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Subterranean ants: summary and perspectives on field sampling methods, with notes on diversity and ecology (Hymenoptera: Formicidae)

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Abstract

Soil organisms represent a key component of most ecosystems, and their study must rely on efficient and standardized methods. In ants, subterranean assemblages are perceived as distinct from those of other strata (e.g., ground surface or canopy ants) and as such deserve particular attention – the value of which has recently been acknowledged in research on ant evolution, systematics, and ecology. In this review, we first compile information on the variety of available field methods for studying subterranean ants and comparatively evaluate their usage. Next, we summarise the taxonomic and ecological diversity of subterranean ants. Finally, we propose future avenues for enhancing knowledge on the biology of these species. We identify seven techniques for sampling subterranean ants, which are categorized under three main methodological approaches: Subterranean Baiting, Soil Sampling, and Direct Sampling. Although subterranean sampling methods are specifically tailored to overcome the logistical challenges of collecting ants from within soil, in general they share similar limitations and sources of bias with conventional sampling methods like leaf litter sampling, surface baiting, and pitfall traps. For example, both subterranean and conventional sampling methods are limited by the amount of time and labour required, and their results may be biased by the exclusion of some species when particular sampling periods or baits are used. In contrast, the usage of subterranean sampling methods can result in the discovery of rare hypogaeic species (e.g., species of *Leptanilla* EMERY, 1870, *Leptanilloides* MANN, 1923, and *Oxyepoecus* SANTSCHI, 1926) as well as unique ecological relationships (e.g., seasonal variation in species richness of subterranean ant communities) and life histories (e.g., distinct foraging patterns of hypogaeic Dorylinae species) that are still poorly understood. Studies show that subterranean ants form a diverse (up to 113 species) and distinct community (up to 44% uniqueness) in comparison with ants collected from higher strata. Systematic subterranean sampling has been used on five continents; however, the distribution and intensity of sampling varies greatly among studies, with most effort concentrated in the Neotropics, while the majority of biomes, such as tropical grasslands and moist forests, remain largely under-sampled. Future studies should address the current under-sampling of subterranean ants by employing standardized and improved methods within the framework of pursuing new research questions. For example, many areas pertaining to the ants' activity patterns, trophic ecology, and contributions to ecosystem function deserve further study. To rapidly advance knowledge on subterranean ants, systematic soil sampling may be employed in comparative diversity assessments across biogeographic and environmental gradients, while alternative field methods such as subterranean baiting could be useful for investigating important aspects of the ants' behaviour and ecology.

Key words: Hypogaeic ants, subterranean pitfall trap, soil core, subterranean diversity, cryptic species, soil organisms, review.

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Introduction

The world's 15,100+ species of ants (Hymenoptera: Formicidae) occupy a remarkable diversity of ecological niches across structurally complex environments. For example, ant communities display vertical stratification in most habitats. Different ant species occupy the subterranean stratum as well as several surface strata (e.g., leaf litter, ground-dwelling / understorey, canopy). While much progress has been achieved in determining the diversity and species composition of aboveground ant communities (e.g., WILSON 1959, LONGINO & NADKARNI 1990, BELSHAW & BOLTON 1994, BRÜHL & al. 1998, FISHER 1999, YANOVIAK & KASPARI 2000, CAMPOS & al. 2008, GROC & al. 2014), subterranean ants remain relatively unexplored.

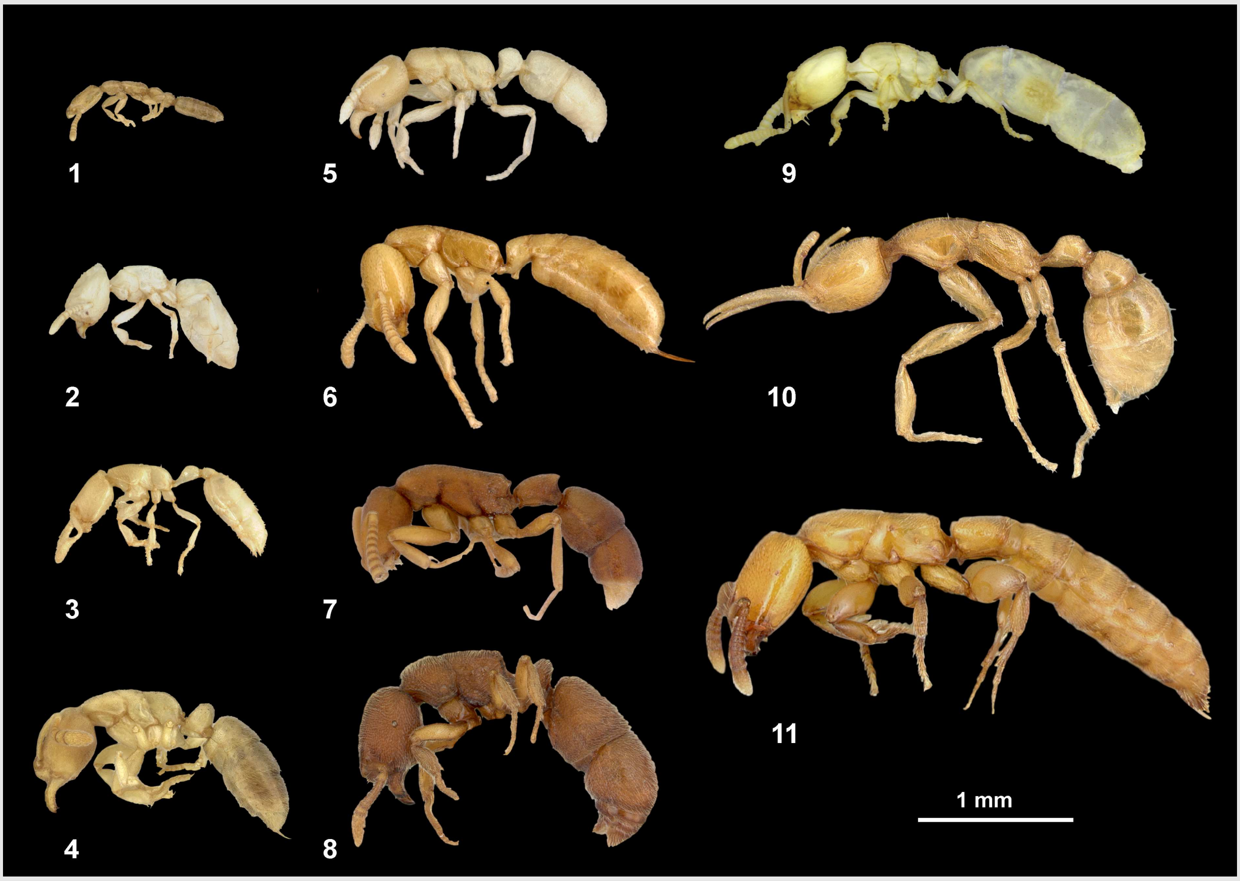
The subterranean microhabitat supports many species key to our general understanding of the behaviour and ecology of early ants. The phylogenetic position of the hypogaeic species *Martialis heureka* RABELING & VERHAAGH, 2008, as well as recent phylogenetic assessments (LUCKY

