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RESEARCH ARTICLE

Hypogaeic pitfall traps: methodological advances and remarks to improve the sampling of a hidden ant fauna

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Abstract Ant assemblages present a great vertical stratification, with microhabitats showing strong differences in relation to species composition. Among the microhabitats, the hypogaeic has been poorly studied. Hypogaeic or subterranean ants live in the deeper soil layers, which make the sampling logistics and operability a difficult work. The fact that the hypogaeic ant fauna is diversified and abundant, with low similarity to ant assemblages in other microhabitats, has promoted the development of several collecting techniques to sample this hidden ant fauna. Here, we verify the ability of hypogaeic pitfall traps to sample subterranean ants. In addition, we propose methodological advances and remarks about the use of this ant sampling technique.

Keywords Collecting techniques · Formicidae · Hypogaeic ants

Introduction

Ants (Hymenoptera: Formicidae) represent an abundant and speciose insect group with a wide geographic distribution and, together with termites, they comprise more than half of the total insect biomass (Wilson and Hölldobler, 2005). At this moment, there are about 12,600 ant species already described (25 January 2010) (Agosti and Johnson, 2005) and this number has been estimated to reach 20,000 species (Hölldobler and Wilson, 1990).

Thus, ants are important elements of terrestrial ecosystems, involved in mutualistic associations with plants, other insects and many kinds of organisms (Schultz and McGlynn, 2000; Oliveira and Del-Claro, 2005). Moreover, ants impressively contribute to edaphic processes such as water and soil movement and nutrient cycling (Lobry de Bruyn, 1999; Sousa-Souto et al., 2007). Therefore, many studies have used ants as models in ecological surveys (Agosti et al., 2000) and suggest the use of these insects as bioindicators of different disturbances (Underwood and Fisher, 2006).

Hence, different collecting techniques have been developed (Bestelmeyer et al., 2000) to offer a representative, repeatable and unbiased sampling effort of ant faunas, allowing safe results about the ecology of ant communities. However, among ant biodiversity and community studies, collecting techniques have often focused mainly on specific microhabitats, particularly the epigaeic and arboreal ones (e.g. Greenslade and Greenslade, 1971; Olson, 1991; Orbist and Duelli, 1996; Agosti et al., 2000; Wang et al., 2001; Ribas et al. 2003a; Ribas et al., 2005; Elisson et al., 2007). Hypogaeic or subterranean ants are seldom studied, because these ants live in the lower soil layers, which make the logistics and operability of the sampling effort a difficult work. Despite these sampling difficulties, studies have shown that species composition of hypogaeic ant assemblages is completely different from the ant fauna sampled in other microhabitats (e.g. epigaeic and arboreal) (Silva and Silvestre, 2004; Wilkie et al., 2007; Schmidt and Diehl, 2008).

Furthermore, as well as ants inhabiting the canopies of tropical forests, hypogaeic ants probably compose a large

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part of ant species that have not been described yet. Recent data, such as the description of *Martialis heureka* Rabeling et al., 2008, also indicate that hypogaeic microhabitats host phylogenetically ancient and conserved ant lineages. This means that hypogaeic habits probably evolved early during the evolution of ants (Rabeling et al., 2008). Thus, ants from hypogaeic microhabitats represent the greatest frontier for the knowledge of ant diversity which has encouraged some authors to develop collecting techniques to sample these ants (Weissflog et al., 2000; Berghoff et al., 2002, 2003a; Wilkie et al., 2007).

The collecting techniques for hypogaeic ants developed in the studies cited above, in general, used baits in small recipients or probes that could be buried into the soil. Thus, ants that live in lower soil layers could be attracted to the baits.

Moreover, modified pitfall traps (hypogaeic pitfall traps) have been developed to sample subterranean ants (e.g. Berghoff et al., 2003b). However, it was proposed as a specific technique, without the character of a standard method to sample subterranean ants. Hence, hypogaeic pitfall traps is a known ant sampling technique, but few efforts have been employed to improve its versatility and standard use.

In this paper, we verify the ability of hypogaeic pitfall traps to sample subterranean ants. In addition, we propose methodological advances and remarks to enhance the standard using of this ant sampling technique. Still, we provide a framework to standardize the technique.

