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Nesting Substrate Characteristics of *Partamona seridoensis* Pedro & Camargo (Hymenoptera: Apidae) in Areas of Dry Forest in Brazil

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Abstract
For the first time the association between *Partamona seridoensis* and *Constrictotermes cyphergaster* is described. *Partamona seridoensis* occurs in xeric areas of Northeastern Brazil, and it is a termitophile species as its nests are built in active and inactive arboreal termite nests of the species *C. cyphergaster*. This study aimed to verify the characteristics of the nesting substrate used by *P. seridoensis* in two areas of dry forest (*caatinga*) in Cariri region, Paraíba state. It has been found that the vertical distribution of termites that contained colonies of *P. seridoensis* varied from 10 cm to 3.60 m, while the height of the nest entrance varied from 20 cm to 3.70 m. *Commiphora leptophloeos*, popularly known as imburana, was the support tree of 22 (43.1%) from 51 observed termite nests that harbored bee colonies. Most (44; 86.2%) of the host termites colonies were active. Most of the colonies showed the nest entrances not directed to the east/southeast. All colonies located were housed in large termite nests, whose volumes exceed 30 liters. In the two areas surveyed, frequently the entrances of the nests were directed to other nearby colonies, suggesting a parental relationship that should be further investigated.

Introduction

Stingless bees (Meliponini) comprise more than 500 described species and possibly 100 more as yet undescribed (Michener, 2013), the real number is unknown because there are many cryptic species (Michener, 2007). The species are pantropical and southern subtropical, make honey as the closely related stinging honey bees, and live in perennial eusocial colonies of a few dozen to more than 100,000 workers (Michener, 2007).

Nesting biology is highly diverse in stingless bees, unknown in many species, and closely related to the biology and behavior of each species (Roubik, 2006). Most species nest in pre-existing cavities in living trees or trunks, although some species establish their colonies in crevices in rocks, aerial nests, underground cavities, roots and plants and active or not nests of ants and termites (Nogueira-Neto, 1997; Roubik, 2006).

The genus *Partamona* is a Neotropical stingless bee comprising 33 described species, many of which are cryptic, with aggressive bees that nest in a wide variety of substrates, and many species are obligatory termitophiles (Camargo & Pedro, 2003; Pedro & Camargo, 2003). Because they present similar morphological traits, nesting behavior and the nest entrance structure are useful traits for species identification (Camargo & Pedro, 2003).

Partamona seridoensis Pedro and Camargo occurs mainly in the dry forest biome, called *caatinga* and nests

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only inside termite nests (Camargo & Pedro, 2003). There are few data on the ecology of species of *Partamona* (Barreto & Castro, 2007; Miranda et al., 2015), and in general the genetics of these species is poorly known (Fernandes et al., 2012). However, *P. seridoensis* gene variation and the degree of differentiation between two populations from Cariri region in the state of Paraíba, Brazil, have been studied studied through allozymic and microsatellite analyses (Fernandes et al., 2012). Furthermore, this species was sampled in studies about the relations of melittophilous plants and bee species at the same region (Aguiar et al. 1995; Aguiar & Martins, 1997 in both cited as *Partamona* sp., Martins et al., 2003). In addition, *P. seridoensis* was the most abundant species in association with pig carcasses exposed in a forensic entomological study in the same area (Santos et al., 2014), and Lorenzon et al. (1999) has preliminarily studied the association of *P. seridoensis* and its host termite *Constrictotermes cyphergaster* (Silvestri) (referred by authors as *P. aff. nigrior* and *Constrictotermes* sp.). As Lorenzon et al. (1999) did not identify both species, Carrijo et al. (2012) in a recent review of published records of bee species as guests in termite nests stated that the *P. seridoensis* host is unknown. Although in a recent short note Oliveira et al. (2016) cited the association between *P. seridoensis* and *C. cyphergaster*, however the citations referred in the note, as reported above, did not identify the species.

