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A preliminary survey of nocturnal ants, with novel modifications for collecting nocturnal arboreal ants

***David Emmanuel M. General[1], Perry Archival C. Buenavente[2], Lillian Jennifer V. Rodriguez[3]**

[1] *University Researcher, Museum of Natural History, University of the Philippines Los Baños, Los Baños, Laguna 4031, Philippines; and Research Associate, National Museum of Natural History, Malate, Manila, Philippines.*
[2] *Researcher, Zoology Division, National Museum of Natural History, Malate, Manila, Philippines.*
[3] *Assistant Professor, Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines.*

(Email: dmgeneral@up.edu.ph)

Abstract

Ants were surveyed in a 100-m horizontal transect by employing three collecting techniques: leaf litter sifting and Winkler extraction, pitfall trapping, and beating of low vegetation. Ants were surveyed during the day and again at night to record the temporal behavior of the ants. In aggregate, 23 species of ants were collected only during the day, 24 species during the night, and 36 species during both day and night. There was a large overlap between diurnal and nocturnal ground-foraging and leaf-litter ant communities. On the other hand, the diurnal arboreal ant community seems to be distinct from the nocturnal arboreal ant community (Jaccard Distance = 0.85). Our results suggest that nocturnal arboreal ants are likely sources of new discoveries. The novel modifications we present here may help address this knowledge gap for ants and other nocturnal arboreal arthropods.

Keywords: *ant survey techniques, nocturnal arboreal ants, novel modification, temporal behaviour of ants.*

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Introduction

The diversity of ants is surveyed using standardized quantitative collecting methods and traps, informing studies of ant communities and assembly rules. Ant surveys are usually conducted during the day. Collecting traps are typically deployed during the day and after some time, also retrieved during the day (Bestelmeyer *et al*. 2000; Delabie *et al*. 2000). Pitfall traps may be kept in place for 48-72 hours or longer, e.g., Lee and Guénard (2019). Malaise traps are usually deployed for several days, depending on how fast the collecting jars are filled by specimens, e.g., Lessard *et al.* (2007).

Leaf litter sifting and Winkler extraction, e.g., Lessard *et al.* (2007), and beating of low vegetation, e.g. Fisher (2002), are usually performed during the day.

Safety, convenience, or habit may be the main reasons for collecting ants during the day. However, the daytime conduct of these surveys commonly overlooks the nocturnal activity of ants and biases the estimation of the diversity, richness, and assemblage of ants in favor of diurnally active ant species. Moreover, ecological and behavioral information may be lost by running traps for several days or collecting ants only during the day.

Nocturnal ant communities are poorly studied, although specimens of nocturnal ants are likely to be among the catch of long-running traps. Unfortunately, there is no infallible way to distinguish diurnal ants from nocturnal ants by

simply examining their morphology.

Rare ants may be more common at night. Wong and Yong (2017) observed the activities of a nest fragment of *Tyrannomyrmex rex* Fernandez, 2003 in a laboratory setting and inferred that the ant species may be nocturnal, based on the increased activity of the ants in the late afternoon. It is possible that nocturnal ants escape the leaf litter sifting and beating methods used by researchers.

In this paper, we introduce modifications to the typical ant collecting methods to accomplish the separate collection of diurnal and nocturnal ants. We also provide an initial data set and analysis of these data. We hope that this paper generates interest in the study of the temporal behavior of ants.

Materials and Methods

This study was conducted in March 2019 in Mt. Isarog Natural Park, in Del Rosario Village, Municipality of Pili, Camarines Sur Province, Luzon Island, Philippines.

The study site, at 600 meters above sea level, is a disturbed second-growth forest, dominated by a climbing bamboo, *Dinochloa* sp. The area is part of the watershed of the Metro Naga Water District (MNWD). MNWD planted native and non-native trees within their administered watershed area. Field collections are covered by Gratuitous Permit R5-105 issued to DEMG by the Department of Environment and Natural Resources. Specimens are deposited in the Entomological Collection of the Museum of Natural History of the University of the Philippines Los Baños.

A modified ALL Protocol (Agosti and Alonso, 2000) was employed to sample the diurnal and nocturnal leaf litter-residing, ground-foraging, and arboreal ants. The ALL Protocol was abbreviated in length and modified with the addition of beating of low vegetation. Ten transect plots, 5-m radius, were established in a 100-m horizontal transect. Within each transect plot, the following collecting techniques were employed: leaf litter sifting and Winkler extraction for leaf litter ants; pitfall trapping for ground-foraging ants; and beating of low vegetation for arboreal ants. The techniques were performed during the day and again at night.

