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RESEARCH ARTICLE - ANTS

Evaluating Efficiency of Different Sampling Methods for Arboreal Ants (Hymenoptera: Formicidae) in A West African Forest-Savanna Mosaic

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Abstract

Ants constitute an important part of arboreal arthropod biomass in rainforests. Nevertheless, there are only a few methods which permit a rapid assessment of these insects in the canopy layer. This study aims at evaluating the efficiency of a new variant type of pitfall trap i.e. "the funnel trap", to sample arboreal ants in a secondary and gallery forest in Lamto reserve (Côte d'Ivoire). This method was compared to standard arboreal pitfall trap and beating. In total, the 3 methods yielded 7072 ant workers belonging to 43 species, 14 genera and 5 subfamilies. Tree beating recorded the highest ant's numerical abundance (3670 workers), with 27 species, 12 genera and 3 subfamilies followed by the "funnel trap" that yielded 2800 ant workers, with 23 species belonging to 12 genera and 5 subfamilies. Finally, arboreal pitfall traps caught the lowest individual with 602 ant workers from 20 species belonging to 9 genera and 3 subfamilies. The composition of species which are caught by arboreal pitfall trap and "funnel trap" was similar at 53 percent. Tree beating showed a distinct species composition compared to arboreal pitfall trap and "funnel trap". The "funnel trap" could be a fast and efficient way to quickly assess ant-biodiversity in forest canopies and agroecosystems as it looks like a non-destructive sampling method.

Introduction

Tropical forest canopies are known to support a high animal diversity. Particularly arthropods can be very diverse and abundant (Klimes et al., 2012). Ants account for 20 to 60 % of total arthropod biomass of tropical forest canopy invertebrates (Floren et al., 2014), and therefore, represent one of the most abundant and ecologically important animal groups in tropical regions (Hölldobler & Wilson, 1990; Lach et al., 2010). Ants are also widely considered as keystone species due to the important ecological role they play in many ecosystems (Del Toro et al., 2012). In tropical forest canopy, ants often exercise a high predation pressure and thus significantly influence structure and dynamics of arboreal arthropod communities (Philpott & Armbrecht, 2006). Ant are also used as indicator taxa for ecological surveys because they are relatively easy to sample in soil litter (Alonso & Agosti, 2000).

Despite this importance, the arboreal ant community remains poorly known for some region in tropics, mainly because the difficulty for scientists to find appropriate sampling method to sample either at three meters above the ground. For example, methods to sample arboreal ants such as the spikes-and-belt method, single-rope technique, baited pitfall traps and so forth, are methods for which one needs an appropriate logistic to access to the canopy and for the installation of all sampling equipments (Basset et al., 1997). Nevertheless, few techniques such as the canopy fogging and beating do not require a lot of equipment in the canopy (Castaño-Meneses, 2014). Currently, pitfall traps (including its variants) are often used for arboreal ant sampling (Powell et al., 2011; Chapin & Smith, 2019).

Although, pitfall trapping is a well-recognized ant sampling technique, an important difficulty remains the installation of these traps above three meters high in the canopy. Usually this installation requires climbing which is difficult and risky. Hence, to sample canopy biota rigorous inventory techniques need to be developed. These techniques should be simple, fast and allow researchers to optimize their sampling in the canopy (Yusah et al., 2012; Yusah et al., 2018; Leponce et al., 2019).

Here, this study reports the results of arboreal ant diversity surveys using different sampling methods in a tropical forest-savanna mosaic habitat at Lamto scientific reserve (Côte d'Ivoire). Overall, it aims at establishing a database on arboreal ant species richness in Côte d'Ivoire. Indeed, the arboreal ant community remains poorly known and the only existing studies go back to the seventies (Delage-Darchen, 1970, 1971, 1972, 1973, 1974; Levieux, 1976). This study aims at testing the efficiency of a new variant of pitfall trap, the "funnel trap" to collect arboreal ants in secondary and gallery forest of Lamto scientific reserve. This trap was compared with arboreal baited pitfall trap and beating of tree leaves, by analysing the differences in numbers of ant workers, species richness and species composition.