Furthermore, many aspects of the association between this stingless bee species and its termite host remain unknown. The objective of this study was to improve our understanding of the nesting substrate characteristics of *P. seridoensis* in areas of dry forest in Northeastern Brazil with emphasis on size, volume, height and substrate of the termite nests, interaction between the bees and termites and the orientation of the nest entrances.

Material and Methods

The study was carried out at two farms located in the dry forest of Northeastern Brazil, called *Caatinga*, in the Cariri region of Paraíba state. The *caatinga* is a xeric biome (Andrade-Lima, 1981) that comprises xeric vegetation, ranging from shrub lands to small forest-like savannas (Prado, 2000). The Almas Farm, comprising a Particular Natural Heritage Reserve - RPPN (7º28`23.7"S; 36º54`17.1"W), is located at the municipalities of São José dos Cordeiros and Sumé, Paraíba, whose vegetation ranges from open shrubby arboreal and dense shrubby arboreal vegetation between flagstones with typical vegetation, with annual average rainfall of 400-800mm/year (Lima & Heckendorff, 1985). The Moreiras Farm (7º23`36.3"S; 36º24`53.9"W) is located at the municipality of São João do Cariri, Paraíba, which has an open shrubby arboreal vegetation, annual average rainfall of 386.6 mm, relative humidity 50%, temperature between 28.5 and 35 °C, and a dry irregular period of eight months, including the months of June to February (Atlas Geográfico da Paraiba, 1985).

Data collection was carried out at monthly intervals between March 2008 to June 2009, and every two months from July 2009 to December 2010. In each area transect lines, measuring 10 m wide by 200 m long each = 2,000 m^2) were plotted and inspected in four consecutive days (two at each area) from 5:00h to 17:00h. In each transect line, the *P. seridoensis* colonies identified inside the arboreal termite nests of *C. cyphergaster* were counted, georeferenced and had these information recorded: the presence or absence of termites (at the time of the colony discovery and throughout the study period); the tree species supporting the termite nest; the height of termite nest and of the colony entrance (relative to ground, using a measuring tape), the colony entrance position (in relation to the cardinal points using the compass of an Etrex® Summit-GPS); the termite nest size, measuring with a wooden ruler the height of the termite nest, the largest diameter, and the smallest diameter. The volumes were obtained using the formula V= 2/3.π.a.b.c/1000, where a = the height of termites; b = ½ the largest diameters; c = ½ smaller diameters (Fontes, 1980).

From each colony, some specimens were collected and placed in lethal chambers, later they were mounted on entomological pins and deposited in the Entomological Collection of the Department of Systematics and Ecology of the Federal University of Paraíba (UFPB). Termites were collected, fixed in 70% ethanol for identification by experts, and subsequently deposited in the Entomological Collection of the Department of Systematics and Ecology of the Federal University of Paraíba (UFPB).

Normality and test t were calculated using Statistica v7.0 and the homogeneity of the distribution of the orientation of the nest entrances in relation to the cardinal and collateral points for each studied area, and for the total, were tested with Rayleigh test using BioEstat v5.

Results

A total of 51 colonies of *P. seridoensis* were found in the study areas (29 at Almas Farm and 22 at Moreiras Farm) all of them inside nests of *C. cyphergaster.* Among these, almost 61% were in termite nests supported by only two tree species: 43.1% (22) in termite nests supported by *Commiphora leptophloeos* (imburana), and 17.6% (9) in *Poincianella pyramidalis* (catingueira) trees (Table 1). Two other colonies were housed in termite nests supported by fences made with dry sticks, while another was on the floor. This last one had been removed probably by bee hunters and was outside from their place of origin.

The vertical distribution of the termite nests that hosted colonies of *P. seridoensis* in the studied areas varied from 10 cm to 3.60 m, considering the height of the base of termites to the ground and was significantly different in both areas ($t= 5.235$; $p<0.0001$; df=47; Fig. 1). No bee colonies were observed in termite nests supported directly on the ground or on stones in any of the studied areas. The height of the bees nest entrance varied from 20 cm to 3.70 m (mean = 1.75 m to 0.80 m in Almas and Moreiras Farms, respectively), as the entrances were located from 0 to 20 cm above the base of the termite nests.