Leaf litter sifting and Winkler extraction: The leaf litter from a randomly placed 1-m^2 quadrat was sifted and the siftate placed in Winkler bags for 24 hours. Leaf litter was collected and sifted during the day and again at night, but from a different part of the plot. Leaf litter collected during the day was labelled with "LLD + plot number", while leaf litter collected at night was labelled with "LLN + plot number". These labels were placed in the collecting vial of the Winkler bag.

Pitfall trapping: A plastic cup (70 mm diameter, 85 mm depth) was placed in the ground, flush to the level of the ground. The cup was half-filled with a weak soapy solution (1-2 drops of liquid dish detergent in 1 litre clean water). For diurnal ants, the pitfall traps were in place from just before sunrise (local time 5:30 a.m.) to sunset (local time 6:00 p.m.). At the end of the trapping period, all the arthropods were collected and immediately placed in labelled 10ml vials containing fresh water. The soapy water in the traps was topped up to the previous level. The rinsed specimens were then transferred to labelled vials containing 95% ethanol. For nocturnal ants, the traps were in place from sunset (local time 6:00 p.m.) to just before sunrise (local time 5:30 a.m.). At the end of the trapping period, all the arthropods were collected and processed as with the diurnal pitfall catch. The diurnal pitfall collection was labelled with "PFD + plot number", while the nocturnal pitfall collection was labelled with "PFN + plot number". These labels were placed in the appropriate vials.

Beating of low vegetation: This method requires a team of 4-6 persons (Fig. 3), composed of: a striker, a person holding the beating sheet, a secretary to keep track of the branches struck, a person to hold the vial for the specimens, and others to manually collect the ants and bycatch from the beating sheet. During the establishment of the transect, five suitable (leafy and accessible) branches were selected within the plot. The branches were labelled with long pieces of flagging tape (~70 cm long) with the plot number and branch number, e.g., a branch labelled "1-1" refers to Plot 1, Branch 1. The long flagging tape allowed the team to locate the proper branches at night (compare Figs. 1 and 2). The labelling of the branches ensured that the

branches sampled during the day would be sampled again at night. Each branch was struck five times over a 1-m^2 beating sheet. Ants and other arthropods that fell on the beating sheet were hand-collected and placed in a vial with 95% ethanol. The collections from the five branches in a plot were pooled into one labelled vial. The diurnal beating collection was labelled "BD + plot number", while the nocturnal beating collection was labelled "BN + plot number". These labels were placed in the appropriate vials.

Two attempts to replicate this study at a different site of the same mountain were frustrated by bad weather.

Figure 1. Daytime view of branches of low vegetation tagged with long flagging tape. (Image courtesy: Emerson Y. Sy)

Figure 2. Nighttime view of same branches of low vegetation tagged with long flagging tape, at a slightly different angle. (Image courtesy: Emerson Y. Sy)

Figure 3. Team effort in collecting specimens from beating of low vegetation, at daytime. DEMG with grey shirt, PACB with dark blue shirt, LJVR as secretary, with notebook. (Image courtesy: Jasmin Meren).

Laboratory Work: The field ethanol was replaced with fresh 95% ethanol within 3 days after collection. The collections were sorted into morphospecies and placed in individual labelled vials. The morphospecies were then identified using available keys. The following keys were used in determining the genus of the specimens: Bolton (1994), Borowiec (2016), General and Alpert (2012), LaPolla *et al.* (2010), Schmidt and Shattuck (2014), and Ward *et al.* (2016). The following species-level references and keys were used for individual genera: *Anochetus* (Brown, 1978); *Crematogaster* (Hosoishi and Ogata, 2016, 2019); *Eurhopalothrix* (Taylor, 1968); *Lordomyrma* (Taylor, 2012); *Myopias* (Probst *et al*., 2015); *Myrmicaria* (Zettel *et al*., 2018); *Myrmoteras* (Zettel and Sorger, 2011); *Odontomachus* (Sorger and Zettel, 2011); *Odontoponera* (Yamane, 2009); *Parasyscia* (Brown, 1975; Borowiec, 2016); *Pheidole* (Eguchi, 2001); *Polyrhachis* subgenus keys: Dorow, 1995; Kohout, 2008; *Polyrhachis (Myrma) cyaniventris* species group: (Sorger and Zettel, 2010); *Ponera* (Wilson, 1957; Taylor, 1967; Leong *et al*., 2019); *Pristomyrmex* (Wang, 2003; Zettel, 2006); *Recurvidris* (Bolton, 1992; Zettel, 2008); *Strumigenys* (Bolton, 2000); *Technomyrmex* (Bolton, 2007); *Tetramorium* (Bolton, 1976, 1977); *Tetraponera* (Ward, 2001); *Vombisidris* (Bolton, 1991). We also referred to online resources to check our determinations (AntWeb, 2019; AntWiki, 2019). The Jaccard Similarity Index, J = a/(a+b+c), where a is the number of species common to both collections and b and c are unique to each collection (Gotelli *et al*., 2011) and its complement, the Jaccard Distance, 1-J, were calculated. The Jaccard Indices were then subjected to cluster analysis to visualize the grouping of the transect plots. Dissimilarity cluster analysis was performed in R v. 3.5.2 (R Core Team, 2018) using the vegan package v. 2.5-5 (Oksanen *et al*., 2019).