Methods

Study site

The study was conducted in Lamto Scientific Reserve, located in central Côte d'Ivoire at 6°13'/6°25' N and 4°97'/5°01' W. The annual precipitation range is 1000–1500 mm/year while the mean monthly temperature is about 27°C. Lamto Scientific reserve contains a great heterogenous vegetation (Abbadie et al., 2006) characterized by a forest-savanna mosaic habitat. The study was carried out in the gallery forest located at the border of Bandama river, and the secondary forest that resulted from an experimental bush fire exclusion since 1962 (Abbadie et al., 2006; Gnaoré et al., 2018).

Sampling design and identification

Ants were sampled in three 100 × 50 m plots in gallery forests and secondary forest. On each plot, 20 trees with a circumference at breast height ≥ 32 cm were examined. Overall samplings were carried out on 120 trees spread over 6 plots in all. On each plot, three sampling methods were used to collect arboreal ants: canopy beating, arboreal pitfall trap (Agosti & Alonso, 2000; Underwood & Fisher 2006; Yeo et al ., 2013) and the "funnel trap", a modified variant of the arboreal pitfall trap (Fig 1).

Fig 1. "Funnel trap" model.

Arboreal pitfall trap: These traps consisted of plastic cups (7.5 cm in diameter, 10.5 cm deep) with water, detergent and baited with tuna (Ribas et al., 2003). They were placed on the central axis of the trees at least at 5 m above the ground thanks to a point. They were installed using a ladder. Pitfall traps remained in service on the trees during 48 h.

The "funnel trap": The latter was made from empty bottles of 33 cl of mineral water or soda, rope and a bamboo shaft. Bottles were cut in half at 8 cm from the top edge. Next, the upper part of the bottle was placed upside down in the second part in order to have a funnel. These two parts were attached to their joints with a transparent adhesive tape. Finally, two small holes were made at 1cm of the top edge of the bottle to insert a fine nylon thread whose length corresponds to the height at which the trap can be attached to the tree. To prevent the thread from mixing, it was previously wind up on small pieces of wood 7 cm. Finally, tuna bait was placed inside the bottle with a little water and was hoisted at 5 meters into the trees using a bamboo of at least 5m height (Fig 2). The traps remained in service on during 48 hours. The sampling was carried out on 120 trees.

Fig 2. Installation of "funnel trap" in tree canopy.

Canopy beating: This method consisted in collecting the ants by beating the foliage of the lowest branches of the trees (between 2m and 3m high). The foliage of the trees was beaten in two sequences of 5 beats. Each sequence was followed by the gathering of the ants that fell on the canvas. In total the foliage was beaten ten times at the same point. Also, it had 20 collection points on a plot, and therefore 120 collection points in all.

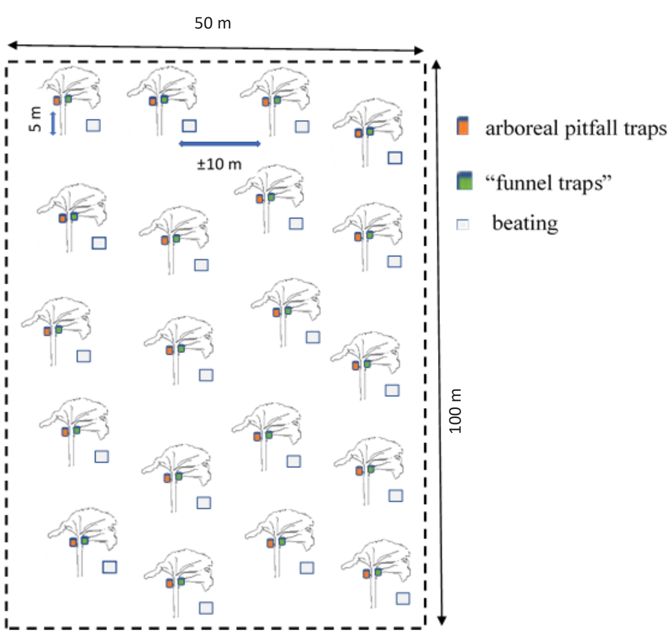

Fig 3. Scheme of the sampling plot in the Lamto Scientific Reserve, Côte d'Ivoire.