Table 1. Plant species used by *Constrictotermes cyphergaster* to support their nests where colonies of *Partamona seridoensis* were found from March 2008 to December 2010.

Plant Family	Plant species	Common name	Number of bee colonies
Burseraceae	*Commiphora leptophloeos* (Mart.) J. B. Gillett	Imburana	22
Caesalpinioideae	*Poincianella pyramidalis* Tul	Catingueira	9
Cactaceae	*Cereus jamacaru* DC.	Mandacaru	4
Anacardiaceae	*Spondias tuberosa* Arruda	Umbuzeiro	3
Euphorbiaceae	*Manihot glaziovii* Müll.Arg.	Maniçoba	2
Cactaceae	*Pilosocereus gounellei* (F. A. C. Weber) Byles & G. D. Rowley	Xique-xique	2
Mimosoideae	*Mimosa tenuiflora* (Willd.) Poir.	Jurema-preta	2
Apocynaceae	*Aspidosperma pyrifolium* Mart.	Pereiro	1
Anacardiaceae	*Myracrodruon urundeuva* Allemão	Aroeira	1
Anacardiaceae	*Schinopsis brasiliensis* Engl	Baraúna	1
Apocynaceae Caesalpinioideae	*Aspidosperma pyrifolium* Mart. *Poincianella pyramidalis* Tul	Pereiro Catingueira	1[1]

[1] *One nest supported by two tree species.*

The presence of termites was recorded in 44 (86.2%) of the host termite colonies of *P. seridoensis*. Only six (11.8%) of the host termite colonies were dead. In one colony at Almas Farm, it was not possible to verify the presence or absence of termites because it had been opened by local inhabitants, probably to collect honey and pollen, and was damaged with very few bees. Agonistic behaviors between the termites and the bees were not observed.

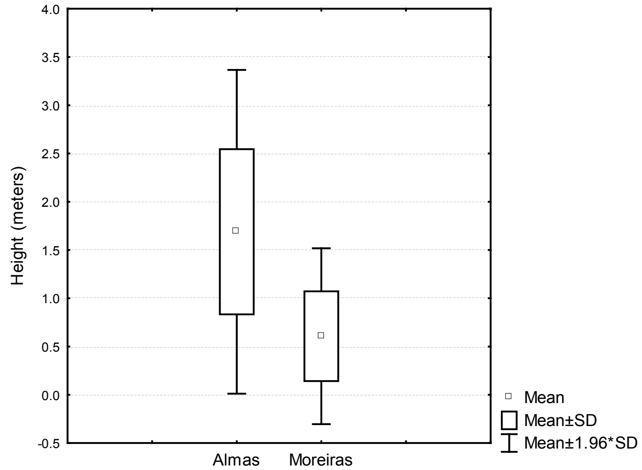

Fig 1. Box plot showing the height (meters) of termitaria nested by *Partamona seridoensis* in Almas (N= 28) and Moreiras Farms (N= 21).

The volume of the termite nests in which the bee colonies were hosted varied from 33.8 liters to 233 liters (mean = 98.2 ± 45.88 liters; Fig 2).

