant difference between trap-nest size, nor among attractants for visitation or nesting, suggesting that the availability of cavities or hollows is more important than odor for nesting. Monthly pooled visitation and nesting events were not correlated with monthly rainfall. Based on our results, we can conclude that, despite the low capture rate, the acquisition of swarms through nest traps is a viable alternative to obtain new colonies of stingless bees for meliponaries.

KEYWORDS: social bees, Meliponini, attractants, obtaining of swarms, meliponiculture

# Eficiência de ninhos-isca na atração de abelhas sem ferrão na Amazônia central brasileira

#### RESUMO

A obtenção de colônias de abelhas sem ferrão na natureza, para a formação ou ampliação de meliponários e outras finalidades, é permitida por lei no Brasil usando recipientes-isca ou ninho-isca, ou outros métodos não destrutivos. Nós testamos a eficiência do ninho-isca feito de garrafa plástica para atração e nidificação de abelhas sem ferrão na região da Amazônia central brasileira. Foram utilizadas garrafas de 2 L e 5 L e três tipos de atrativos (cerume e geoprópolis de *Melipona seminigra, M. interrupta* e uma mistura dos dois). Foram utilizados 216 ninhos-isca distribuídos em três áreas experimentais durante 13 meses. Foi registrada a visitação de seis espécies de abelhas sem ferrão em 58 (26,9%) dos ninhos-isca e a nidificação de três espécies em 12 (5,6%) dos ninhos-isca em duas áreas próximas a meliponários. Não houve diferença significativa entre o tamanho do ninho-isca, nem entre os atrativos na visitação ou nidificação, sugerindo que a disponibilidade de cavidades ou ocos é mais importante do que o odor para nidificação. Os dados mensais conjuntos de eventos de visitação e nidificação não foram correlacionados com a precipitação mensal. Baseados nos nossos resultados podemos concluir que, apesar da baixa taxa de captura, a aquisição de enxames por meio de ninho-isca é uma alternativa viável para obtenção de novas colônias de abelhas sem ferrão para meliponários.

PALAVRAS-CHAVE: abelhas sociais, Meliponini, atrativos, obtenção de enxames, meliponicultura

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# INTRODUCTION

Among the essential ecosystem services, pollination stands out for its function in providing food for humans and animals. Bees are among the most abundant pollinators (Imperatriz-Fonseca *et al.* 2012) and it is estimated that between 40 and 90% of Brazilian native plants are pollinated by stingless bees (Apidae, Meliponini) (Kerr *et al.* 2001). At least 75% of Meliponini occur in tropical regions (Melo 2020) and their greatest diversity (238 of the 550 species known worldwide) is found in Brazil, especially in the Amazon (Oliveira e Nogueira 2022), where they are commonly reared for the production of honey and pollen, a practice known as meliponiculture (Carvalho-Zilse *et al.* 2012; Carvalho-Zilse 2013).

Meliponiculture is currently expanding in Brazil and has recently been regulated by federal and state legislation (e.g., CONAMA 2020; CEMAAM 2017, 2021), which allow the use of bait containers (also known as trap nests) and other non-destructive methods to obtain matrix colonies for meliponiculture. Trap-nests are empty containers of different materials installed in the natural habitat of stingless bees mimicking a natural cavity to attract nesting swarms (Oliveira et al. 2013). The trap nests usually contain attractants made from parts of wild nests of bee colonies, considering that stingless bee swarms may have a preference to occupy cavities used by other colonies (Nogueira-Neto 1954; Wille e Orozco 1975). It remains unclear, however, whether bees prefer such cavities because of the odor of the previous occupants or because of the suitability of the cavity for nesting (Gruter 2020). In this context, the efficiency of the trap-nest method has not yet been thoroughly tested for stingless bees, especially in the Amazon biome, where the high rainfall rates of up to 3500 mm per year (Fisch et al. 1998) may influence the nesting activity of stingless bees (Slaa 2006; Oliveira et al. 2013).