Box 1: Hypogaeic ant species.

The soil habitat and cryptobiotic morphology of hypogaeic ant species

Although most ecological parameters of soils such as temperature, moisture, organic content, and pH levels vary with geography and topography, the soil habitat may be broadly characterized and distinguished from most other microhabitats (e.g., forest canopies and leaf litter) by two factors: low levels or the complete absence of light, and, severe spatial limitations that correspond to the natural pore system and substrate grain size (Eisenbeis & Wichard 1987). Accordingly, most soil-dwelling arthropods including hypogaeic ant species are likely to have developed distinct morphological and physiological adaptations as evolutionary responses to this unique environment. Having little need for visual sensing in a low-light environment with a shortage of visual stimuli, many hypogaeic ant species either possess drastically reduced eyes or lack eyes completely, and have depigmented bodies that are pale yellow to almost white in colour (Eguchi & al. 2006, 2010, Zryanin 2015). The physical constraints that the natural pore system places on the maximum size and effective mobility of hypogaeic ants also accounts for their frequently small body sizes, slender forms, as well as associated reductions in their extremities and spinescence (or an absence of spinescence altogether) (Wilson 1959). While the morphologies of hypogaeic ant species may not always conform to the parameters described above, hypogaeic species belonging to a wide range of subfamilies do often display – either selectively or in combination – the above cryptobiotic characters of small body size, reduced or absent spinescence, short legs, depigmented bodies, and reduced or absent eyes (Wilson 1959, Andersen & Brault 2010).

Examples of hypogaeic species of eleven ant subfamilies



(1) Leptanillinae: *Leptanilla oceanica* Baroni & Urbani, 1977, (2) Formicinae: *Acropyga smithii* Forel, 1893, (3) Myrmicinae: *Anillomyrma decamera* (Emery, 1901), (4) Ponerinae: *Cryptopone fusciceps* Emery, 1900, (5) Ectatomminae: *Typhlomyrmex pusillus* Emery, 1894, (6) Amblyoponinae: *Adetomyrma venatrix* Ward, 1994, (7) Proceratiinae: *Probolomyrmex watanabei* Tanaka, 1974, (8) Heteroponerinae: *Heteroponera microps* Borgmeier, 1957, (9) Dolichoderinae: *Anillidris bruchi* Santschi, 1936, (10) Martialinae: *Martialis heureka* Rabeling & Verhaagh, 2008, (11) Dorylinae: *Acanthostichus punctiscapus* Mackay, 1996. Images from AntWeb.

& al. 2013), present a compelling case for the evolution of ants from soil-dwelling, subterranean ancestors (Wilson & Hölldobler 2005). The ecology of subterranean ants also deserves further study in as much as species assemblages of the subterranean stratum are often distinct from those aboveground (Wilson 1959, Silva & Silvestre 2004, Ryder Wilkie & al. 2007, Schmidt & Diehl 2008, Yeo & al. 2017). Ant genera across different subfamilies are predominantly, if not fully, comprised of hypogaeic species. Examples include *Prionopelta* Mayr, 1866 (Amblyoponinae), *Leptanilla* EMERY, 1870 (Leptanillinae), *Acanthostichus* MAYR, 1887 (Dorylinae), *Acropyga* ROGER, 1862 (Formicinae) and *Centromyrmex* MAYR, 1866 (Ponerinae).

Conventional ant sampling techniques such as litter extractions, ground baiting and pitfall traps (reviewed in BESTELMEYER & al. 2000) have been reported to under-sample species foraging within soil, and may fail to capture strictly hypogaeic species that do not forage near the ground surface (LONGINO & COLWELL 1997, FOWLER & al. 2000, LUBERTAZZI & TSCHINKEL 2003, ANDERSEN & BRAULT 2010, YEO & al. 2017). It is likely that current records of ant diversity, as well as knowledge on the biology of many understudied and enigmatic hypogaeic species, can be substantially increased with effective, targeted sampling in the subterranean stratum (RYDER WILKIE & al. 2007). Despite this, techniques specifically aimed at collecting subterranean ants are excluded from the majority of biotic surveys, often due to the perceived logistical difficulties associated with their usage (RYDER WILKIE & al. 2007, SCHMIDT & SOLAR 2010).

The overarching goal of this review is to consolidate and present information that will be useful to the study of subterranean ant communities, which are possibly the "final frontier" of ant diversity research (RYDER WILKIE & al. 2007). In the first section, we catalogue, describe, and evaluate techniques employed in the collection of ants from the subterranean environment. Next, we opportunistically review information on the diversity and ecology of subterranean ant communities so as to shed light on this understudied group. Finally, we outline sampling strategies with the potential to rapidly advance the knowledge of subterranean ants and highlight important themes to explore in future research.

