Ant (Formicidae) Assemblages Associated with *Piper* spp. (Piperaceae) in the Undergrowth of an Atlantic Rainforest Remnant in Southeastern Bahia, Brazil

by

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ABSTRACT

We studied ant assemblages associated with plants of genus *Piper* (Piperaceae) in the undergrowth of a fragment of Atlantic Forest in Southeastern of State of Bahia. The study was conducted in Serra Bonita Natural Reserve, in Camacan and Pau-Brazil municipalities. Hand collections of ants were made on four species of *Piper* during seven months; 56 ant species were collected. The genus *Camponotus*, *Pheidole* and *Crematogaster* were the most abundant. We highlighted the occurrence of *Linepithema* spp. given their scarce records in the region. Few ants were observed simultaneously (up to five) per plant during each survey. Rarefaction curves showed that collections were not exhaustive. The Shannon index showed that *Piper caldense* presented the largest ant diversity occurrence, perhaps due to its higher amount of resources available. The ant community in the undergrowth of the Atlantic forest appears to be diverse, although the species found in *Piper* constitute only a fraction of the diversity that really occurs in this stratum of the forest.

Keywords: Diversity, *Linepithema*, RPPN Serra Bonita.

INTRODUCTION

Ants have numerous attributes and are an example of ideal organisms for diversity studies, for their high numerical diversity and biomass dominance in almost every terrestrial landscape of the World (Brown 2000). These organisms are especially important as predators, prey, anti-herbivore agents, or as seed

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dispersers; the last aspect, for instance, has a relevant ecological function for the maintenance and regeneration of the forests (Ferreira 1998). Ants have a wide range of interactions with plants and other animals (Hölldobler & Wilson 1990, Beattie 1985, Jolivet 1996, Rico-Gray & Oliveira 2007), with which they show several types of associations: casual, obligatory, mutualistic or antagonistic. An example of these associations with plants is the use of domatia for the construction of ant nests; another is nutritional substances secreted by specialized structures in plants (secretory trichomes, extrafloral nectaries, pearl bodies, etc) given as an award for ant protection against herbivory (Freitas & Oliveira 1996, Gaume *et al.* 1997, Delabie & Ospina 2003, Rico-Gray & Oliveira 2007).

In addition to these associations, it is possible to find ants in virtually all tropical wet forest strata, from the litter and undergrowth, to the canopy of larger trees (Espírito Santo *et al.* 2007).

The Angiosperm family Piperaceae is Pantropical and typical of the undergrowth. It has approximately 2,000 species belonging to 10 genera. In the New World, species of Piperaceae are found from southern Mexico to Argentina (Figuereido & Sazima 2000). In Brazilian forests, the genus *Piper* (L.) includes 265 species (Silva *et al.* 2009). They are aromatic shrubs or small trees up to 10m high, with alternate leaves, fruit-shaped drupe and nodes in the articulations (Guimarães & Silva 2004).

Some species of the genus, such as *P. cenocladum* (C. D.C.) or *P. fimbriulatum* (C. D.C.), develop interactions with ants, in which the amount of secretor cells producing nutrients is directly related to the number of ants foraging on plants: a greater number of ants are related with a greater number of secretor structures (Risch & Rickson 1981, Letorneau & Dyer 1998, Fischer *et al.* 2003).

The shrub-herbaceous vegetation of the undergrowth stratus is important because it represents up to 50% of all plant species found in a tropical forest area (Gentry & Dodson 1987). This fact influences the diversity of ants and consequently the foraging strategies that they can present. The present study was developed in order to characterize the assembly of ants that live in the undergrowth stratum in a remaining area in the northern part of the Brazilian Atlantic forest.