Preliminary studies with trap nests of various materials have demonstrated the feasibility of plastic bottles for the nesting of Meliponini in the Atlantic Forest (Oliveira *et al.* 2013; Arena *et al.* 2018). Herein, we evaluated the efficiency of plastic-bottle trap nests for attracting and nesting of stinglessbee swarms in the Amazon rainforest. We tested the preference of bees for different trap sizes and different attractants, and evaluated the relation of visitation and nesting frequency with precipitation.

# MATERIAL AND METHODS

We built trap nests from PET plastic bottles used for mineral water, which are cost-effective and easily available. We used 216 bottles, 108 with a volume of 2 liters (2 L) and 108 with a volume of 5 liters (5 L). The two sizes were chosen to suit different bee species, which have a great variation in both body size and size of swarming population. Half of the bottles of each size class were fitted with attractants used by local beekeepers, who mix propolis from bee species from the region (Oliveira *et al.* 2009). We prepared our attractants based on information acquired from Dr. Ademilson Espencer Egea Soares, with adaptations. We chose materials (cerumen + geopropolis) produced by two species of native bees that are commonly bred in the regional meliponiculture: *Melipona seminigra* Friese 1903 (A1), *Melipona interrupta* Latreille, 1811 (A2), and a mix of extracts A1 and A2 in a 1:1 proportion (A3). A1 and A2 were composed of 300 g of chopped cerumen and 50 g of geopropolis macerated in 700 mL of ethyl alcohol 92.8°. The solution was kept in the dark for 30 days, stirred occasionally, and then filtered. Each trap nest with attractant was internally impregnated with 5 mL of the respective solution, which was renewed every two months.

Each trap nest was prepared (Supplementary Material, Figure S1) by inserting a L-shaped PVC connexion tube (25 mm in diameter) with one end facing outwards and the other facing inwards towards the bottom of the bottle. The inner wall and the entrance hole of all the trap nests were then coated with macerated geopropolis, to ease the locomotion of the bees, as in Pereira and Sousa (2015). The geopropolis was not considered as an attraction factor. Five small holes were drilled into the bottom of the bottle, which was then covered with 20 g of macerated geopropolis for absorption and outflow of moisture. Finally, the bottle was wrapped into newspaper, covered with black plastic sheet and labeled with an individual identification code.

#### **Experimental areas**

The trap-nests were tested in three experimental areas in the state of Amazonas, Brazil. The choice of areas was based on logistical criteria. Two areas were nearby meliponaries and one in a primary forest reserve: (1) Menino Deus community (2°32'43.5"S, 56°31'25.9"W), located in an area of floodplain forest (várzea forest) in the municipality of Parintins (an island in the Amazon River), where meliponiculture has been practiced for over 10 years, currently comprising 20 colonies of M. interrupta and one colony of Cephalotrigona femorata (Smith, 1854); (2) Valeria community (2°28'33.5"S, 56°26'36.4"W) is also located in Parintins, in an area of terra firme forest and floodplain forest (várzea), where meliponiculture has been practiced for less than five years, currently comprising 19 colonies of M. interrupta; and (3) Adolpho Ducke Forest Reserve (2°57'44"S, 59°55'19"W), a 10,000-ha area of terra firme forest located in the municipality of Manaus, with no history of meliponiculture.

The climate of the three areas is of the Am type (tropical rainy, with monsoon rains and a short dry season) according to the Köppen classification (Köppen 1936). In Parintins, average annual minimum and maximum temperatures are 22 °C and 33 °C, respectively, with average relative humidity of 52%, highest precipitation in February and April, and lowest precipitation between August and October. In Manaus, average annual minimum and maximum temperatures are 24

°C and 32 °C, respectively, average relative humidity is 41%, precipitation is highest in February, March and May, and lowest between June and August. The meteorological data were obtained from the database of the Brazilian National Institute of Meteorology – INMET for 2019 (https://portal.

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inmet.gov.br/dadoshistoricos).

Trap nests were grouped in experimental points to test each attractant. Each experimental point consisted of 12 traps grouped in three test sets. Each test set consisted of four traps: two 2-L and two 5-L traps. Of each pair, one trap contained one of the attractants and the other did not contain attractant. The test set was attached to a tree 1.80 m from the ground (Figure 1). The distance between test sets within each experimental point varied according to tree disposition, but was of at least 2 m.