Results

A total of 1,253 ants were collected, representing 83 species in 40 genera and five subfamilies. In aggregate, 23 species were collected only during the day, 24 species were collected only during the night, while 36 species were collected during both day and night (Table 1).

Table 1. Aggregate list of ant species collected during diurnal and nocturnal sampling, using pitfall trapping, leaf litter sifting, and beating of low vegetation, Mt. Isarog Natural Park, San Isidro Village, Municipality of Pili, Province of Camarines Sur, Philippines. (sp. nr. = species near; cf. = compared with).

Diurnal Only (n=23)	Diurnal and Nocturnal (n=36)	Nocturnal Only (n=24)
Camponotus sp5	*Anochetus graeffei*	*Brachyponera* sp1
Carebara sp3	*Anochetus incultus*	*Camponotus* sp2
Cataulacus catuvolcus	*Brachyponera obscurans*	*Camponotus* sp3
Centromyrmex feae	*Camponotus* sp1	*Camponotus* sp4
Colobopsis leonardi	*Camponotus* sp6	*Colobopsis* sp3
Colobopsis sp1	*Carebara macca*	*Ectomomyrmex* sp1
Crematogaster rothneyi	*Carebara* sp1	*Hypoponera* cf. *opaciceps*
Dilobocondyla sp1	*Carebara* sp2	*Lordomyrma diwata*
Lepisiota cf. *chapmani*	*Colobopsis horrens*	*Myopias bidens*
Leptogenys diminuta	*Crematogaster philippinensis*	*Myrmecina* sp1
Odontoponera denticulata	*Dolichoderus thoracicus*	*Paraparatrechina* sp2
Parasyscia rufithorax	*Eurhopalothrix philippina*	*Pheidole cariniceps*
Pheidole sp. nr. *bugi*	*Hypoponera* sp1	*Pheidole quadricuspis*
Polyrhachis cyaniventris	*Hypoponera* sp2	*Pheidole* sp1
Polyrhachis parabiotica	*Myrmicaria* sp1	*Pheidole tjibodana*
Polyrhachis saevissima	*Myrmoteras mcarthuri*	*Polyrhachis* cf. *hippomanes*
Ponera sp. nr. *wui*	*Odontomachus banksi*	*Polyrhachis illaudata*
Recurvidris sp1	*Paraparatrechina* sp1	*Strumigenys eggersi*
Strumigenys pedunculata	*Pheidole aglae*	*Strumigenys euryale*
Syllophopsis sp2	*Pheidole fervens*	*Strumigenys* sp1
Temnothorax sp1	*Pheidole* cf. *hortensis*	*Strumigenys* cf. *synchysis*
Tetramorium sp1	*Pheidole kikutai*	*Tapinoma* sp1
Tetraponera allaborans	*Pheidole* sp. nr. *parva*	*Tetramorium pacificum*
	Pheidole rabo	*Vombisidris* sp1
	Pheidole sp. nr. *sayapensis*	
	Plagiolepis sp1	
	Ponera sp1	
	Prenolepis sp1	
	Pristomyrmex collinus	
	Solenopsis sp1	
	Strumigenys koningsbergeri	
	Strumigenys mirifica	
	Syllophopsis sp1	
	Technomyrmex sundaicus	
	Tetramorium aspersum	
	Tetramorium khnum	

Leaf litter ants: Fifty-one species were collected from the leaf litter (Table 2). Twelve species were collected only during the day, 15 species were collected only during the night, while 24 species were collected from both day and night sampling of the leaf litter. Figure 4 illustrates the dissimilarity index between diurnal and nocturnal leaf litter collections.