Important definitions: We use the term "subterranean" to refer to the environment that lies immediately beneath the organic surface layer of living vegetation and loose plant and woody debris (i.e., leaf litter). In most cases, this environment comprises the O, A, E, B, and C soil horizons (ISBELL 2016). Our definition of "subterranean" differs from other applications of the term, such as in reference to underground cave systems (e.g., HOWRATH 1993). More importantly, although the term "subterranean" may generally refer to organisms found within or collected from a specific habitat (e.g., a "subterranean ant" and "the diversity of subterranean communities"), we distinguish this from the term "hypogaeic". Henceforth we only use "hypogaeic" to describe the biology of a species that predominantly lives and forages within the subterranean environment, and / or possesses cryptobiotic morphological characteristics (see Box 1) that would otherwise suggest such a life history. Therefore, the terms "subterranean ant" and "subterranean ant diversity" do not equate to "hypogaeic species" and "diversity of hypogaeic species", respectively. We propose establishing the above definitions of "subterranean" and "hypogaeic" for the field of myrmecology. While some studies do recognize the differences between the two terms and apply them consistently (e.g., RYDER WILKIE & al. 2007, OSUNKOYA & al. 2011, PACHECO & VASCONCELOS 2012), the terms have been used interchangeably in reference to habitats, sampling methods, and biology in other studies (e.g., EGUCHI & al. 2006, BRANDÃO & al. 2008, SCHMIDT & al. 2014) or used for all purposes, perhaps to avoid confusion in other publications (e.g., LYNCH & al. 1988, LUBERTAZZI & TSCHINKEL 2003, ANDERSEN & BRAULT 2010, RYDER WILKIE & al. 2010, ANDERSEN & al. 2012). By elucidating the subtle yet important differences between these terms, and sharpening their definitions for consistent and precise usage, we therefore hope to facilitate more efficient communication within myrmecology.

Although the current review focuses on sampling methods to collect ants from the subterranean environment as a whole, and while not all ants collected from the subterranean environment are necessarily hypogaeic species, the relevance of hypogaeic species to this specific habitat warrants separate treatment from other species. This approach follows previous studies on subterranean ants, where hypogaeic species are distinguished on the basis of their cryptobiotic morphology (Box 1) or relative abundance in subterranean collections (RYDER WILKIE & al. 2007, ANDERSEN & BRAULT 2010, BERMAN & ANDERSEN 2012, PACHECO & VASCONCELOS 2012). As such, the distinction between hypogaeic and non-hypogaeic species will be highlighted in this review where relevant.

Methods

We performed a literature search for scientific articles reporting on subterranean ants using three separate approaches. First, articles were identified during the development of the GABI database (see details in GUÉNARD & al. 2017), which includes over 8800 publications. Second, to be certain that no publications were overlooked, the terms "Ants" or "Formicidae" in addition to one of the following terms "Subterranean", "Underground", "Hypogaeic", and "Soil" were incorporated into a search in Google Scholar. Finally, literature cited in previously identified publications were scrutinized for relevant material that might have been missed. In total, fifty publications were incorporated into this review (Tables S1 - S3 as digital supplementary material to this article, at the journal's web pages.)

Techniques for sampling subterranean ants

An unexpected variety of techniques have been employed to collect ants from the subterranean stratum. With the exception of the technique used by ESTEVES & al. (2008) who trialled collecting subterranean ants from the gut contents of myrmecophagous lizards, with limited success (yielding only five ant species), the current array of subterranean sampling techniques available to myrmecologists are classified under three dominant methodological approaches: (I) Subterranean Baiting, (II) Soil Sampling, and (III) Direct Sampling (summarised in Tab. 1).

Subterranean Baiting: Subterranean baiting involves placing an attractive substance underground to recruit foraging ants for collection. Unlike conventional surface baiting where ants may be observed and collected directly from the ground surface, the process of subterranean baiting is often completely obscured from the investigator's view, preventing real-time observation and direct manual collection. Instead, in subterranean baiting a receptacle is used to contain recruited specimens until recovery of the whole baiting device. To date, two relatively similar devices for subterranean baiting have been used: the Subterranean Baited Container (SBC) and Subterranean Pitfall Trap (SPT). While their designs vary considerably among stud-

Tab. 1: Classification of the main subterranean sampling methods and their respective techniques.

Sampling method	Specific technique(s)	Acronym
Subterranean Baiting	Subterranean baited container	SBC
	Subterranean pitfall trap	SPT
Soil Sampling	Manual sifting of soil samples	SSm
	Berlese extraction of soil samples	SSb
	Winkler extraction of soil samples	SSw
	Lavage de terre, extraction of soil samples	SSldt
Direct Sampling	Direct sampling from soil	DS

ies (Fig. 1), both SBCs and SPTs generally comprise some bait placed within a container that is buried underground. Multiple perforations in the container's wall facilitate the entry of ants into the device. In SBCs, the containers are commonly empty or may otherwise be filled with soil medium (see Fig. 1g and WEISSFLOG & al. 2000, BERGHOFF & al. 2002, 2003), which allows recruited ants to remain alive until the device is retrieved. Alternatively, in SPTs the ants are killed and preserved in solution at the base of the container. SBCs and SPTs probably do not differ extensively in their potential for collecting subterranean ants, although in SBCs there is a greater risk of specimen damage / loss from live ants preying on each other. As such occurrences are not well documented, however, this subject will be omitted from subsequent discussion.

Bait types: To attract subterranean ants, most studies employ combinatory baits comprising both proteins and carbohydrates (e.g., FOWLER & DELABIE 1995, RYDER WILKIE & al. 2007, ANDERSEN & BRAULT 2010), or proteins and lipids (e.g., SCHMIDT & DIEHL 2008, PACHECO & VASCONCELOS 2012). Protein is the most popular substance used in subterranean baiting; often in the form of processed fish meat; and has been incorporated into all subterranean baiting protocols (see Tab. S1, available as an electronic supplement on the journal's web page). Such approaches possibly reflect the notion that subterranean and leaf-litter species are predominantly carnivorous in comparison to arboreal species relying on carbohydrate-rich diets (DELABIE & FOWLER 1995, DAVIDSON & al. 2003). However, baiting strategies may seriously bias descriptions of community composition because baits are selective and prone to being monopolized by dominant, mass-recruiting species (BESTELMEYER & al. 2000). On the other hand, solitary foraging species and specialist predators may be underrepresented in collections obtained using non-specific, foreign bait such as fish meat. As such, the use of both protein-based (sardines) and carbohydrate-based (honey) baits did not result in a significant difference in species richness and composition between SBCs used in a Brazilian sugarcane plantation (SOUZA & al. 2010). Surprisingly, no other attempts have been made to investigate the relationships between specific bait types (i.e., protein vs. carbohydrates vs. lipids) and observed subterranean ant diversity or composition. However, there is some evidence to suggest that using a variety of bait types promotes diversity in collections. For example, among small trials of carbohydrate (cookie crumbs) and protein (tuna) incorporated into a wider sample of SBCs baited with palm oil (N = 170), species of *Probolomyrmex* MAYR, 1901