Six experimental points (totalling 72 trap nests) were installed in each experimental area. In the two areas with a history of meliponiculture, three points were installed at 400 m from the meliponary, and three less than 10 m from the meliponary. All points were at least at 400 m from each other. In the Ducke Reserve, all six points were distributed at least at 400 m from each other. The distance of 400 m was chosen to avoid overlapping of swarm areas based on the



**Figure 1.** Disposition of a test set of trap nests for stingless bees (two 2-L and two 5-L plastic bottles around a tree trunk at 1.8 m above the ground. This figure is in color in the electronic version.

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swarm movement of 200 m estimated for *Melipona scutellaris* Latreille, 1811 (Carvalho-Zilse and Kerr 2004).

The trap-nests remained in the field from December 2018 to December 2019 and were monitored every 20 to 25 days, between 08:00 and 14:30. Visitations were recorded in real time by visual observation during monitoring or inferred from traces of visitors (dead specimens) in the trapnests, both of which were considered as successful visitation events. A successful nesting event was recorded when we observed the formation of a nest entry (partial or complete), the construction of internal cerumen structures (pots or remains of brood discs) or the establishment of the colony in the trap-nest. The stingless bee swarm that remained in the trap-nest from detection to the subsequent monitoring, i.e., 20-25 days after detection, was transferred to a standardized wooden box sensu Oliveira and Kerr (2000) and taken to the local meliponary. Containers that were occupied by other animals or damaged were cleaned or exchanged.

Specimens of Meliponini that visited or nested in the trap-nests were collected and identified to genus using identification keys for the Brazilian genera of stingless bees (Silveira *et al.* 2002) and to species by specialist Dr. David Silva Nogueira, from Instituto Federal de Educação, Ciência e Tecnologia do Amazonas (IFAM) Manaus, Brazil. Vouchers of the collected specimens (bees, wasps and ants) were deposited in the INPA invertebrate collection. Sampling and transport of Hymenoptera were authorized by license SISBIO # 70576-1/2019. Other invertebrate visitors in the trap-nests were photographed for later identification by experts.

#### Statistical analysis

The frequency of visitation and nesting by stingless bees was compared between trap volumes (2 L and 5 L), presence/ absence of attractant and distance from the meliponary (400 m and <10 m, only in the Parintins areas) with chi-square tests (X<sup>2</sup>). The pooled data on monthly number of visitation and nesting events, and on monthly rainfall were tested for normality using the Shapiro-Wilk test, and for homogeneity of variance using the Levene test. Based on the results, the relation between monthly visitations/nestings and rainfall was analyzed with the Spearman correlation test. We used only rainfall data for Parintins data. A significance leve of 5% was used for all analyses. The statistical analysis was performed using R Core Team, version 4.2.1 (©2022 R Studio).

# RESULTS

#### Visitation of stingless bees

Visits to trap-nests were recorded in all experimental areas. Overall, of the 216 trap-nests, 58 (26.9 %) were visited by stingless bees in 14 (78%) of the 18 experimental points. There was a significantly higher frequency of visited trap-nests in experimental points distant from the meliponary in the



Parintins communities ( $X^2_{(2, 144)} = 17.505$ , p = 0.0036) (Table 1). The largest number of visited trap-nests was recorded in the Menino Deus community. We recorded significantly more bee visitations in traps without attractant ( $X^2_{(3, 216)} = 13.954$ , p = 0.0030), but visitation of bees was recorded with all attractants tested in all experimental areas, mostly for the A1 attractant (Table 1).

Stingless bees visited traps of both sizes in the three experimental areas. We recorded visits in 33 (56.9%) of the 5-L traps and 25 (43.1%) of the 2-L traps (Table 1). The two bottle volumes did not differ significantly in visitation frequency ( $X^2_{(1,216)} = 0.589$ , p = 0.4427).

#### Nesting of stingless bees

Nesting of stingless bees was recorded in 12 (5.6%) of the 216 trap-nests, eight (66.7%) in the Menino Deus community and four (33.3%) in the Valeria community (Table 2). No nesting was recorded in Ducke Reserve.