Table 2. List of leaf litter-inhabiting ant species encountered during diurnal and nocturnal leaf litter sifting (LL) and Winkler extraction, Mt. Isarog Natural Park, San Isidro Village, Municipality of Pili, Province of Camarines Sur, Philippines. (sp. nr. = species near).

Diurnal LL (n=12)	Diurnal and Nocturnal LL (n=24)	Nocturnal LL (n=15)
Carebara sp3	*Anochetus graeffei*	*Brachyponera* sp1
Centromyrmex feae	*Anochetus incultus*	*Camponotus* sp1
Colobopsis horrens	*Brachyponera obscurans*	*Hypoponera opaciceps*
Parasyscia rufithorax	*Carebara macca*	*Lordomyrma diwata*
Pheidole hortensis	*Carebara* sp1	*Myopias bidens*
Pheidole kikutai	*Carebara* sp2	*Odontomachus banksi*
Ponera sp. nr. *wui*	*Crematogaster philippinensis*	*Pheidole aglae*
Recurvidris sp1	*Eurhopalothrix philippina*	*Pheidole cariniceps*
Strumigenys mirifica	*Hypoponera* sp1	*Pheidole fervens*
Strumigenys pedunculata	*Hypoponera* sp2	*Pheidole tjibodana*
Syllophopsis sp2	*Myrmicaria* sp1	*Strumigenys eggersi*
Temnothorax sp1	*Myrmoteras mcarthuri*	*Strumigenys euryale*
	Paraparatrechina sp1	*Strumigenys synchysis*
	Pheidole sp. nr. *sayapensis*	*Tetramorium aspersum*
	Pheidole rabo	*Tetramorium pacificum*
	Plagiolepis sp1	
	Ponera sp1	
	Prenolepis sp1	
	Pristomyrmex collinus	
	Solenopsis sp1	
	Strumigenys koningsbergeri	
	Syllophopsis sp1	
	Technomyrmex sundaicus	
	Tetramorium khnum	

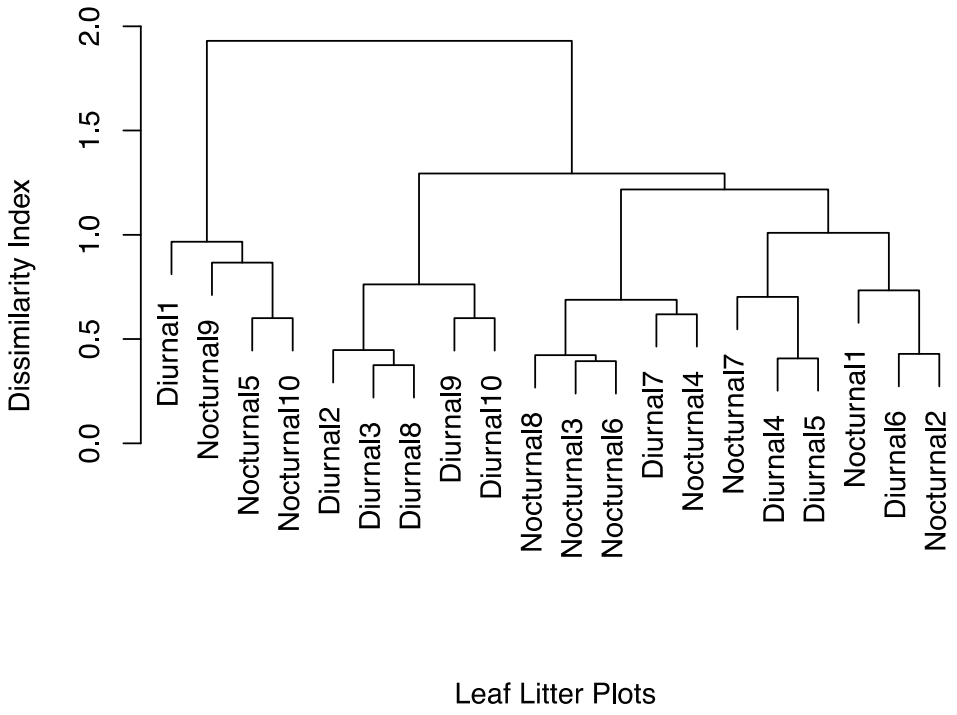

Figure 4. Dendrogram illustrating the dissimilarity index between diurnal and nocturnal leaf litter collections, by transect plot. Numbers refer to the transect plot.