Materials and methods

Hypogaeic pitfall trap design and installation

The pitfall traps consist of plastic recipients (diameter 8 cm; height 12 cm) with four radial holes (1 cm diameter) made at a height of 6 cm in the plastic recipient, which allow ants to access the interior of the trap (Fig. 1). In the inner of the pitfall trap, if the study aims to use attracting baits, a smaller plastic recipient may be attached (the bottom of a 500 ml water pet bottle cut at 7 cm height) where we set two small plastic pots (e.g. empty boxes of photographic films, 3.3 cm diameter; 5.0 cm height). One of the small plastic pots is filled with sardine (5 cm^3) and the other with honey (10 cm^3). In the space between the inner recipient and the pitfall wall we put 200 ml of a liquid solution, water, glycerol (5%) and salt (0.9%), which kept and killed the ants and prevented them from reaching the baits. The traps were closed with their plastic lids to prevent the soil from entering into the pitfall and disturbing the sampling.

A string with a colored ribbon was tied to the pitfall to help find the place where the hypogaeic pitfall was buried in the field. The hypogaeic pitfall traps were buried in the soil, in such a way that the recipient bottom is fixed at 20 cm depth (but see "Methodological remarks"). We made a uniform hole by the use of a post hole digger to prevent huge alterations on the soil structure.

We removed the pitfall traps after 48 h with the help of a small shovel. Furthermore, to avoid the spilling of the

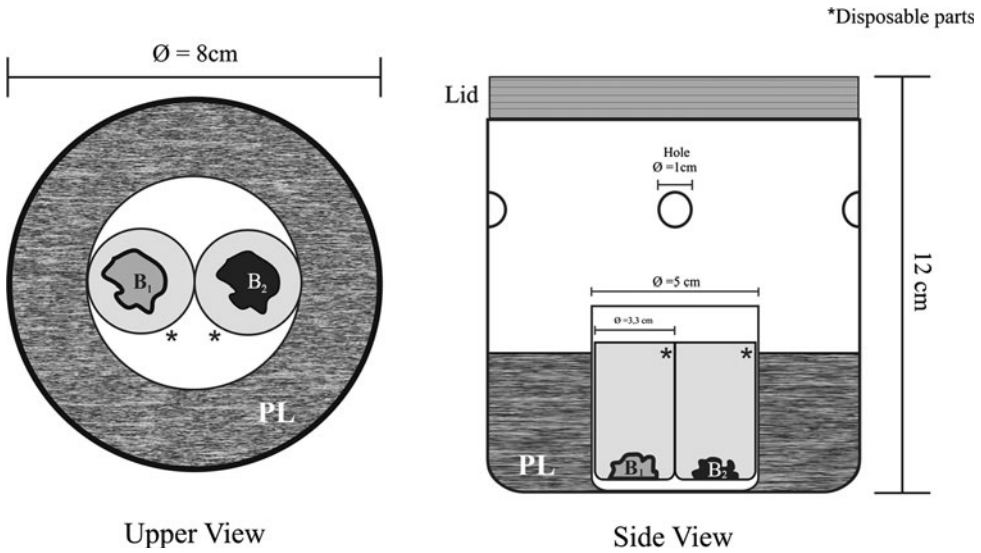

Fig. 1 Hypogaeic pitfall trap design. Upper view Larger plastic recipient (diameter 8 cm) and in the inner part, the smaller plastic recipient (bottom of 500 ml mineral water pet bottle cut at 7 cm height) where we set two small plastic pots (e.g. empty boxes of photographic film, 3.3 cm diameter; 5.0 cm height) each one contain the baits, represented by B_1 and B_2 (in this case, sardine and honey). Side view Large plastic recipient (height 12 cm) with four radial holes (1 cm diameter) made at 6 cm height, which allow ants to access the trap. Asterisks represent the disposable parts, if it is decided not to use the baits. PL preservative liquid

solution with ants, it is necessary to pass an adhesive strap in the holes of the trap. Otherwise, the content can be transferred to a hermetic recipient.

Sampling ants

We verified the ability of hypogaeic pitfall traps to sample subterranean ants in Viçosa, Minas Gerais State, southeastern Brazil. The landscape of the region is characterized by forests fragments described as semideciduous seasonal rainforest (Veloso et al., 1991), a forest formation of Atlantic Rain Forest biome (IBGE, 2004), crop areas (mainly coffee), pasture and forestry (Valverde, 1958; Fundação Centro Tecnológico de Minas Gerais—CETEC, 1983; Ribas et al., 2003b).

Between January and February 2007 the pitfall traps were placed at eight areas, one pasture and seven forest remnants at distinct restoration time (years) ranging from 7 to 120 years. In each area, we set the pitfall traps in ten sampling units along a 100 m transect and the distance between one sampling unit and the other was 10 m. The pitfall traps remained in the field for 48 h.