Most nestings were recorded in traps with A1 attractant, followed by traps with A2, traps without attractant, and traps with A3. However, there was no significant difference among the groups ( $X^2_{(3,216)} = 6.353$ , p = 0.0957). Nine (75%) nestings occurred in 5-L traps and three (25%) in 2-L traps, but the difference was non significant ( $X^2_{(1,216)} = 2.206$ , p = 0.1375).

**Table 1.** Number of trap nests visited by stingless bees in three experimental areas in the central Brazilian Amazon from December 2018 to December 2019, according to trap volume, type of attractant used and distance to meliponaries. N = total number of trap nests; TV = number of traps visited by stingless bees (independently of the number of visitation events per nest); Attractant (cerumen and geopropolis): A1 = *Melipona seminigra*, A2 = *M. interrupta*, A3 = mixture of A1+A2, NA = no attractant. MDC = Menino Deus community; VC = community of Valeria; DR = Ducke Reserve.

Area	N	TV	Distance to meliponary* (m)		Attractant				Trap volume (L)		Visiting species	
			400	< 10	A1	A2	A3	NA	2	5	51	
MDC	72	35	17	7	6	3	2	12	10	13	Melipona interrupta Latreille, 1811	
			1	2	2	1	0	0	1	2	Trigona branneri Cockerell, 1912	
			4	2	1	2	1	3	2	5	Cephalotrigona femorata (Smith, 185	
			1	1	1	1	0	0	2	0	Unidentified **	
VC	72	11	2	5	3	1	2	1	3	4	M. interrupta	
			1		0	1	0	0	1	0	T. branneri	
			2	1	2	0	1	0	0	3	Tetragona clavipes (Fabricius, 1804)	
		12			1	0	0	0	0	1	Lestrimelitta aff. spinosa Smith, 1854	
DR	72				0	0	1	0	1	0	Ptilotrigona lurida (Smith, 1854)	
					1	4	2	3	5	5	Unidentified **	
Total	216	58	28	18	17	13	9	19	25	33		

\* The condition only applies to MDC and VC, as there were no meliponaries in DR.

\*\* Specimens not identified to species or genus level, as they were found in pieces inside the containers. According to the wings, we were able to recognize that they were stingless bees.

**Table 2.** Nesting records of stingless bees in 144 trap nests, grouped in 12 experimental points in two experimental areas in Parintins, Amazonas state, Brazil, from December 2018 to December 2019. MDC = Menino Deus community; VC = Valeria community. Volume = trap-nest volume; Attractant = cerumen extract + geopropolis of *Melipona seminigra* (A1); *M. interrupta* (A2); *M. seminigra* + *M. interrupta* (A3); or no attractant (NA).

Area	Point	Distance from meliponary (m)	Volume (L)	Attractant	Species	Month	Observation	
	EP3	406.1	5	NA	Melipona interrupta	June	Interrupted by ant attack	
	EP2	405.2	2	A1	M. interrupta	August	Interrupted - cause unknown	
	EP2	404.8	2	A2	M. interrupta	August	Interrupted - cause unknown	
MDC	EP3	406.1	5	NA	M. interrupta	August	Trap with nest stolen	
MDC	EP6	5.5	5	A1	Cephalotrigona femorata	March	Interrupted by ant attack	
	EP4	9.1	5	A1	M. interrupta	July	Interrupted - cause unknown	
	EP5	13.7	5	NA	M. interrupta	October	Colony transferred to the meliponary	
	EP5	8.5	5	A2	M. interrupta	November	Interrupted by ant attack	
	EP1	405.5	5	A3	Species not collected	September	Interrupted - cause unknown	
VC	EP1	405.5	2	A3	M. interrupta	October	Interrupted by ant attack	
	EP6	3.4	5	A1	Tetragona clavipes	June	Interrupted by Phoridae attack	
	EP5	4.5	5	A2	M. interrupta	December	Interrupted - cause unknown	

Nine of the 12 nestings were by *M. interrupta* and, among these, six occurred in 5-L and three in 2-L traps. All colonies in the trap nests, except one, were attacked by other animals or abandoned before being transferred to the breeding boxes (see Table 2). Only one *M. interrupta* colony was not attacked and there was time for it to be properly transferred to the breeding box and had a good development in the meliponary. There was no significant difference in nesting frequency between points distant and close to meliponaries ( $X^2_{(2, 144)} = 1.090, p = 0.9549$ ).