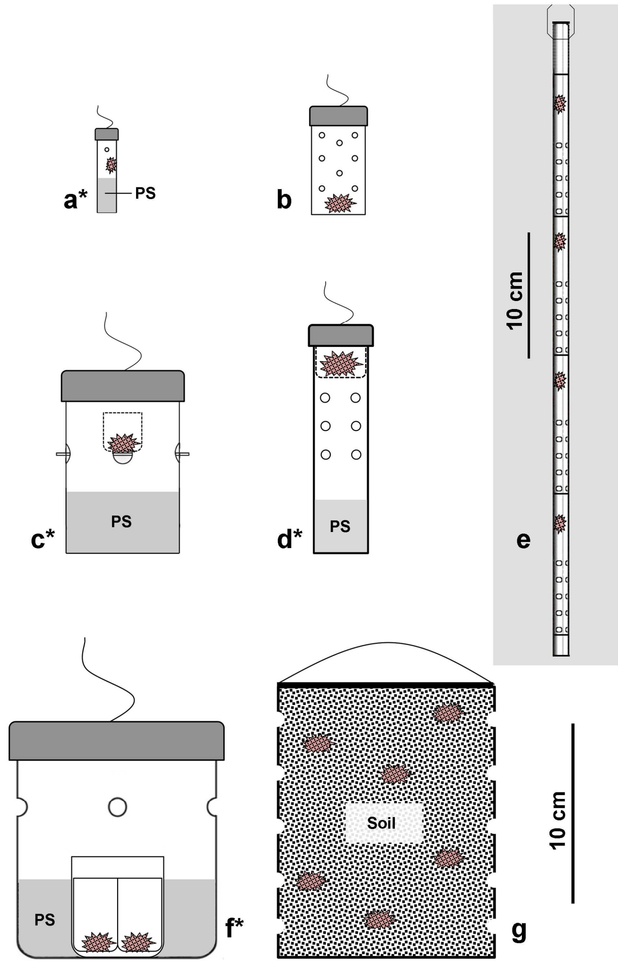


Fig. 1: Diversity of designs of subterranean baiting devices used, drawn to scale. Subterranean Pitfall Traps (SPTs) are represented by designs a, c, d & f and are shown with preservation solution (PS); designs b, e, g represent Subterranean Baited Containers (SBCs). The respective positions of baits (pink) are illustrated for individual designs. In SBCs, the bait is simply placed inside the container, or mixed in with the soil medium when one is present (design g). In SPTs, the bait often has to be separated from the preservation solution. This is either achieved by placing the bait in a smaller holding container (designs c, d, f) or by smearing it as a paste on the container's inner wall (design a). All devices are capped at the top to impede the entry of epigaeic species into the containers. Strings or handles attached to the caps facilitate retrieval of the buried devices. The studies represented by the designs shown are as follows: (a) ANDERSEN & BRAULT (2010), OSUNKOYA & al. (2011), ANDERSEN & al. (2012), BERMAN & ANDERSEN (2012); (b) MORINI & al. (2004), SOUZA & al. (2010); (c) PACHECO & VASCONCELOS (2012); (d) WONG & GUÉNARD (2016a, b); (e) RYDER WILKIE & al. (2007, 2010); (f) BRANDÃO & al. (2008), SCHMIDT & SOLAR (2010), SCHMIDT & al. (2016); (g) BERGHOFF & al. (2002, 2003).

only recruited to protein baits (BERGHOFF & al. 2003). In the only reported instance of subterranean baiting with live prey to date, YAMAGUCHI & HASEGAWA (1996) installed SBCs containing different soil invertebrates (e.g., earthworms, beetle larvae, woodlice etc.) in an urban, disturbed site and collected large numbers of generalist species such as *Solenopsis japonica* WHEELER, 1928 and *Tetramorium caespitum* (LINNAEUS, 1758) as well as the possible specialist *Ponera scabra* WHEELER, 1928. For future studies, subterranean baiting may be trialled for collecting specific hypogaeic ant species by using the known or hypothesized prey as bait. For example, geophilomorph centipedes may be trialled as baits for recruiting hypogaeic species of the Leptanillinae (e.g., *Leptanilla*) and Amblyoponinae (e.g., *Stigmatomma* ROGER, 1859 and *Xymmer* SANTSCHI, 1914) subfamilies.

Setting period: The setting period is the duration for which a baiting device is placed in the field to recruit subterranean ants. Among sixteen studies using subterranean baiting, setting periods vary from short periods of five or eight hours (e.g., FOWLER & DELABIE 1995, EGUCHI & BUI 2009) to one week (PACHECO & VASCONCELOS 2012), with the majority of studies (N = 11) using a setting period of two days (Tab. S1). Based on the findings from a handful of studies investigating the effects of setting period on observed subterranean species richness (Australia, ANDERSEN & BRAULT 2010; Borneo, BERGHOFF & al. 2003; Brazil, PACHECO & VASCONCELOS 2012; and Ecuador, RYDER WILKIE & al. 2007), longer setting periods (i.e., four to seven days) are unlikely to yield significant differences in species richness from comparatively shorter ones (i.e., one to two days). Similarly, a marginal increase in observed species richness with duration of setting period is reported for surface baiting (DELABIE & al. 2000). While setting periods as short as 60 to 90 minutes are deemed sufficient to recruit dominant ground-foraging species in surface baiting (BESTELMEYER & al. 2000), this is unlikely to be the case for subterranean ants. Instead, ANDERSEN & BRAULT (2010) recommend a setting period of 24 hours for obtaining an effective sample, a duration in which almost 80% of species can been collected (RYDER WILKIE & al. 2007). It is worth noting, however, that setting periods which are too short to encompass changes in daylight may exclude species with specific activity patterns. For example, EGUCHI & BUI (2009) observed only one species from daytime SBC trials (10:00 - 15:00) but up to five from overnight trials (15:00 - 09:00). If clear circadian patterns are indeed common among subterranean ants, a more mechanistic approach may be considered to understand the abiotic factors (e.g., temperature and humidity) or biotic factors (e.g., competition and predation) that limit species activity. Similarly, the identification of cues used by ants to detect circadian rhythms within an aphotic environment represents an interesting research question.