MATERIALS AND METHODS

The study was conducted at the Private Reserve of Natural Heritage (RPPN) Serra Bonita (15°23'28.5"S 39°33'52.0"W), altitudes between 300 and 960 m (Amorim *et al.* 2009). The study area is located in the municipalities of Camacan and Pau-Brazil, in the State of Bahia, Brazil (Fig. 1). This area comprises one of the last remaining regions of altitude forest of the Atlantic Forest Central Corridor (Ministério do Meio Ambiente 2006), with 7,500 hectares. About 50% of the area is covered by primary forest and the remainder is formed by a mosaic of vegetation in various stages of succession, including secondary forest, cabruca agrosystem (agro-forestry, where the small vegetation is removed but the largest trees maintained to provide shade to the cacao trees, see Schroth *et al.* 2011) and pastures. The vegetation of the area ranges from tropical rain forest with elements of semideciduous

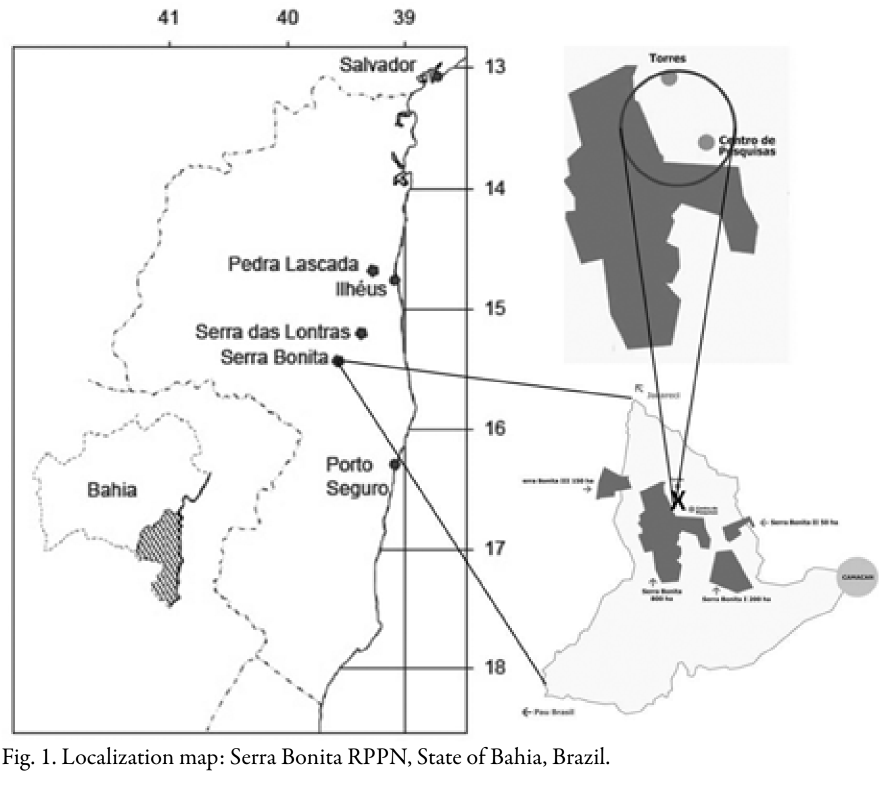

Fig. 1. Localization map: Serra Bonita RPPN, State of Bahia, Brazil.

forest in the lower parts, to submontane rainforest (cloud forest) high in the mountains (Amorim *et al*. 2009).

120 individuals of the plants *Piper caldence* C. D.C., *P. cernuum* Vell., *P. hispidum* Sw. and *P. umbellatum* L. (30 individuals per species) were randomly selected and labeled. *P. caldense* is characterized by having plants up to three meters high, with leaves glabrous, bright, up to 18 cm in length; *P. hispidum* has leaves of similar size to *P. caldence*, but are distinguished by their opaque color and pubescence. In addition, plants of *P. hispidum* may grow up to five meters high. This species is able to expand laterally more than any other species studied. *P. umbellatum* grows up two meters high, and presents few umbeliform leaves (up to six leaves per plant); *P. cernuum* was the largest species in the population studied here, with up to 8 meters high, and the species presents larger leaves (up to 40 cm long), with domatia at the base of the petiole, a feature found only in this species among those here studied.

The plants were selected with an interval of 20 m between successive plants aiming to decrease the probability of ants collected in two neighboring plants belonging to the same colony. The plants were labeled with the purpose of making observations on the same plants during all sampling series.