Visitation and nesting of stingless bees in the trap nests was higher between June and November 2019, which were the months with lowest precipitation. However, the correlation between monthly number of nestings/visitations and rainfall was not significant (r = -0.460, p = 0.131).

We recorded the presence of other animals in 157 (72%) trap nests (Supplementary Material, Table S1), especially in the experimental points near the meliponaries. In each experimental area, more than 50% of the trap-nests were occupied by other animals at some point during the experiment period.

# DISCUSSION

All the bee species we recorded visiting the trap nests use tree hollows for nesting, except *Trigona branneri* Cockerell, 1912, which makes an external or aerial nest (Camargo and Posey 1990; Roubik 2006; Rasmussen and Camargo 2008). The *T. branneri* visiting our trap nests were possibly in search of nest material (perhaps the material of the hardened attractant). Likewise, *Lestrimelitta* aff. *spinosa* Smith, 1854, which is a kleptobiotic species, i.e., it raids other stingless-bee colonies to get food or building material (Sakagami *et al.* 1993; Nogueira-Neto 1997), were also probing the traps for the hardened material inside or as potential nesting place. The species that were recorded nesting in the traps [*M. interrupta, C. femorata* and *Tetragona clavipes* (Fabricius, 1804)] are among the bees that are most commonly bred in the Brazilian Amazon (Cortopassi-Laurino *et al.* 2006; Carvalho-Zilse *et al.* 2012).

Visitation in trap nests occurred in all experimental areas, indicating the presence of natural bee nests in these places and their search of bees for available cavities. The visitation frequency of 26.9% to trap-nests in 78% of the experimental points indicates that the trap nests were effective in attracting stingless bees in the three experimental areas, even if it was not possible to establish whether the visiting bees (*M. interrupta* and *C. femorata*) came from natural swarms or from nearby meliponaries in the Menino Deus and Valeria communities. The higher visitation and nesting frequency in trap nests in Menino Deus may be due to that the bee population in that area is larger than that in Valéria, either due to the longer presence of meliponaries in this community, or to a greater natural density of bees in the area. Another possibility is a lower availability of natural nesting cavities, leading to a greater interest of the bees in the artificial cavities represented by the trap nests. In the Ducke Reserve, the visitation without nesting may be related to a greater availability of natural nesting substrates in the area, reducing the attractiveness of trap nests (O'Neill and O'Neill 2018). The reduction in the number of trees with adequate nesting sites directly affects the dispersion of stingless bees in nature, as observed by Hubbell and Johnson (1977) and Samejima et al. (2004) in forests of Costa Rica. As there was no preference for any particular attractant, we can infer that the type of attractant, or even the presence of an attractant, may not be determinant for the visitation of bees to the trap nests. In particular the visits to traps without attractant suggest that the workers inspect any available hollow and, if it is suitable, they may start the nesting process, as has been observed in other studies (Michener 1946; Schwarz 1948; Kerr et al. 1996).

There was no significant difference in visitation frequency by stingless bees between the two tested volumes, confirming that bees scour all available cavities for nesting sites (Kerr *et al.* 1996). As the expected visitation and nesting rate is naturally low (Oliveira *et al.* 2013; Arena *et al.* 2018), the sampling period and the number of traps at each area may not have been sufficient to detect statistically significant differences.

As nesting occurred only in the Menino Deus and Valeria communities, it may have been influenced by the presence of the meliponaries, considering that most nestings were by species raised in the meliponaries, although the same species also occur naturally in the region (ICMBio 2021). Although the vast majority of visitation and nesting events in the Parintins areas were by species raised in the meliponaries, the significantly higher visitation frequency at points more distant from the meliponaries suggests that wild bees were also attracted to the traps. The points further from the meliponary were likely less disturbed, allowing higher density of wild bees. The nesting frequency did not vary significantly with the distance from the meliponaries, however, the sample size may not have been large enough to infer site effects.