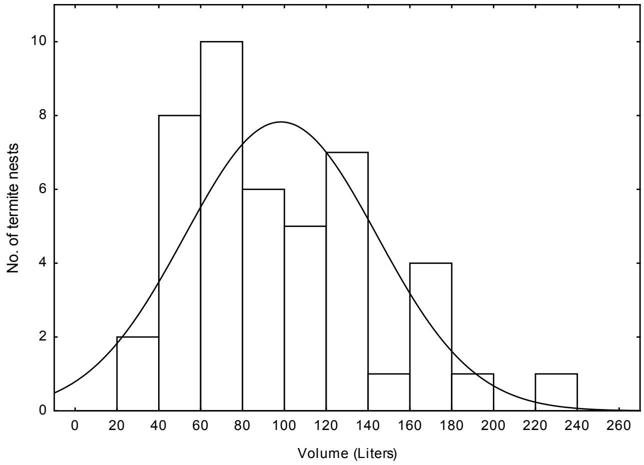

Fig 2. Frequency distribution of the number of termite nests inhabited by bee colonies by volume (Liters). The line shows the observed normal distribution of the size of large termite nests occupied by bee colonies (Kolmogorov-Smirnov d=0.09902, Chi-Square=3.53640, df=3, p=0.31607, N=45).

The Rayleigh's test showed that the orientation of the nest entrances of the bees was not uniformly distributed, with a significant concentration around the mean angle of 205.5° in Almas Farm (South, R= 10.2426, p<0.05, N=28) and the mean angle of 211.4° (Southwest, R=13.3206, p<0.05, N=50) in total sample (Figs. 3A,C). At Moreiras Farm, the Rayleigh's test showed no significant difference regarding the orientation of the nest entrances of the bees (R= 3.3059, p>0.05, N=22), although Southwest was also the direction with the highest number of entrances directed (Fig. 3B). It is noteworthy that in Moreiras Farm there is a small mountain blocking the East side.

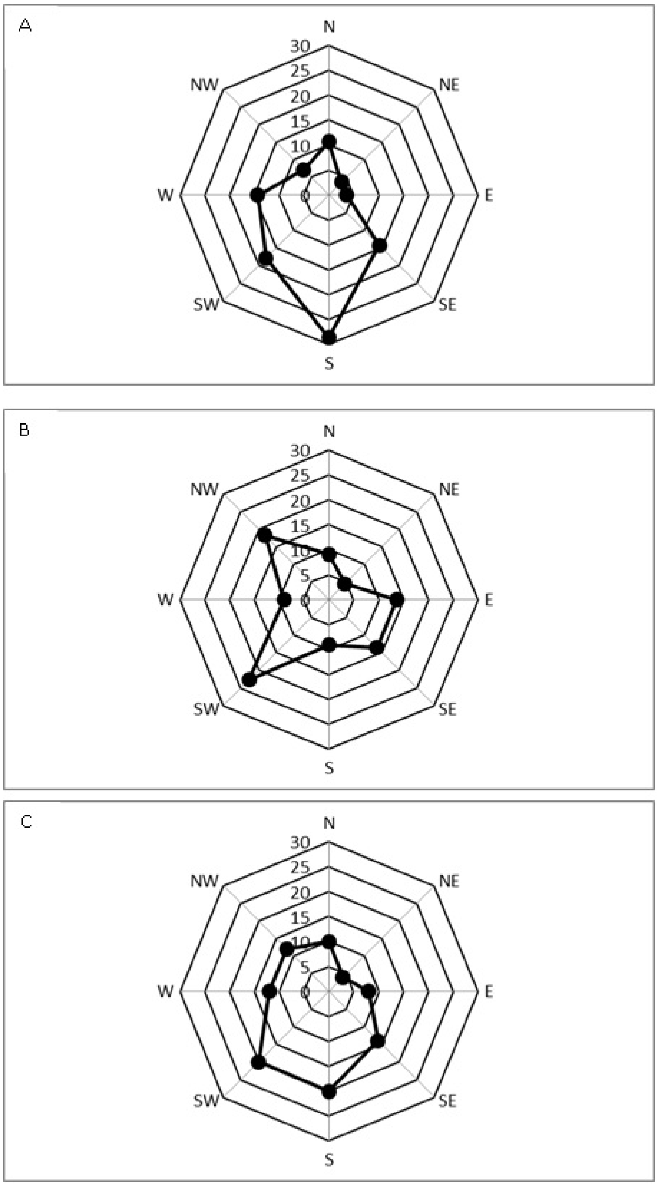

Fig 3. Number of bee nests (percent) sampled at Almas and Moreiras Farms accordingly to orientation of entrances to cardinal and collateral points, **A** Almas Farm, **B** Moreiras Farm, and **C** total.