Designs of baiting devices: Individual designs of subterranean baiting devices vary extensively among studies (Fig. 1). In terms of size, both SBCs and SPTs are typically constructed with small containers, such as film canisters used for SBCs (SOUZA & al. 2010) (Fig. 1b), as well as SPTs made from 3cm^3 vials half-filled with preservation solution and baited with a paste smeared on the vial's inner wall (ANDERSEN & BRAULT 2010, OSUNKOYA & al. 2011, ANDERSEN & al. 2012, BERMAN & ANDERSEN 2012) (Fig. 1a). Larger containers have also been used, notably soil-filled SBCs made from 12cm-wide sieve buckets (BERGHOFF & al. 2003) (Fig. 1g), SCHMIDT & SOLAR's (2010) "multiple bait containers within trap" design of SPTs (Fig. 1f), as well as a 50cm-long SBC intended to be a "subterranean probe" (RYDER WILKIE & al. 2007, 2010) (Fig. 1e). Due to the small number of studies, the considerable variation in geography, habitat and sampling effort between individual studies (Tab. S1), as well as the absence of controlled comparative trials, the relative sampling efficiencies of the different devices are unknown. The influence of varying SBC / SPT dimensions and configurations on overall sampling efficiency remains an important aspect to determine. For instance, in the collection of ground foraging ants (and other arthropods), the overall size of surface pitfall traps influences both the richness and composition of the species collected (ABENSPERG-TRAUN & DION 1995, WORK & al. 2002, LANGE & al. 2011). Conceivably, small-sized subterranean baiting devices would be easier to replicate and install, thus maximizing sampling effort (i.e., the combined exposed surface area of all setups in the soil) while lowering the energetic cost of the sampling process. In addition, smaller containers should suffice for holding hypogaeic species, which tend to be small in body size (WILSON 1959, ANDERSEN & BRAULT 2010). However, the space afforded by larger containers may be advantageous in accommodating a wider variety of bait types (e.g., solid vs. liquid baits) and positions (e.g., bottom vs. top of container). Options for positioning baits are more relevant to SPTs as baits need to be separated from the preservation solution (e.g., Fig. 1c, 1d, 1f).

Aside from the overall size of the container, other specifications of subterranean baiting devices should ultimately depend on the nature of the research question. For example, an SBC filled with soil of specific properties (e.g., organic matter content, particle size, or moisture) may facilitate observations on colony movement and behaviour (e.g., BERGHOFF & al. 2002) or ecological requirements and sociometry, while a partitioned SBC will be useful for investigating vertical stratification among soil-dwelling species (e.g., RYDER WILKIE & al. 2007). The potential applications of subterranean baiting have certainly not been fully explored within myrmecology, and to this end it is worthwhile examining how subterranean baiting is employed within other fields. For example, a new species of hypogaeic beetle was collected from SPTs that facilitate the repetitive sampling of subterranean fauna at the same location over a full year (ORTUÑO & al. 2014). These SPTs consist of a collection jar positioned at the bottom of a wire mesh shaft that is permanently installed in the soil column; the jar is simply retrieved and replaced with minimal soil disturbance during the quarterly collection of samples (ORTUÑO & al. 2014).

Sampling depth: In subterranean baiting, sampling depth corresponds to the point at which the ants enter the baiting container. The majority of studies target depths between 10 and 20 cm (Tab. S1), with occasional subterranean baiting occurring as deep as 50 cm. Only a handful of studies incorporate a variety of sampling depths in their baiting protocol, with collections from within the 10 - 20 cm range achieving the greatest overall species richness and / or greatest number of hypogaeic species (e.g., PACHECO & VASCONCELOS 2012). Likewise, among SBC collections from depths of 12.5, 25, 37.5, and 50 cm, maximum species richness (42 out of a total of 47 species) was recorded at the depth of 12.5 cm (RYDER WILKIE & al. 2007). Among SPTs recruiting at 5, 10, and 15 cm, ANDERSEN & BRAULT (2010) attribute the high observed species richness at 5 cm to "substantial contamination by epigaeic species", and recommend sampling at a minimum depth of 10 cm when targeting hypogaeic species. Apart from the selection of appropriate depths to maximize the overall diversity of subterranean collections, sampling depth may be tailored for taxon-specific studies of hypogaeic species according to previous collection records so as to maximize collection success (see Tab. S2 for collection data including sampling depths and bait types for ant genera collected from previous studies).

Notable species and taxonomic accounts from subterranean baiting: Subterranean baiting is a relatively novel sampling method and has only been incorporated into ecological studies on ants in the last two decades (e.g., YAMAGUCHI & HASEGAWA 1996, BERGHOFF & al. 2002, LUBERTAZZI & TSCHINKEL 2003, RYDER WILKIE & al. 2007, 2010, ANDERSEN & BRAULT 2010). Nevertheless, several uncommon ant genera were collected via subterranean baiting. For example, individuals of *Anillomyrma* EMERY, 1913 were found in SBCs baited with pork at depths of 10 cm in Vietnam (EGUCHI & BUI 2009), while *Prionopelta* MAYR, 1866 and *Oxyepoecus* SANTSCHI, 1926 were collected in SPTs baited with a mix of sardines and vegetable oil at depths of 20 cm in Brazil (PACHECO & VASCONCELOS 2012). A survey of taxonomic literature also reveals several species accounts based on specimens collected via subterranean baiting. These include the rediscovery of *Simopelta minima* (BRANDÃO, 1989) (BRANDÃO & al. 2008) and the range extension of *Anillidris bruchi* SANTSCHI, 1936 (SCHMIDT & al. 2014) in Brazil, as well as the description of two new hypogaeic species from Singapore: *Leptanilla hypodracos* WONG & GUÉNARD, 2016, and *Aenictus seletarius* WONG & GUÉNARD, 2016.