The nesting frequency did not vary significantly with the distance from the meliponaries, however, the sample size may not have been large enough to infer site effects The nesting frequency obtained in our study (5.6%, 12 nests in 216 available traps in 13 months) was similar to that recorded in Atlantic forest by Oliveira *et al.* (2013) also using trap nests made from plastic bottles (4%, 48 nests in 1,200 available traps in 12 months), Silva *et al.* (2014) (3.5%, 25 in 720 traps in 30 months), and Arena *et al.* (2018) (5.5%, 4 in 72 traps in eight months). The nesting pattern in a given year can influence subsequent nesting occasions (Oliveira *et al.* 2013), and bees usually carry out a single yearly process of nest formation when conditions are favorable (Roubik 2006; Oliveira *et al.* 2013). Therefore, the effectiveness of trap nests in attracting nesting is independent of the frequency of nesting

in the traps. Considering that legislation recommends that matrix colonies for meliponiculture are obtained by means of harvesting in nature using bait containers (CONAMA 2020), even a low nesting success in traps may be considered efficient, as the resolution envisages obtaining few colonies. However, in economic terms, during the implantation of a meliponary, when a rapid increase in the number of colonies is required, the generally low swarm capture rate of trap nests may be a problem.

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Both sizes of trap nests were used for nesting, supporting the notion that stingless bees are opportunistic in their selection of nesting sites and tend to colonize any tree that offers a suitable cavity (Eltz et al. 2003). While the minimum cavity diameter for nesting is species-dependent in stingless bees, they are not limited to a maximum diameter (Hubbell and Johnson 1977). For example, species that form less populous colonies or whith brood cells that are organized into bunches tend to occupy smaller cavities (Silva et al. 2014). Although the difference between trap sizes was non significant, possibly due to the small sample size, it was notable that most nestings (nine of 12) occurred in 5-L trap nests, which was also recorded by Oliveira et al. (2013). There may be a positive relationship between the size of stingless bees and the minimum diameter of trees where they nest (Kleinert 2006). More robust species, such as bees of the genus Melipona (which are commonly bred in the Amazon region), seem to choose larger cavities (Martins et al. 2004; Silva et al. 2014). Tetragona clavipes, which forms large populations within the hive (Rodrigues et al. 2007) and was recorded in our study, also nested in a 5-L trap, as recorded by Oliveira et al. (2013). This apparent tendency by stingless bees for nesting in larger artificial cavities should be further investigated in future studies.

Nesting events of stingless bees tend to occur at the end of the dry season and the number of swarms increase during the warmer months in southeastern Brazil (Oliveira *et al.* 2013) and for 14 species in Costa Rica, regardless of the habitat (Slaa 2006). The lack of statistical significance in the correlation of our visitation and nesting data with rainfall is likely due to the low overall number of visitation and nesting events in our study. The dry season in the Amazon region is associated with peaks in flowering (Haugaasen and Peres 2005), which provide favorable environmental conditions for the establishment of new nests (Nogueira-Neto 1997).

The occupation of more than half of the available trap nests by other animals at some point during the study period in the three experimental areas probably influenced the onset and evolution of the nesting events by stingless bees. Opportunistic animals such as spiders and ants are the most common competitors for nesting sites in the Atlantic Forest (Oliveira *et al.* 2013). Wasps (Silva *et al.* 2014), termites, frogs

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and rodents (Arena *et al.* 2018) were also recorded occupying trap nests in the Atlantic Forest.

# CONCLUSIONS

We demonstrated that plastic trap nests were visited and accepted for nesting by Amazonian stingless-bee species regardless of the presence or type of attractant and bottle size, whith a tendency (though non-significant) for more frequent use of 5-L over 2-L bottles for nesting. The higher frequency of visitation in traps further away than close to a meliponary indicates that naturally occurring bees are attracted to the traps. However, the nesting frequency was not related to the distance or proximity of the meliponaries. Our results show that, despite the low capture rate, acquiring swarms through nest traps is a viable alternative for obtaining new stingless bee colonies for meliponaries. The performance of trap-nests may improve by increasing the spatial and/ or temporal sampling effort, and by monitoring the traps at intervals shorter than 20 days for more efficient cleaning and removal of other animal occupants that may prevent or interrupt the nesting process of bees.