Arboreal pitfall traps and funnel traps were installed on the same trees. As for the beating, they were generally carried out on the same trees or in thickets directly connected to the trees in which the traps were installed (Fig 3).

Ants were identified to genus level using the guide of the genera to Fisher and Bolton (2016) with a Leica MZ6 microscope. At species level, the keys of Bolton (1980, 1982 and 1987); Rigato, 2016), and reliable digital keys (antweb.org) were used. When species-level identification was impossible, distinct specimens were sorted according to morphospecies. Morphospecies were numbered according to the ant reference collection for Côte d'Ivoire at Lamto ecological research station. The specimens were added to Lamto ecological research station collection.

Data analysis

Samples of each plot were pooled to obtain a total of 120 samples for each sampling method. Taxonomic structure (subfamily), species richness, abundance, and species composition were compared for each sampling method. The Chao 2 index species richness estimator was calculated to extrapolate the species richness from our data. Sample coverages were determined to estimate sampling efficiency based on different sampling method using EstimateS v.9.1.0. Comparative analysis was carried out on the species richness and numerical abundance of the ants collected by the different trap types. Kruskall-Wallis and Man-Whitney pairwise comparison tests were used to test the differences across the trap types using species richness and numerical abundance. The comparison of species composition was conducted calculating Jaccard similarity index.

Results

General results

Overall, the three combined methods yielded 7072 ant workers belonging to 43 species, 14 genera and 5 subfamilies. The subfamilies were Dolichoderinae, Myrmicinae, Formicinae, Ponerinea and Pseudomyrmicinae. All five subfamilies were collected with the funnel trap, whereas only three out of five subfamilies were collected with arboreal pitfall traps and beating, respectively (Fig 4).

Sampling efficiency

Table 1 shows that the sample coverage varied between 71 and 93 %, illustrating that all three methods were suitable to investigate arboreal ant community. Observed species

Table 1. Numbers of species caught by the trapping methods during study.

	Observed species	Estimated species (Chao 2)	Sampling coverage	Unique species	Doubletons
Arboreal pitfall	20	21,49	93%	4	3
Funnel trap	23	32,26	71%	8	2
Beating	27	31,17	86%	7	4

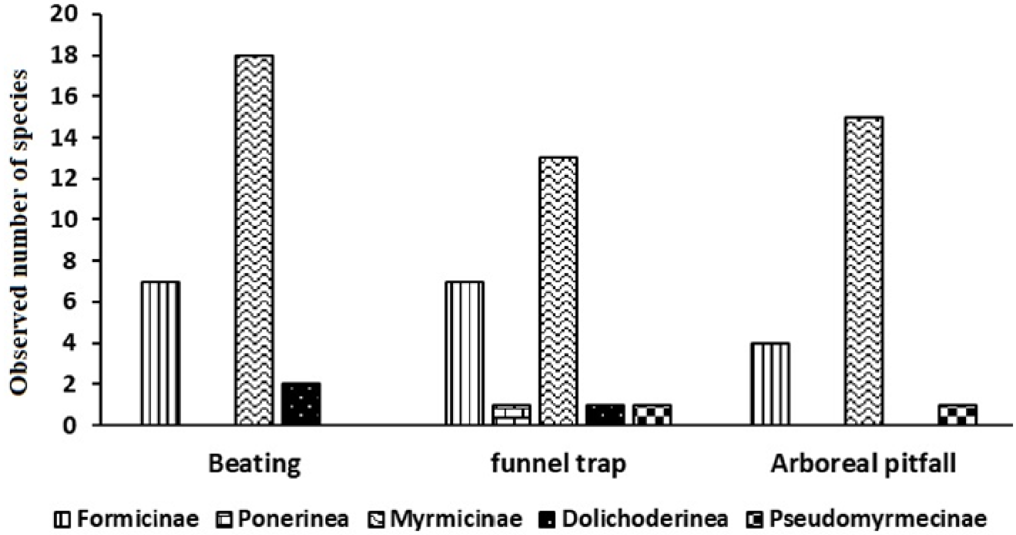

Fig 4. Ant species distribution within the different subfamilies encountered for each sampling method.

accumulation curves evolved towards asymptotic lines for all sampling methods. However, the Chao 2 estimated species accumulation curves for "funnel trap" and beating increased steadily with sampling size (Fig 5). In addition, the tree beating method collected the highest number of species (27 species), followed by funnel trap (23 species) and arboreal pitfall trap (20 species). Funnel traps caught more unique species (8 species) followed by beating (7 species) and arboreal pitfall (4 species) (Table 1).