Soil Sampling: Soil sampling represents another commonly employed method for collecting subterranean ants, involving the excavation of soil from the ground and subsequent processing to retrieve the ants within samples, either manually or with various extraction techniques. Soil sampling paired with manual collection (SSm) involves hand-sifting the soil samples onto trays so as to reveal ants for collection (e.g., DELABIE & FOWLER 1995, WATANASIT & NHU-EARD 2011). Apart from SSm, three extraction techniques are used to retrieve ants from soil samples, namely the Berlese extraction (also referred to as the Tullgren funnel) (SSb), Winkler extraction (SSw), and the Lavage de Terre (SSldt), a technique traditionally employed in the collection of other hypogaeic insects (LÓPEZ & al. 1994). To the best of our knowledge, SSldt has not been used to systematically sample whole communities of subterranean ants (i.e., unlike the first three techniques). This lack of use is most likely due to the logistically challenging and time-consuming two-step SSldt extraction process where the organic matter is first separated from large quantities of excavated soil through repeated washing and filtering, and then subject to a Berlese extraction to retrieve the specimens.

Unit size and sampling depth: Compared to the sizes of individual SPT and SBC devices used in subterranean baiting, the volume of a single unit used in soil sampling tends to be large, with most requiring an excavation of 1500 to > 15,000 cm^3 of soil per unit (Tab. S1). As such, soil sampling represents the most laborious method to execute, although the two methods function very differently and cannot solely be compared on the basis of labour. Like subterranean baiting, most soil sampling occurs within the range of 10 to 20 cm below ground (Median = 15 cm) (Tab. S1). However, while SBCs and SPTs allow the collection of ants from depths up to 50 cm (RYDER WILKIE & al. 2007, 2010, PACHECO & VASCONCELOS 2012), most studies that use soil sampling have not collected ants at depths greater than 30 cm (Tab. S1). In addition, among studies using soil sampling, the distinction of sampling depths at which specimens are collected has not been effective. This contrasts the vertical stratification of samples afforded by SBCs and SPTs (e.g., RYDER WILKIE & al. 2007, 2010), which may be used to target specific soil depths (i.e., by limiting the positions of entrances to the collection containers). While not yet tested in a standard sampling protocol for an ant-specific study, HARADA & BANDEIRA (1994) successfully divided blocs of excavated sandy soil into subsamples according to depth, and determined species composition for soil-dwelling arthropods in distinct strata. Although this method may work well with sand- and clay-based soils, we are doubtful that such a neat division of excavated soil will be feasible when working with porous soils of poor structure or comprising large proportions of soft organic matter.

Notable species and taxonomic accounts from soil sampling: In assessments of subterranean ant diversity, rare hypogaeic species have been collected from systematic soil sampling, notably two species of *Leptanilloides* MANN, 1923 from SSm in Brazil (VASCONCELOS & DELABIE 2000) and *Tatuidris tatusia* BROWN & KEMPF, 1968 from SSm in Ecuador (JACQUEMIN & al. 2012). Opportunistic soil sampling has also catered for taxonomic accounts on rare and important hypogaeic ant species. The first two specimens of the very rare and phylogenetically distinct *Martialis heureka* were collected from soil samples (RABELING & al. 2008), with other examples including the original description of the rare ponerine *Simopelta minima* from four workers collected via SSb in Brazil (BRANDÃO 1989), as well an unexpected hypogaeic species of the genus *Meranoplus* SMITH, 1853 recently described from 41 specimens also collected via SSb in Vietnam (ZRYANIN 2015). While we did not encounter any studies systematically using SSldt techniques in diversity assessments, a survey of taxonomic literature suggests that collection by SSldt significantly contributes to species discoveries. For example, using SSldt in a survey of soil-dwelling beetles, soil samples taken to a depth of 50 cm from under a deeply embedded stone revealed multiple workers of the endemic Moroccan myrmicine *Stenamma punctiventre* EMERY, 1908, representing the first record of the putatively extinct species in over 90 years (ESPADALER & HERNANDO 2012). By processing large samples of excavated soil (ca. 40 kg) with SSldt, LÓPEZ & al. (1994) collected multiple species of the uncommon hypogaeic genus *Leptanilla* from the Iberian Peninsula in surprisingly large numbers, including 84 workers of *L. zaballosi* BA-

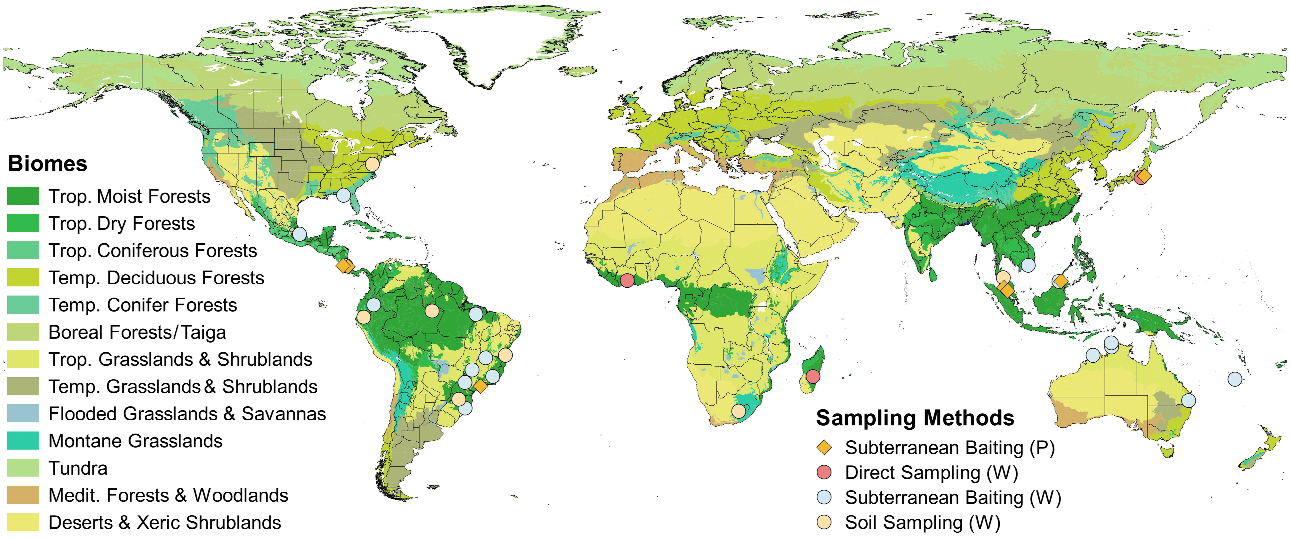


Fig. 2: Global sampling locations of studies using subterranean sampling techniques as part of a standard sampling protocol (N = 36), with respect to the distribution of major biomes (after OLSON & al. 2001). The majority of studies (n = 30) aimed to collect whole subterranean communities (W), that is, as many ant species as possible from subsurface soil without targeting a specific taxon. These include studies that were fully dedicated to sampling subterranean ant communities, as well as studies that incorporated subterranean sampling into a wider sampling protocol. In contrast to whole community studies, partial studies (P) used subterranean sampling to collect a targeted, specific group of ants (e.g., Dorylinae in KUMAR & O'DONELL 2009). Excluded from this diagram are taxonomic accounts (i.e., papers focusing on new species descriptions or distribution records) where sampling can be considered as opportunistic. The complete list of studies used is found in Table S3.