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**SUPPLEMENTARY MATERIAL** (only available in the electronic version) Cruz *et al.* Efficiency of trap nests in attracting stingless bees in the central Brazilian Amazon



**Figure S1.** Preparation of the trap nests for stingless bees. A – PET bottles used with a volume of 2 and 5 liters; B – Drilling a hole in the side of the bottle; C–D – PVC connection tubes used; E – Spraying of PVC connection with geopropolis; F – Coupling of the PVC connection to the bottle; G – Drilling of holes in the base of the bottle; H – Bottle with geopropolis layer at the inner bottom; I – Bottles wrapped in newspaper; J – Bottle wrapped in black plastic folie over the newspaper; K – Layer of glue featured on the outside of the side nozzle; L – Geopropolis on the outside of the nozzle; M – Identified trap nest ready for field deployment; N – Application of the attractant; O – Trap nests fixed to a tree trunk at the experimental point in the field.

**Table S1.** Records of occupation by animals other than stingless bees\* in trap-nests in three experimental areas (Area) in the central Brazilian Amazon throughout the period from December 2018 to December 2019. At each area, trap nests were grouped in six experimental points (Point). Each point contained 12 traps. When detected, occupied traps were replaced. MDC = Menino Deus community; VC = Valeria community; DR = Ducke Reserve. N available = number of available traps at the point; N occupied = number of traps occupied by animals other than stingless bees at some point of the period.

Area	Point	Recorded animals	N available	N occupied
MDC	EP1	Ants (Camponotus atriceps); frogs (Scinax aff. x-signatus); cockroaches	12	9
	EP2	Spiders; ants (Camponotus atriceps)	12	2
	EP3	Ants (Camponotus atriceps; Dolichoderus diversus); cockroaches; frogs (Scinax aff. x-signatus)	12	12
	EP4	Spiders; cockroaches; ants (Camponotus atriceps; Dolichoderus diversus); frogs (Scinax aff. x-signatus)	12	12
	EP5	Ants (Camponotus atriceps); cockroaches; frogs (Scinax aff. x-signatus)	12	12
	EP6	Spiders; cockroaches; ants (Camponotus atriceps; Dolichoderus diversus); frogs (Scinax aff. x-signatus)	12	12
VC	EP1	Frogs (Scinax aff. x-signatus); ant eggs; spiders; cockroaches	12	7
	EP2	Parasitoid (Cryptinae - Ichneumonidae); cockroaches; frog (Scinax aff. x-signatus); ants (Solenopsis geminata)	12	6
	EP3	Frog (Scinax aff. x-signatus); ants (Solenopsis geminata); spider; cockroach	12	8
	EP4	Ants (Cephalotes atratus; Dolicoderus diversus; Pheidole sp.); frog (Scinax aff. x-signatus)	12	10
	EP5	Ants (Dolichoderus diversus; Camponotus prox. simillimus); frog (Scinax aff. x-signatus); rodent's nest (unidentified); spiders	12	10
	EP6	Ants (Dolichoderus diversus; Dolichoderus lutosus; Camponotus atriceps); frog (Trachycephalus typhonius); rodent nest; cockroaches	12	9
DR	EP1	Ants (Crematogaster limata); frog (Osteocephalus taurinus); cockroaches; spiders	12	8
	EP2	Fly larvae; spiders; frog (Osteocephalus taurinus); wasps (Agelaia mymercophila)	12	6
	EP3	Ants (Crematogaster tenuicula); spiders; frogs (Osteocephalus taurinus)	12	8
	EP4	Ants (Crematogaster tenuicula); frog (Osteocephalus taurinus); cockroaches; spiders; larvae	12	10
	EP5	Frog (Osteocephalus taurinus); spiders; fly larvae; wasps (Agelaia pallipes)	12	7
	EP6	Ants (Crematogaster tenuicula; Camponotus rapax); fly larvae; spiders; frogs (Osteocephalus taurinus)	12	9

\*Scientific names are provided when the animal was collected and identified.