Species richness and diversity

Tree beating recorded the highest number of ant species (27 species) with 3670 workers, followed by the "funnel trap" (23 species) for 2800 workers. Arboreal pitfall traps caught the lowest number of ant species (20 species) and workers (602). The mean ant species number found in traps differed significantly between the 3 sampling methods (Kruskall-Wallis: $X^2 = 89.66$; df = 2; p = 0.0001). Tree beating method recorded the highest mean number of ant species (2.59 species/trap) followed by "funnel trap" method (1.63 species/trap) and arboreal pitfall trap method (0.98 species/trap).

The Man-Whitney pairwise comparison test showed that "funnel trap" caught more ant species than arboreal pitfall trap (U = 4670; $p < 0.001$). On the other hand, tree beating method caught more ant species than "funnel trap" (U = 4216; $p < 0.0001$) and arboreal pitfall trap (U = 2335; $p < 0.001$) (Such as Fig 6).

The three sampling methods yielded a high diversity, but the value of the Simpson diversity index was higher for the beating method (0.89), followed by that of the funnel trap (0.86) and finally the arboreal pitfall trap (0.84). On the other hand, evenness values were low, with E=0.34 for the beating and E=0.36 for both funnel trap and arboreal pitfall trap, respectively (Table 2).

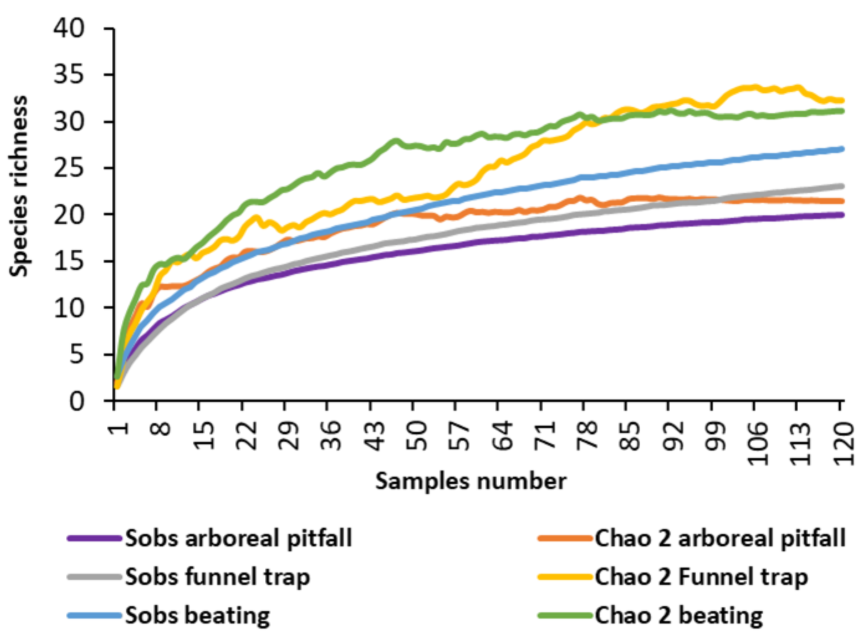

Fig 5. Species accumulation curves of the three sampling methods.