Ground-foraging ants: Twenty-one species were collected from pitfall traps (Table 3). Four species were found only during the day, eight species were collected only at night, while nine species were collected from both day and night pitfall trapping. Figure 5 illustrates the dissimilarity index between diurnal and nocturnal pitfall collections.

Table 3. List of ground-foraging ant species encountered during diurnal and nocturnal pitfall trapping (PF), Mt. Isarog Natural Park, San Isidro Village, Municipality of Pili, Province of Camarines Sur, Philippines. (sp. nr. = species near).

Diurnal PF (n=4)	**Diurnal and Nocturnal PF (n=9)**	**Nocturnal PF (n=8)**
Camponotus sp5	*Brachyponera obscurans*	*Ectomomyrmex* sp1
Dolichoderus thoracicus	*Carebara macca*	*Eurhopalothrix philippina*
Odontoponera denticulata	*Crematogaster philippinensis*	*Myrmecina* sp1
Recurvidris sp1	*Hypoponera* sp2	*Pheidole cariniceps*
	Myrmicaria sp1	*Pheidole rabo*
	Odontomachus banksi	*Strumigenys mirifica*
	Pheidole hortensis	*Strumigenys* sp1
	Pheidole sp. nr. *parva*	*Technomyrmex sundaicus*
	Solenopsis sp1	

Figure 5. Dendrogram illustrating the dissimilarity index between diurnal and nocturnal pitfall collections, by transect plot. Numbers refer to the transect plot.

Table 4. List of arboreal ant species encountered during diurnal and nocturnal beating (BG) of low vegetation, Mt. Isarog Natural Park, San Isidro Village, Municipality of Pili, Province of Camarines Sur, Philippines. (sp. nr. = species near; cf. = compared with).

Diurnal BG (n=18)	Diurnal/Nocturnal BG (n=5)	Nocturnal BG (n=14)
Camponotus sp1	*Camponotus* sp6	*Camponotus* sp2
Camponotus sp5	*Colobopsis horrens*	*Camponotus* sp3
Cataulacus catuvolcus	*Crematogaster philippinensis*	*Camponotus* sp4
Colobopsis leonardi	*Dolichoderus thoracicus*	*Colobopsis* sp3
Colobopsis sp1	*Pheidole aglae*	*Paraparatrechina* sp1
Crematogaster rothneyi		*Paraparatrechina* sp2
Dilobocondyla sp1		*Pheidole cariniceps*
Lepisiota cf. *chapmani*		*Pheidole quadricuspis*
Leptogenys diminuta		*Plagiolepis* sp1
Myrmicaria sp1		*Polyrhachis hippomanes*
Pheidole sp. nr. *bugi*		*Polyrhachis illaudata*
Pheidole kikutai		*Prenolepis* sp1
Polyrhachis cyaniventris		*Tapinoma* sp1
Polyrhachis parabiotica		*Vombisidris* sp1
Polyrhachis saevissima		
Tetramorium aspersum		
Tetramorium sp1		
Tetraponera allaborans		