RANDICA, LÓPEZ, MARTÍNEZ & ORTUÑO, 1994 from one sample, and a queen with 34 workers of *L. charonea* BARANDICA, LÓPEZ, MARTÍNEZ & ORTUÑO, 1994 from another. Their collection also represented a significant discovery of cryptic diversity in the *Leptanilla* genus – with four species found within a small geographic area of central Spain (LÓPEZ & al. 1994). Similarly, SANTSCHI (1915) described *Leptanilla nana* SANTSCHI, 1915 and recorded *L. theryi* FOREL, 1903 from Tunisia from specimens obtained via SSldt, while PERRAULT (1998) described *Heteroponera georgesi* PERRAULT, 1999 from French Guyana – a species considered as hypogaeic due to the drastically reduced ommatidia observed in the worker caste.

Direct Sampling (DS): DS generally encompasses a progressive excavation of soil layers in situ, which is simultaneously accompanied by a visual search and direct manual collection of ants from the gradually exposed layers of soil and the excavated soil, without the use of a hand sieve or secondary extraction device. In contrast to soil sampling and subterranean baiting techniques, the results of a DS are entirely dependent on the investigator's ability to detect ants in the sampling site, thus making it an "active sampling method" (see BESTELMEYER & al. 2000). Due to such heavy reliance on the skill level of personnel involved in the sampling, it is difficult to establish consistency both within and between studies using DS – not to mention the physically demanding nature of this technique. Hence, it is unsurprising that DS has only been adopted in the systematic sampling protocols of three studies on subterranean ants (i.e., FISHER & ROBERTSON 2002, MASUKO 2010, YEO & al. 2017) (Tab. S1). Although DS is challenging to standardize and laborious to execute, this subterranean sampling method may be advantageous to research that involves the observation of ants in their natural habitat and / or the immediate recording of information in situ. For example, DS was used to discover and map, in great detail, the spatial distribution patterns of subterranean nests belonging to twenty different ant species across thirty 1 m^2 quadrats in a Japanese broadleaf forest (MASUKO 2010). The less intrusive nature of DS also facilitates the identification and collection of soft-bodied immature life stages that are likely missed by subterranean baiting or damaged in soil sampling methods (FISHER & ROBERTSON 2002).

Notable species collected by direct sampling: When using DS to uncover the spatial nesting patterns of subterranean ants in Japan, MASUKO (2010) collected colonies of the tiny and uncommon hypogaeic species *Leptanilla japonica* BARONI URBANI, 1977. Recently, several rare and potentially hypogaeic genera such as *Apomyrma* BROWN, GOTWALD & LÉVIEUX, 1971, *Dolioponera* BROWN, 1974, and *Fisheropone* SCHMIDT & SHATTUCK, 2014 were collected from a systematic DS in the Cote d'Ivoire (YEO & al. 2017). As an example from taxonomic literature, the hypogaeic species *Dolopomyrmex pilatus* COVER & DEYRUP, 2007, which represented a new genus upon its discovery, was first collected by opportunistic soil excavation and searching in situ (COVER & DEYRUP 2007). It is very possible that numerous other species accounts – especially those of hypogaeic species – are likewise based on specimens collected by DS, albeit this would be difficult to confirm when most are simply described as being "collected from soil".

Notes on the diversity and ecology of subterranean ant communities

The geographic distribution of studies sampling subterranean ants is patchy and biased, with a concentration of sampling effort in the Neotropics (Fig. 2). Most studies have been situated within tropical regions, possibly a re-

Tab. 2: Research topics and focal areas of studies using subterranean sampling techniques in a standard sampling protocol (n = 36). Individual studies can comprise several research topics and focal areas. The complete list of studies used can be found in Table S3.

Research topics	**Focal areas**	**Technique used** (No. of studies)
Methods	Development and/or trial of new SBC or SPT designs for subterranean baiting	SBC (2), SPT (3)
	Comparing sampling efficiency and / or complementarity of multiple sampling methods, including subterranean sampling techniques	SBC (2), SSm (2)
Diversity	Assessment of subterranean ant diversity only	SBC (2), SPT (3)
	Assessment (and comparison) of subterranean and aboveground ant diversity	DS (1) SBC (3), SPT (1), SSm (3) SSw (1)
Ecology	Comparing ant communities (including subterranean) within different habitats and / or land use practices	DS (1), SBC (2), SPT (1), SSm (2)
	Investigating vertical stratification of subterranean ants in soil	SBC (2)
	Investigating biogeographic and / or temporal patterns in distributions of subterranean ant communities	DS (1), SBC (1), SSm (1), SSb (1)
	Responses of subterranean ant diversity to changes in abiotic or biotic factors (e.g., soil properties, relative ground cover) in a single habitat	SBC (1), SPT (3), SSm (1)
Foraging ecology	Mapping foraging patterns of a single hypogaeic species	SBC (1)
	Dietary preferences and/or resource partitioning among subterranean (and aboveground) ant communities	SBC (3)
	Responses of foraging rates of subterranean ant communities to changes in habitat, climate, disturbance etc.	SBC (2)

flection of the anticipated higher diversity of subterranean ants in these areas, and only a handful of studies have taken place in temperate ecosystems of Japan and Eastern USA. Subterranean ants of the majority of biomes have not been sampled, especially within the vast grasslands and shrub lands in tropical and temperate regions, as well as in temperate deciduous and coniferous forests. Even tropical moist and dry forests, the biomes which are potentially sampled most frequently by other methods, have been extensively under-sampled for their subterranean ant communities. For example, the Afrotropical region is almost devoid of studies (with the exception of YEO & al. 2017), despite this region's recognition for its important subterranean fauna (e.g., termites, EGGLETON 2000). Similarly, no studies have been undertaken in the tropical forests of the Indian subcontinent, and only a handful of studies have been conducted in the Indomalayan region. Subterranean baiting and soil sampling are the most commonly used methods in standard sampling protocols for subterranean ants, though by contrast, and to the best of our knowledge, direct sampling has only been incorporated in the standard sampling protocols of three studies (see previous section). Common research topics and focal areas for studies employing subterranean sampling techniques are highlighted in Table 2, while preliminary information on the ecology and diversity of subterranean ant communities is discussed below.