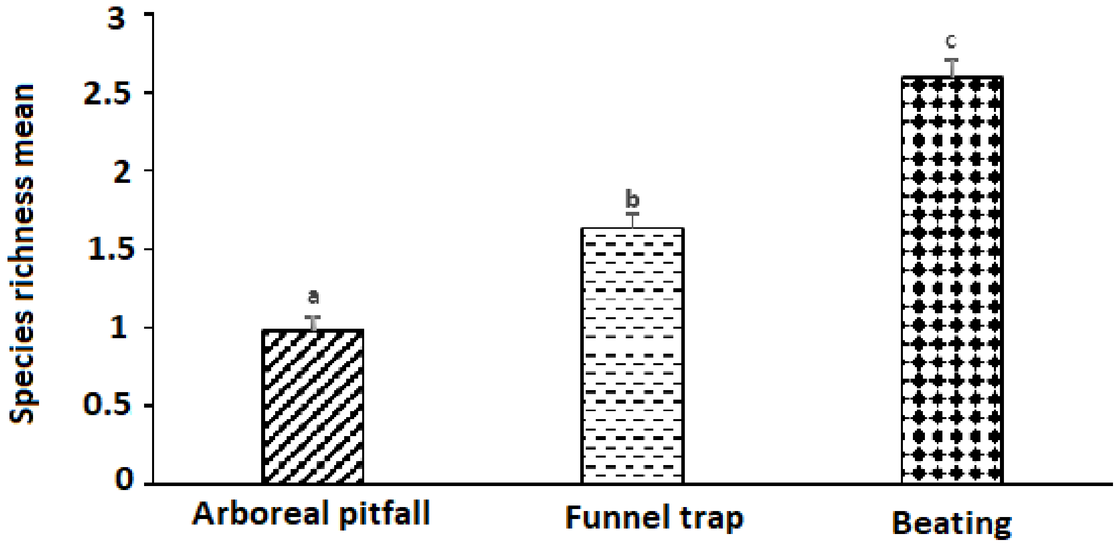

Fig 6. Sample species richness of each trap.

Table 2. Measure of diversity in the different sampling method.

	Species richness	Simpson index	Evenness
Beating	27	0,89	0.34
Funnel trap	23	0,88	0.36
Arboreal pitfall	20	0,86	0.36

Ant abundance and species composition

Overall, ant abundance (individuals) varied significantly among each sampling method (ANOVA of Kruskal-Wallis test: $X^2 = 85.23$; df = 2; p = 0.0001; Such as Fig 7). On average, the tree beating method (29.93 individuals/trap) and "funnel trap" (23.33 individuals/trap) caught the highest numbers of ant workers, whilst arboreal pitfall trap caught 5.02 individuals/trap. Mann-Whitney pairwise comparison test indicated that the funnel trap caught more ant workers than the arboreal pitfall trap (U = 3931; p = 0.001). However, the tree beating method caught more ant workers (individuals) than the funnel trap (U = 5686; p = 0.004), and arboreal pitfall trap (U = 2307; p = 0.001).

Globally, beating yielded a different species composition that funnel trap and arboreal pitfall trap. Otherwise, Jaccard similarity index showed that the arboreal pitfall trap and funnel trap had approximately a similar species composition with a similarity percentage at 53% of shared species. Tree beating shared 30% species with arboreal pitfall trap and 22% with the funnel trap (Table 3). However, funnel trap caught species that were not found in the arboreal pitfall trap and beating (Table 4).