Identification of ant species

We sorted out and identified the collected ants by genera with the help of the identification keys of Bolton (1994) and Palacio and Fernandéz (2003). We adopted the subfamily classification suggested by Bolton (2003).

Species identification was carried out by comparing species to the Formicidae reference collection of the Laboratório de Ecologia de Comunidades—UFV, where all voucher specimens were deposited, and according to taxonomic keys and genera revision papers available at antbase.org (Agosti and Johnson, 2005).

Results and discussion

In all areas (pasture and forests) we collected 29 ant species from 14 genera and 7 subfamilies. The more speciose subfamily was Myrmicinae (20 species), followed by Formicinae (3 species), and Ecitoninae (2 species). Cerapachyinae, Dolichoderinae, Ectatomminae and Ponerinae were each represented by only one species (Table 1).

The apparently low number of ant species (29) sampled by the hypogaeic pitfall trap is within the range of the number of species (16–47) reported in other studies in the hypogaeic microhabitat (Fowler and Delabie, 1995; Fowler et al., 2000; Wilkie et al., 2007; Schmidt and Diehl, 2008). Nevertheless, Delabie and Fowler (1995), Berghoff et al. (2003a, b) and Silva and Silvestre (2004) reported a higher number of ant species (from 71 to 113) in areas where vegetation has an expressive structural complexity such as the Atlantic Coastal Rainforest and the Equatorial Forest.

Even though we had collected a relatively small number of ant species with hypogaeic pitfall traps, compared to epigaeic/arboreal samples, this technique allows the sampling of some remarkable ant species. For example, *Acanthostichus laticornis* Forel, 1908, belongs to a group of predominantly predatory hypogaeic ants that is rarely recorded by other ant colleting techniques (MacKay, 1996) and *Carebara urichi* (Wheeler, 1922) and *Carebara* gr. *lignata*, ants of a group, the identification of which is considerably difficult because of the small size of the specimens. Some other remarkable samplings have been made with the use of this trap in different sites of Minas Gerais State, as *Simopelta curvata* (Mayr, 1887), *Cheliomyrmex* pr. *morosus* Mayr, 1870, ants of genus *Acropyga* Roger, 1862, and at least two other species of the genus *Acanthostichus* Mayr, 1887 (Solar and Schmidt, pers. comm.).

Moreover, the greatest surprise about the ant fauna sampled by hypogaeic pitfall trap was the sampling of *Simopelta minima* (Brandão, 1989). This species was collected for the first time in 1986–1987 and has never been recorded since then. This fact encouraged Brazilian environmental institutions to consider *S. minima* as extinct (MMA, 2007), consisting in the first register of an extinction of an ant species in the world by human activities.

The records of the ant taxa described above, which are considered rare and collected with difficulty mainly by the use of epigaeic sampling techniques, highlight the discussion about the geographical distribution and conservation status of ant species (Brandão et al., 2008). Thus, the possible biological and collecting rarity of some ants could be just a methodological sampling artifact. Since the hypogaeic pitfall trap began to be used systematically, rare ant taxa have been amply and constantly sampled, because this collecting technique is more suitable to sample ants with a subterranean lifestyle.

Hence, knowledge about rarity, status of conservation of subterranean ant species and their response to ecological or environmental processes and human activities, could be improved by sampling techniques, such as hypogaeic pitfall traps, which offer a representative, repeatable and unbiased sampling effort of subterranean ant fauna. Moreover, this technique permits to test more intricate hypotheses about the role of hypogaeic microhabitat on the structure and dynamic of local ant communities and about the natural history of this hidden ant fauna.

Methodological remarks

The installation of the hypogaeic pitfall traps in our study followed some standard parameters. However, different parameters can be adopted, depending on the aim of the ant

Table 1 Ants collected by hypogaeic pitfall traps at one pasture (0) and seven forest remnants at distinct restoration time (years) ranged from 7 to 120 years, in Viçosa, State of Minas Gerais, southeastern Brazil