Ant sampling

Field work was conducted monthly from September to December of 2010, and March to May of 2011. In each field trip we made surveys of four days and four nights, for a total of eight days per field trip. In each case, surveys began at 08:00 hour (day), or 18:00 (night).

We collected only foraging ants found on plants manually. The ants were identified and vouchers were deposited in the collection of the Myrmecology Laboratory (CPDC) of the Centro de Pesquisa do Cacau (CEPLAC) at Ilhéus, Bahia, Brazil.

Data Analysis

We estimated the richness and diversity of ant communities on plants using the Shannon and Chao1 indexes (Colwell 2006). The results were analyzed using the statistical software Past 1.74 (Hammer *et al*. 2001). In addition, we constructed rarefaction curves using Mau Tau, Bootstrap and Chao2 indexes, in order to estimate and compare the ant richness on the plants. The data were analyzed using the statistical program EstimateS 8.2 (Colwell 2006). Finally, we compared ant assemblages with a dendrogram of similarity of Bray-Curtis using Past 1.74 (Hammer *et al.* 2001).

RESULTS

We collected 56 species of ants on the 120 individuals of *Piper* belonging to the four species sampled in this study. The ants collected are distributed into four subfamilies and 16 genera. Myrmicinae showed the largest number of occurrences, followed by Formicinae, Dolichoderinae and Ponerinae (Table 1). *P. caldense* and *P. hispidum* showed the greatest richness with 48.2% and 44.6% respectively of the species collected (Table 1).

Pheidole, *Camponotus* and *Solenopsis* were the genera with the larger numbers of species, corresponding to 19.6%, 17.9% and 12.5% of the ant diversity respectively. Other significant genera were *Camponotus, Pheidole, Crematogaster* and *Linepithema* representing respectively 8.2%, 8.2%, 19.8% and 31.0% of individual occurrences.

Only a few ants were found nesting on the plants, except *Wasmannia auropunctata*, *Linepithema iniquum* and *L. neotropicum*, in domatia of *P. cernuum*; *Dolichoderus attelaboides* was found establishing initial nests in the abaxial region of leaves of *P. cernuum* forming a concave structure where the ants nested. *Crematogaster longispina* was found nesting in the abaxial region of the leaves of *P. hispidum*, but in this case without forming a concavity below the leaves.

The Dolichoderinae *D. attelaboides* and *L. iniquum*, and the Myrmicinae *W. auropunctata* were common on the four species of *Piper*. It must be observed that the occurrence of *L. iniquum*, *L. leucomelas*, *L. neotropicum* and *L. pulex* represents the first reports of these ants foraging in plants of the genus *Piper* in the Atlantic forest, as well as the first record of most of these species for the region.

The Shannon index showed higher diversity in *P. caldense* and *P. hispidum*, respectively (Table 1). Regarding the $Chao_1$ index, *P. hispidum* and P. *cernuum* showed a very high number of expected species (187. 9), which is expected to be an artifact due to the large number of singletons obtained in the series of data.

Rarefaction curves obtained did not reach asymptotes for any of the plants studied (Fig. 2).

Table 1. Ant species collected, and related ecological characteristics, on four species of *Piper*, September 2010 to May 2011, Serra Bonita RPPN, Bahia, Brazil. *probable artifacts due to the large proportion of singletons in the sampled series.