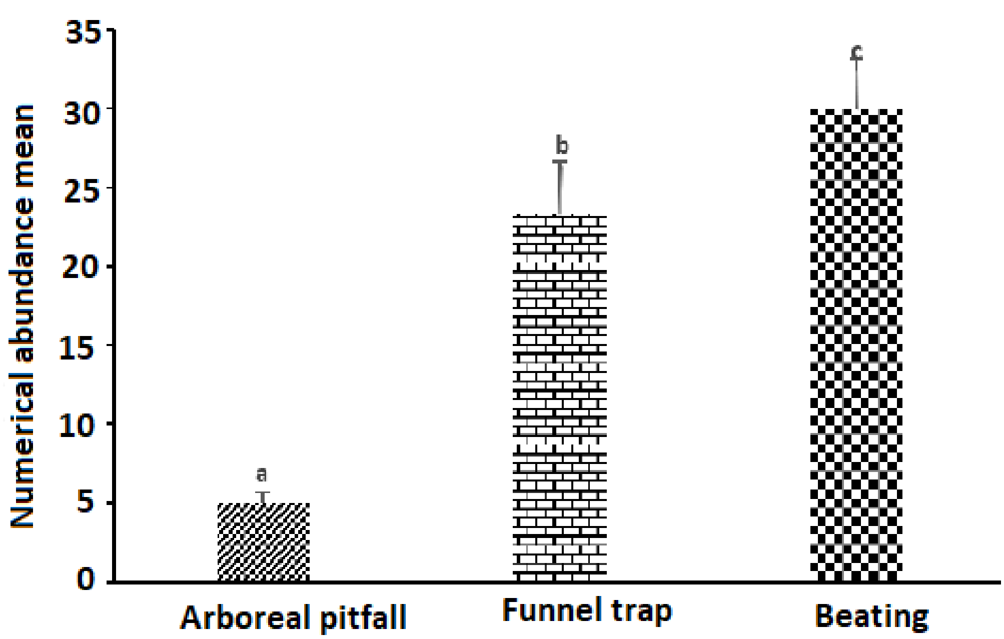

Fig 7. Ants numerical abundance mean (± SE) collected in different traps (numerical abundance per sample).

Table 3. Similarity index (Jaccard) between ant assemblages collected by the three sampling methods.

	Arboreal pitfall	Funnel trap	Beating
Arboreal pitfall		0.53	0.30
Funnel trap			0.22

Discussion

Several studies already focused on the comparison of different sampling techniques to collect arboreal ant (Kaspari, 2000; Yusah et al., 2012; Garcia-Martinez, 2018; Leponce et al., 2019). Here, we demonstrate that the "Funnel trap" can also

Table 4. Arboreal ant species composition between three sampling method.

Subfamily	Espèces	Arboreal pitfall (n=120)	Funnel trap (n=120)	Beating (n=120)
Formicinae	*Camponotus puberulus* Emery,1897	0	10	0
	Camponotus compressiscapus André, 1889	0	62	0
	Camponotus solon Forel, 1886	3	26	0
	Camponotus acvapimensis Mayr, 1862	0	0	2
	Camponutus maculatus Fabrius, 1782	2	47	0
	Lepisiota sp.1	92	181	18
	Oecophylla longinoda Latreille, 1802	122	1538	488
	Plagiolepis alluaudi Emery, 1894	0	0	90
	Plagiolepis sp.2	0	0	21
	Plagiolepis sp.3	0	0	3
	Polyrachis sp.1	0	1	4
Dolichoderinae	*Tapinoma lugubre* Santschi, 1917	0	9	0
	Tapinoma sp.1	0	0	2
	Tapinoma sp.2	0	0	16
Myrmicinae	*Cataulacus traegaohdi* Santschi, 1914	2	2	47
	Cataulacus guineensis Smith, 1853	0	0	1
	Crematogaster solenopsides Emery, 1899	163	284	1558
	Crematogaster striatula Emery, 1892	1	28	42
	Crematogaster africana Mayr, 1895	38	371	0
	Crematogaster sp.9	23	0	1
	Crematogaster nigronitens Santschi, 1917	18	1	0
	Crematogaster sp.14	66	23	115
	Crematogaster sp.22	0	0	25
	Crematogaster sp.17	0	0	692
	Crematogaster sp.21	0	0	1
	Monomorium dolatu Bolton, 1987	1	0	5
	Monomorium floricola Jerdon, 1851	9	97	10
	Monomorium inquietum Santschi, 1926	37	57	287
	Monomorium pharaonis Linnaeus, 1758	0	13	0
	Monomorium sp.2	5	0	63
	Monomorium sp. 3	0	0	1
	Pheidole megacephala Fabricius, 1793	13	32	0
	Pheidole sp.2	0	10	0
	Pheidole sp.6	0	2	0
	Pheidole sp.7	0	0	11
	Pheidole sp.8	0	0	2
	Terataner velatus Bolton, 1981	0	0	1
	Tetramorium lucayanum Wheeler, 1905	1	4	0
	Tetramorium quadridentatum Stitz, 1910	1	0	0
	Tetramorium sp.3	4	0	0
	Tetramorium sp.4	0	0	2
Ponerinae	*Platythyrea conradti* Emery, 1899	0	1	0
Pseudomyrmicinae	*Tetraponera mocquerysi* André, 1890	1	1	0
	Total	**602**	**2800**	**3670**

be an efficient sampling method which requires a simple logistic, to assess arboreal ant diversity. The funnel trap" was able to catch more species from different subfamilies than the arboreal pitfall trap during a sampling campaign. On contrary, it caught less ant species than trees beating method. Probably beating also collects terricolous ant species that were encountered in the foliage. The fact that the "funnel trap" has caught more ant species than the arboreal pitfall trap might be due to the low chance of escape for ants once entering the trap. Also, this could explain the relative high rate of sampling coverage of 70% and the high number of unique species collected in comparison to the other sampling methods.