Discussion

The results record formally for the first time the nesting of *P. seridoensis* in termite nests of *C. cyphergaster*. Although Lorenzon et al. (1999) observed this relationship, they did not identify the species and referred them as *P. aff. nigrior* and *Constrictotermes* sp. In a recent short note, Oliveira et al. (2016) cited the relation between *P. seridoensis* and *C. cyphergaster* however using the same citations referred above. Thus, here it is the first time that this specific relationship is formally recorded. Furthermore, *P. seridoensis* is considered a termitophile species *sensu stricto* (*sensu* Carrijo et al., 2012) because all the nests were found exclusively in termite nests. In addition, the distribution area of *P. seridoensis* is endemic to *caatinga* (Camargo & Pedro, 2003) and inserted in the distribution area of *C. cyphergaster*. *C. cyphergaster* is also commonly found in the central savanna of Brazil, Paraguay, Bolivia, and northern Argentina (Constantino, 1998), and is reported in the *caatinga* as the main species of termite that builds conspicuous nests in this biome (Godinho et al., 1989; Melo & Bandeira, 2004). However, it is noteworthy that recently Oliveira et al. (2016) found in the extreme northeast of the caatinga *P. seridoensis* colonies in association with termite nests of the genus *Microcerotermes* (14 bee colonies, 13 associated with *Microcerotermes indistinctus* Mathews and one with *Microcerotermes strunckii* Sörensen). As these authors did not find *C. cyphergaster* in the studied area, results showed that the occurrence of *P. seridoensis* is not at all restricted to the local availability of *C. cyphergaster*, and that the bee may occupy opportunistically nests of other termite species available in the environment, at least in the absence of *C. cyphergaster*.

Our data show that the termite nests hosting bees were supported by the larger species of trees in the area (mostly *Commiphora leptophloeos* and *Poincianella pyramidalis* = *Caesalpinia pyramidalis*). Barreto and Castro (2007) also observed in an arboreal *caatinga* other *Partamona* species (*P. rustica* Pedro & Camargo, and *P. cupira* (Smith)) nesting in *C. cyphergaster* termitaria supported mainly by *Commiphora leptophloeos, Psidium* sp., and *Poincianella pyramidalis (*= *Caesalpinia pyramidalis*) trees. *Commiphora leptophloeos* and *Poincianella pyramidalis* were recorded among the most common and phytosociologically important tree species at Almas Farm and São João do Cariri (Barbosa et al. 2007). Furthermore, Bezerra-Gusmão et al. (2013) demonstrated that in São João do Cariri, *C. cyphergaster* nests in the plant species with the higher values of importance, cover, volume, basal area, and density, *Poincianella pyramidalis (= Caesalpinia pyramidalis*), which was the species with the highest index of importance value and also that supported the greatest number of large-sized nests. This is related to the observation in this study that *P. seridoensis* nested only in large termite nests (as defined by Mélo & Bandeira, 2004), above 30 liters, which need large trees for support and comprise between 7-14% of total nests (Bezerra-Gusmão et al., 2013).

Another important observation is that *P. seridoensis* nests in active termite nests as noted by Camargo and Pedro (2003). All bee colonies found by Oliveira et al. (2016) were also nesting in active arboreal nests of termites of the genus *Microcerotermes*. However, in São João do Cariri, among 22 termite nests examined, Lorenzon et al. (1999) recorded 12 *P. seridoensis* nests (cited by authors as *P. aff. nigrior* and *Constrictotermes* sp.), half of them in inactive termite nests. Lorenzon et al. (1999) suggested that the occupation of the termite nests by the bees could cause the abandonment of the nest by the termites because the bees occupy the central position of the nests and they observed termite soldiers attacking the bees when the separation between the nests was destroyed. Although this needs to be tested, the low (6%) proportion of inactive termite nests with *P. seridoensis* nests, and the absence of agonistic behavior between the species, when the nests are not disturbed, suggests that both species live for a long time in association. It is noteworthy that *C. cyphergaster* produces seasonal polycalic nests, satellite nests connected to the large or "mother" nest by tunnels, thus, when the bees occupy the central position of the termite nests, the reproductives could stay in the other nest. In addition, we observed bee colonies that died after the death of termites, and inactive termite nests begin to crumble due to lack of maintenance. Thus, because they have perennial colonies, it is expected that the bees have been selected for nesting in enduring structures (Roubik, 1989, 2006).