Taxon	***P. caldense***	***P. hispidum***	***P. cernuum***	***P. umbellatum***
Formicidae				
Ponerinae				
Odontomachus haematodus	0	0	0	1
Odontomachus hastatus	0	1	8	1
Pachycondyla crenata	4	1	0	0
Pachycondyla schultzi	0	1	0	0
Dolichoderinae				
Dolichoderus attelaboides	5	1	5	6
Linepithema iniquum	6	1	31	11
Linepithema leucomelas	1	0	1	2
Linepithema neotropicum	14	0	3	9
Linepithema pulex	0	4	1	0
Tapinoma sp.1	0	0	3	0
Tapinoma sp.2	1	0	0	0
Formicinae				
Brachymyrmex sp.1	0	1	1	0
Brachymyrmex heeri	3	3	4	0
Crematogaster sp. gp. *heeri*	0	0	1	1
Camponotus (Myrmaphaenus) sp.3	2	0	0	0
Camponotus (Myrmaphaenus) sp.4	2	1	0	0
Camponotus (Myrmaphaenus) sp.5	0	0	0	1
Camponotus (Tanaemyrmex) sp.1	0	2	0	1
Camponotus (Tanaemyrmex) sp.2	1	1	0	0
Camponotus agra	0	1	0	0
Camponotus cingulatus	0	3	0	2
Camponotus crassus	1	0	0	0
Camponotus depressus	0	1	0	0
Camponotus nidulans	3	0	0	0
Myrmelachista sp.	0	0	0	1
Nylanderia guatemalensis	0	0	1	1
Paratrechina longicornis	0	0	2	0
Myrmicinae				
Cephalotes angustus	0	0	0	1
Cephalotes pavonii	1	0	0	0
Crematogaster brasiliensis	0	1	0	0
Crematogaster (gr. *limata*) sp.	2	0	5	0

Table 1. Ant species collected, and related ecological characteristics, on four species of *Piper*, September 2010 to May 2011, Serra Bonita RPPN, Bahia, Brazil. *probable artifacts due to the large proportion of singletons in the sampled series (continued).

Taxon	***P. caldense***	***P. hispidum***	***P. cernuum***	***P. umbellatum***
Crematogaster longispina	5	9	15	0
Crematogaster nigropilosa	3	7	6	0
Pheidole (gr. *flavens*) sp.1	2	1	0	0
Pheidole (gr. *fallax*) sp.2	0	0	1	7
Pheidole sp.4	1	0	0	0
Pheidole (gr. *fallax*) sp.5	1	0	0	0
Pheidole sp.6	0	1	0	0
Pheidole sp.7	2	0	0	0
Pheidole sp.8	0	1	0	0
Pheidole sp.9	0	0	1	0
Pheidole sp.10	0	0	1	0
Pheidole sp.11	0	0	0	1
Pheidole sp.12	1	0	1	0
Procryptocerus goeldii	1	0	0	0
Procryptocerus hylaeus	0	1	0	0
Procryptocerus pictipes	0	1	0	0
Procryptocerus sp.	3	4	1	0
Solenopsis sp.1	0	0	0	1
Solenopsis sp.2	0	0	1	0
Solenopsis sp.3	0	1	0	0
Solenopsis sp.4	1	0	0	0
Solenopsis sp.5	1	0	0	0
Solenopsis sp.6	0	0	1	0
Solenopsis sp.7	1	0	0	0
Wasmannia auropunctata	3	19	5	1
Observed ant species / host plant	27	25	23	18
Observed species compared with the whole study (%)	48.2	44.6	41.1	32.1
H’	3.0	2.9	2.4	2.3
Exclusive species	12	9	6	6
Exclusive/Observed species (%)	21.4	16.1	10.7	10.7
Expected ant species ($Chao_1$)/ host plant	41	187*	95*	47
Sampling exhaustivity (%)	66.0	13.4	26.3	38.3

Although small differences among the studied plants were observed, Fig. 3 shows that the composition of ant assemblages around the four *Piper* species has a high degree of similarity, especially between *P. caldense* and *P. cernuum*.

DISCUSSION

In our study, we used only hand collection because the structure and size of plants (never higher than 2.50 m for those observed) allowed ant observation and collection, and evaluation of the whole plants, without the use of another kind of sampling methodology. In comparison with similar studies, such as Espirito Santo *et al.* (2007), Sirqueira *et al.* (2006) or Schütte *et al.* (2007), the observed diversity was lower. It is then rather possible that these differences are due to the methodology used, because these studies combined manual collection, chemical shock and/or entomological umbrella, increasing the number of sampled species.