The difference observed in species richness between the different sampling methods could be explained by the voracity of the ants during the dry season when there is an extra need for water and food. Indeed, baits from funnel trap and arboreal pitfall were entirely consumed or transported by the ants outside the traps. Sousa-souto et al. (2016) already mentioned that the dry season could have negative effects on the arboreal ant species richness because the loss of leaves in most tree species decreases the connectivity between tree canopies with a consequent reduction of resource availability. Sometimes traps were monopolised by a single species, leading less competitive species giving up the bait and not been collected (Garcia-Martinez, 2015).

We found a high diversity for all methods, but on contrast low values for evenness. This finding matches with the study of Basset et al. (2003a) and Yusah et al. (2018) who reported that ant assemblages of tropical forest canopy are often characterized by a high diversity. Concerning the low values of evenness, a possible explanation may be that bait food attracts more species of ants that have a high recruitment rate and consequently the monopolization of baits by some dominant species (Leponce et al., 2019). These findings demonstrated that, the "funnel trap" could be therefore considered as a suitable trap to estimate the diversity of canopy ant communities and to study the structure of the canopy ant mosaic in natural and modified habitats.

Beating caught more ants than the "funnel trap", followed by arboreal pitfall trap. A plausible explanation is that generally beating was done between 2 and 3m above the ground in the foliage of trees or shrubs. At this height also, it is possible ant species that nest on the ground and occasionally forage in the trees being collected (Klimes 2017, and Vasconcelos et al., 2019).

The three sampling methods generally yielded similar ant species composition. Although some studies showed that the specific composition of canopy ants varies with habitat heterogeneity (Dambros et al*.,* 2018), here, the similarity of ant species composition observed after comparison of the three different sampling methods may be explained by the similar plant compositions of the two forest formations (Gnaoré et al., 2018). Nevertheless, the funnel trap method caught a greater number of unique species than the other methods. Therefore we would recommend it to study arboreal ants in both natural and disturbed habitats as the "funnel trap" can capture some larger ant species such *as, Camponotus puberulus* Emery, 1897*, Camponotus compressiscapus* André, 1889*, Platythyrea conradti* and smaller ant species like *Pheidole sp.2, Monomorium floricola* Jerdon, 1851*, Plagiolepis alluaudi* Emery, 1894 and *Tapinoma lugubre* Santschi, 1917.

Interestingly, 48 hours after bait placement in "Funnel trap", some ant species workers were still active and alive in the traps although the bait was totally consumed. Thus, it is possible the "funnel trap" also offers unique possibility to observe the existence of competition and interactions between ant species. For example, in some trap, we have observed a high number of killed workers of both *Camponotus solon* Forel, 1886 and *Oecophylla longinoda* Latreille, 1802, suggesting a strong competition between these two ant species in canopy.

The funnel trap is an efficient sampling technique to the study of arboreal canopy ant communities. In addition, it allows to capture several other orders of insects like Blattodea, Diptera, other Hymenoptera, Coleoptera, Orthoptera and Lepidoptera. Funnel trap can also be used for sampling in the agroecosystem canopy or in other natural area besides savanna or forest. Funnel traps collect also ant species that will not easily be collected with the usually used arboreal pitfall trap.

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Authors' Contributions

Christine Dakélé YODÉ: Design of the work, methodology, data collection, data analysis, interpretation of data for the work and writing the original draft.

Wouter DEKONINCK, Kolo YEO: Contributed to revising draft, and Final approval of the version to be published.

Lombart M. Maurice KOUAKOU, Kanvaly DOSSO, Souleymane KONATE, Phillipe Kouassi KOUASSI: Contributed to conceptualization, revising draft critically for important intellectual content and final approval of the version to be published.

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