Partamona rustica and *P. cupira* were also observed nesting in *C. cyphergaster* termitaria (Barreto & Castro, 2007; Miranda et al., 2015). In addition, Barreto and Castro (2007) observed that the nesting cavities were initially constructed by a parakeet called "jandaia" or "gangarra" (*Eupsittula cactorum* (Kuhl) = *Aratinga cactorum* (Kuhl) for reproduction. Despite *E. cactorum* occurs in Almas Farm (Araujo et al., 2012) it was not observed their reproductive nesting behavior in *C. cyphergaster* termitaria. Miranda et al. (2015) report that although termite nests with hollows made by this bird were found, no nesting activity of *P. rustica* in such cavities was observed. It is interesting to note that the number of large termite nests observed in the two study areas was higher than the number of colonies of bees, suggesting that the local availability of nesting sites is not a limiting factor in the area (*e.g.* Hubbell & Johnson, 1977; Eltz *et a*l., 2002). However, the behavior of the parakeet or other agent that makes the cavities in the termite nests can be a limiting factor and deserves further investigation.

The results showed that the nesting features of *P. seridoensis* are similar in the two areas. In Almas Farm the height of the termite nests with bee colonies were higher and this is related to the better conservation state of the Particular Natural Heritage Reserve of Almas Farm, and, consequently, higher vertical distribution of this area when compared to Moreiras Farm.

In our study, *P. seridoensis* presented the entrance less directed to the east/southeast which are the predominant wind directions in Cariri region (INMET, 2016). Preference for nest entrances non-oriented to the prevailing wind directions have also been observed in another stingless bee species, *Melipona bicolor schencki* Gribodo (Witter et al., 2010) and in solitary bees and wasps nesting in pre-existing cavities in trap nests (Martins et al., 2012). It is possible that the entrances protected from wind also have a relation with the rain and luminosity.

One interesting observation was that frequently the entrances of the nests were directed to other nearby colonies. Within the transect lines many neighboring colonies were observed that each colony successively had the entrance of its nest directed to the nearest colony, suggesting they maybe sister colonies. Further genetic analysis may confirm this fact.

Recently, four colonies of *Melipona subnitida* Ducke were observed nesting in *C. cyphergaster* termitaria (Carvalho et al., 2014) in the *caatinga*. Because this species nests in hollows of live trees (Martins et al., 2004), Carvalho et al. (op. cit.) discuss whether or not this opportunistic nesting habit of *M. subnitida* is an adaptation to shortage of pre-existing cavities in trees locally.

Caatinga ecosystem is one of the most vulnerable and threatened environments in Brazil. In addition to the severe climate conditions, deforestation, inadequate land use practices, and the use of firewood and charcoal, have increased the risk of desertification. The natural vegetation remains at only 54% of the original area, and the average rate of deforestation is 2,700 km^2 per year (MMA 2011). Nevertheless, Miranda et al. (2015) suggest that bee hunters are the most serious threat to *P. rustica* in caatinga, and intense human pressure is likely the main cause that nests have become rare or even absent from some areas of its distribution. Because pollination services provided by bees are important to the maintenance of ecosystem diversity and to agricultural production, many aspects of the nesting biology, ecology and genetics of bee species require detailed investigation, mainly in endemic areas, for applying this knowledge to practical species conservation and land management in the near future.

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