Of the 56 ant species collected, 59% were found on a single plant species (Table 1), 21.4% of these occurred only on *P. caldense,* and 16.1% only on

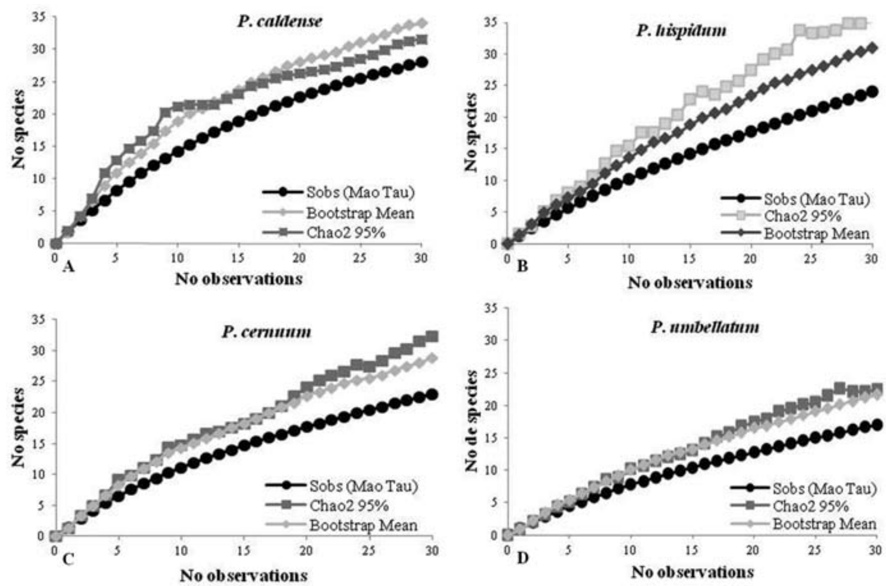

Fig. 2. Rarefaction curves for the plants studied in the Serra Bonita RPPN, Bahia, Brazil. September 2010-May 2011: A) *P. caldense*, B) *P. hispidum*, C) *P. cernuum*, D) *P. umbellatum*.

P. hispidum. Our results may be related to random foraging ants on plants at the time of collection or, possibly due to the occurrence of some kind of interaction between ants and plants, related with the greater occurrence of secretory structures (Rickson & Risch 1984) that would not be present in the other two plant species studied.

Repeated collections of the same species of ants in *Piper* suggest a non-randomized behavioral pattern, reflected in the development of ecological relationships with these plants. Similar results were found by Rickson & Risch (1984) and Fischer *et al.* (2002, 2003), where close relationships among *Piper cenocladum*, *P. fimbriulatum*, *P. obliquum* and *P. sagittifolium,* and ants of the genus *Pheidole* were found; plants provide shelter (domatia) and food (food bodies) (Risch & Rickson 1981), while, in exchange for those services, the ants protect the plant against predators.