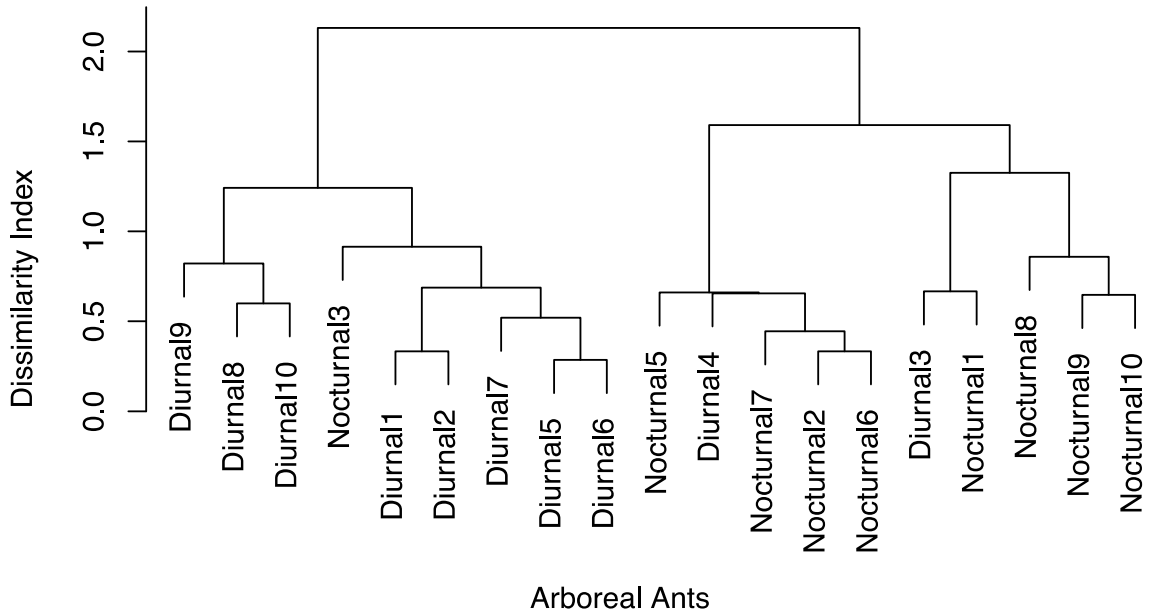

Figure 6. Dendrogram illustrating the dissimilarity index between diurnal and nocturnal beating collections, by transect plot. Numbers refer to the transect plot.

Arboreal ants: Thirty-seven species were collected from beating of low vegetation (Table 4). Eighteen species were found only during the day, 14 species were collected only at night, while 5 species were collected from both day and night beating of low vegetation. Figure 6 illustrates the dissimilarity index between diurnal and nocturnal beating collections.

Comparison between diurnal and nocturnal collections

Jaccard indices and Jaccard distance values were computed for the collections by method as well as for the pooled collection (Table 5). The most distinct ant communities were recorded by the Beating method, while the most similar communities were recorded by the leaf litter sifting.

Table 5. Jaccard similarity indices and Jaccard distance values of the diurnal and nocturnal ant communities, by collecting method and pooled collection.

Collection Method	Jaccard Index	Jaccard Distance
Leaf litter sifting	0.47	0.53
Pitfall trapping	0.43	0.57
Beating	0.15	0.85
Pooled collection	0.43	0.57

Discussion

The novel modifications to the beating technique involve: (a) forming a team and assigning a "secretary", a team member to keep track of which branches have been sampled (Fig. 3); and (b) labeling the branches with long, numbered flagging tape. Having a "secretary" prevents confusion and mistakes in sampling the labelled branches. Labeling the branches allows not only finding the branches at night and keeping track of what had been sampled, but more importantly, the direct comparison of the arboreal ants occupying the low vegetation during the day and at night. Exactly the same branches were sampled during diurnal and nocturnal sampling (Figs. 1 and 2). Since

branches are used by ants as highways, sampling the same branches during the day and at night captures the change of occupancy of the highway.

The results of this preliminary study clearly suggest the presence of a nocturnal subset of the ant community of a forest patch. All three collecting methods support this finding, even though there is a large overlap between diurnal and nocturnal leaf litter and ground-foraging ant communities.

The ants collected by pitfall trapping and leaf litter sifting showed a large overlap of species, thus there may not be enough reason to particularly sample the nocturnal subset of ground-foraging and leaf litter-inhabiting ants.

The arboreal ants, however, exhibit the largest difference between the diurnal and nocturnal assemblages (Jaccard distance = 0.85). This value implies the existence of a distinct assemblage of nocturnal arboreal ants. It is believed that efforts to sample the nocturnal arboreal ants may be rewarded with discoveries of new species and the capture of the temporal behavior of known species.

In sum, the presence of a distinct nocturnal subset of the ant community of a forest patch suggests that the extra effort to collect this subset would be rewarded with discoveries or a better understanding of the ant community as a whole. Nocturnal leaf litter ants may include species that are rare in collections, such as species of the genus *Tyrannomyrmex* (AntWiki, 2019). Of particular interest is the subset of nocturnal arboreal ants, including *Vombisidris*, because these ants are poorly studied.

Beetles, spiders, and other arthropods, e.g., Archaeognatha, were collected by these methods as by-catch but the specimens were pooled. It is possible that these methods may also capture the temporal behavior of other insects and arthropods.

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