Taxa	0	7	20	30	40	50	80	120
Cerapachyinae								
Acanthostichus laticornis Forel, 1908	–	–	–	–	X	–	–	–
Dolichoderinae								
Linepithema aztecoides Wild, 2007	X	X	–	–	X	–	–	–
Ecitoninae								
Labidus mars Forel, 1912	–	–	–	–	X	–	X	–
Labidus praedator (Fr. Smith, 1858)	–	–	–	–	X	X	–	–
Ectatomminae								
Ectatomma edentatum Roger, 1863	–	–	–	X	X	–	–	X
Formicinae								
Brachymyrmex sp. A	–	–	X	–	–	–	–	X
Camponotus (*Myrmothrix*) *cingulatus* Mayr, 1862	–	–	–	–	–	–	X	–
Camponotus (*Myrmaphaenus*) *novogranadensis* Mayr, 1870	–	X	–	–	–	–	–	–
Myrmicinae								
Atta sexdens rubropilosa Forel, 1908	–	–	X	–	X	–	–	–
Carebara urichi (Wheeler, 1922)	–	–	–	–	X	–	–	X
Carebara gr. *lignata* sp. A	–	–	–	X	–	–	–	–
Octostruma simoni (Emery, 1890)	–	–	–	X	–	–	–	–
Pheidole sp. A	–	–	–	–	–	X	–	–
Pheidole sp. B	–	–	–	X	–	–	–	–
Pheidole sp. D	X	–	X	–	–	–	–	–
Pheidole sp. J	–	–	–	–	–	–	–	X
Pheidole sp. N	–	–	–	–	–	–	X	–
Rogeria micromma Kempf, 1961	–	–	–	X	–	–	–	–
Solenopsis saevissima (Fr. Smith, 1855)	X	X	–	–	–	–	–	–
Solenopsis gr. *geminata* sp. A	X	–	–	–	–	–	–	–
Solenopsis sp. B	–	–	X	–	X	–	–	–
Solenopsis sp. C	–	–	X	X	X	X	X	X
Solenopsis sp. E	X	–	–	–	–	–	X	X
Solenopsis sp. F	–	–	–	–	–	–	–	X
Solenopsis sp. L	–	–	–	X	–	–	–	–
Solenopsis sp. H	–	–	X	X	–	–	–	X
Solenopsis sp. M	–	–	–	–	X	–	X	X
Wasmannia auropunctata (Roger, 1863)	–	X	–	–	X	X	–	–
Ponerinae								
Simopelta minima (Brandão, 1989)	–	X	–	–	–	–	–	–

survey. Therefore, we list some parameters of the installation of the hypogaeic pitfall traps that could be changed according to the aim of the study.

The installation of the traps at 20 cm depth

Samplings of hypogaeic ants have been done at 5–25 cm depth (Delabie and Fowler, 1995; Fowler and Delabie, 1995; Fowler et al., 2000; Berghoff et al., 2002; Berghoff et al., 2003a; Silva and Silvestre, 2004). However, Wilkie et al. (2007), sampling subterranean ants at different depths (from 12.5 to 50 cm), reported a pronounced stratification of the subterranean ant fauna at different soil depths. Thus, as our aim was to verify if hypogaeic pitfall trapping is suitable to sample subterranean ants, we chose as standard depth 20 cm, a soil layer that has been reported to host a variety of hypogaeic ants. Nevertheless, the physical dimensions of hypogaeic pitfall traps permit their use also in studies focused on subterranean ant stratification in soil depths in a pile at a single site, as Wilkie et al. (2007) or randomly placed among sites at different depths.

The use of attractive baits

We used attractive baits in pitfall traps to increase the ant fauna sampling. Although in lower soil layers the smell of baits could play an even less pronounced role for ants, their use is still recommended by other studies (Berghoff et al., 2003a; Wilkie et al., 2007). A shortcoming of baits is that they are not attractive to all of the ant species (Porter, 2005). However, unbaited pitfalls could be recommended for more unbiased samplings (T. Marques, pers. comm.).

The use of killing solution

We used a killing solution to ensure that all ant species attracted will be inside the trap at the moment of removal. The use of a killing solution does not allow the ants to reach the baits and to recruit afterwards; however, our aim was merely to sample and account for the number of ant species. Thus, for the sake of species registration, a single ant individual attracted to the trap is ultimately sufficient. However, the trap could also be used without a killing solution, but in this way it will have the same methodological framework of buried baits in small recipients or probes as described by Berghoff et al. (2002) and Wilkie et al. (2007).

The time pitfall traps remained in the field (48 h)

In general pitfall traps used at ground surface are left in the field for 2 or 3 days, which allows a sufficient capture of ant species and in the case of hypogaeic microhabitat this time is enough for the ants to rebuild their tunnels. Thus, as we used hypogaeic pitfall traps in association with others pitfall traps installed at epigaeic and arboreal microhabitats, the 48 h was used as a standard time for ant sampling in all microhabitats during our study. However, other research groups have left the hypogaeic pitfall traps in the field for a longer time (e.g. 7 days) and they used a solution of glycerol (5%) and ethanol (70%) in the inner part of the traps to promote a better conservation of ant specimens (Vasconcelos H.L., pers. comm.).

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