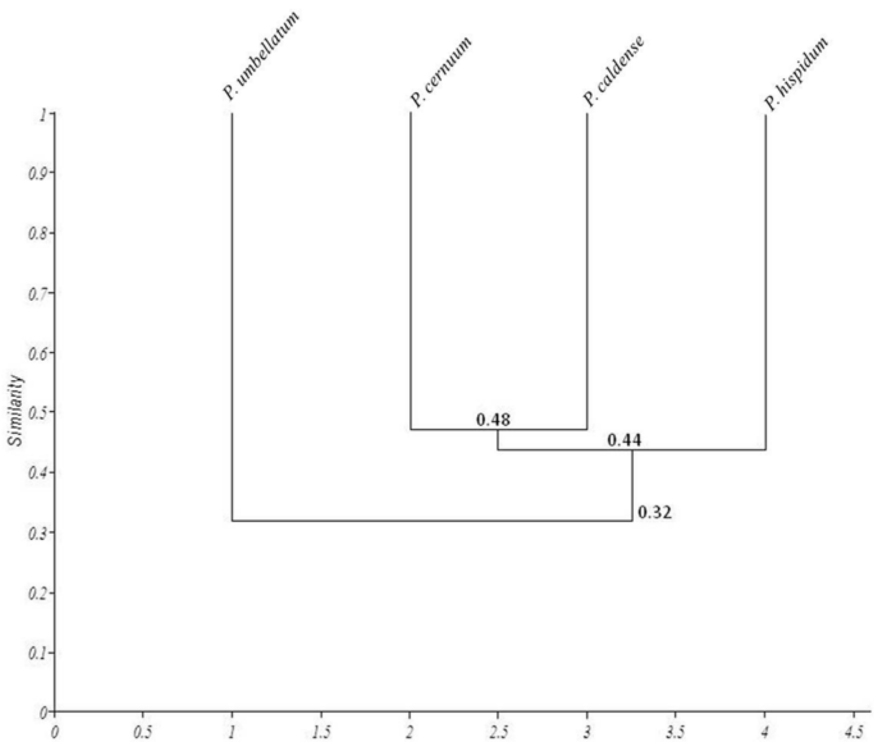

Fig. 3. Bray-Curtis similarity dendrogram comparing the ant assemblages according with their occurrences on four species of the genus *Piper*: *P. caldense*; *P. hispidum*; *P. cernuum* and *P. umbellatum*. Serra Bonita RPPN, Bahia, Brazil, September 2010 – May 2011.

Three species of ants were common to all plants (Table 1): *D. attelaboides*, *L. iniquum* and *W. auropunctata*. The genera *Wasmannia* and *Dolichoderus* were reported by Agosti *et al.* (2000) as generalists foragers present in most tropical and temperate regions. Ants of the genus *Linepithema* are common in the Neotropical ant-fauna, and they can be found in a wide variety of environments such as grasslands and montane habitats along the Neotropic Region (Wild 2007). *L. iniquum* (Mayr), *L. leucomelas* (Emery), L. *neotropicum*, and *L. pulex* were reported by Wild (2007) as widely distributed generalist ants, but these ants are scarcely found in this region of Brazil. We observed ants of the genus *Dolichoderus* foraging in the infructescenses of *P. umbellatum*, but active behavior of seed dispersal was not detected. However, judging by the size of seeds, it is presumed that this interaction is possible, mainly by generalist species, such as *D. attelaboides*.

On the other hand, the diversity of ants observed on the four *Piper* species was not significantly different. This is due to the fact that plants of this genus prefer open habitats of the undergrowth, especially open areas or clearings inside the forest (Risch & Rickson 1981). The preferences for analogous environments may explain the similarity between the diversity of ants collected in four plant species studied here.

The major difference between the diversity of ants was observed between *P. caldense* (H'=3) and *P. umbellatum* (H'=2.3). Given that these species have similar sizes, the differences found could be explained by the greatest number of observations of occurrences of ants in the leaves of *P. caldense* where they seem to be collecting some unidentified substances, behavior which was not observed in *P. umbellatum*. Some species of *Piper* are known to possess secretor trichomes and pearl bodies that can provide nutrients (Fisher *et al.* 2002) attractive to ants.

According the Bray-Curtis dendrogram (Fig. 3), there exists a greater similarity between *P. caldense*, *P. cernuum* tan between *P. cernuum* and *P. hispidum*. This fact highlights that characteristics such as the occurrence of domatia at the base of the petioles in *P. cernuum,* or the occurrence of food bodies in *P. caldense* and *P. hispidum* as well as in other species of *Piper* (Fisher *et al.* 2002) have a significant influence on time of foraging of ants in these species.

As the rarefaction curves do not stabilize (Fig. 2), the ant diversity found in the samples represents only a fraction of the total number of species that might be found on the plants. Consequently, hand collection of ants is certainly not the appropriate methodology to quantify the diversity in this type of habitat (Itô *et al.* 1998).

The assemblage of ants associated with *P. umbellatum* possessed the lowest diversity, and the least similarity of the four species of *Piper*. This could be associated with the reduced occurrence of differentiated structures for feeding ants, or for the presence of potentially toxic or repellent substances in the leaves of this plant (i.e. Isoasarone) (Checksum & Fasihuddin 2002), that could cause ants to avoid foraging in this species.

The active foraging behavior of ants observed on the four species of *Piper* can be considered as a study case of ant-plant interactions in the undergrowth of a tropical forest. The ants of the undergrowth may be especially important to the regeneration, development and maintenance of the rainforest, given their ecologically important role as predators, prey and seed dispersers (see also Dáttilo *et al.* 2009, Leal 2005, Rico-Gray & Oliveira 2007).

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