Diversity of ants in subterranean collections: Despite the relatively limited use of subterranean sampling techniques and their biogeographic biases (Fig. 2), 125 genera have been collected using these techniques, representing about a third of the 334 genera currently described globally. With the exception of the Aneuretinae, Myrmeciinae, and Paraponerinae subfamilies, the majority of ant subfamilies are well represented at the generic level in subterranean samples (Fig. 3). Furthermore, a quarter to nearly half of the genera known from the five most diverse subfamilies has been recorded from subterranean samples. Differences in the relative proportion of genera collected among subfamilies most likely reflect the biology of individual species, though they may also represent potential biogeographic bias of subterranean sampling. For instance, the absence of Aneuretinae in subterranean samples may simply be due to the complete lack of subterranean sampling in Sri Lanka where this subfamily is endemic. The overall shortage of subterranean sampling in Asia (Fig. 2) may also explain the low generic diversity observed in subterranean records of the hypogaeic subfamily Leptanillinae (Fig. 3), for which seven of the eight known genera are restricted to this continent (ANTMAPS.ORG 2016, JANICKI & al. 2016); subterranean sampling within Asia may thus promote capture of leptanilline species (e.g., WONG & GUÉNARD 2016a). Conversely, extensive subterranean sampling within the Neotropics, complemented by some collections from the Australian region (Fig. 2), may have favoured the collection of all genera in the Ectatomminae subfamily (Fig. 3) which are distributed throughout these regions. Notably, a recent and unique study in West Africa allowed for the capture of seven ponerine genera never before collected through subterranean sampling, with four of them endemic to the Afrotropical region (YEO & al. 2017). In summary, an important diversity of genera, representing both ancestral and derived clades of ant species, may be collected through subterranean sampling methods.

Most individual studies using subterranean sampling methods aim to assess the diversity of the subterranean ant communities. For each study, the respective measures of species richness and composition (by subfamily) are summarised in Table S1. Among the studies reviewed, the two

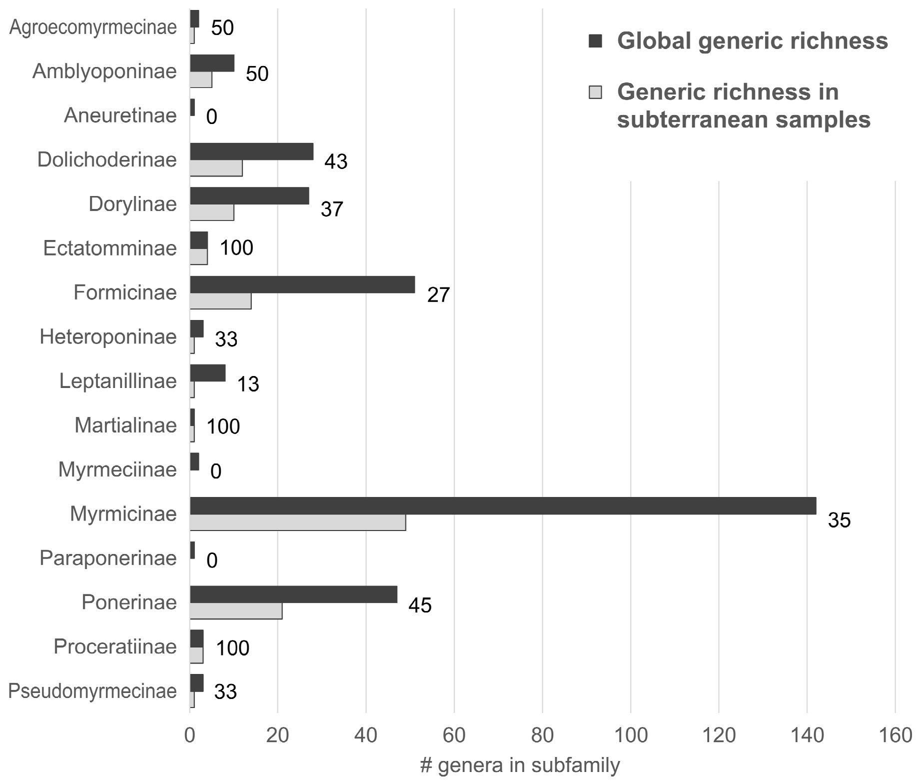


Fig. 3: The sixteen extant ant subfamilies and the respective proportions of their generic diversity recorded in collections from subterranean sampling techniques. For each subfamily, the percentage (%) of total genera represented in subterranean samples is shown. Genus records from both systematic and opportunistic sampling efforts have been included in this figure. Refer to Table S2 for a full list of subterranean collection records for the different ant genera.

most speciose subterranean collections were both obtained by soil sampling (SSm) in Brazil. At a cocoa plantation (8 ha) in Ilhéus (Bahia), 113 species in nine subfamilies were recorded from 4,131 soil samples (DELABIE & FOWLER 1995), while at Manaus (Amazonas), 106 species in four subfamilies were recorded from 324 soil samples in a fragmented tropical forest (< 100 ha) (VASCONCELOS & DELABIE 2000). The lowest recorded diversity of subterranean ants was also observed in Brazil, but through subterranean baiting in 30 ha of fragmented subtropical forests at Rolante City (Rio Grande do Sul), where 13 species in four subfamilies were collected from 80 SBCs (SCHMIDT & DIEHL 2008). The lowest recorded ant diversity from soil sampling (SSm) originates from a subtropical grassland of South Africa (sampled area not reported), where 14 species in three subfamilies were recorded from 36 soil samples (LINDSEY & SKINNER 2001). Importantly, the variation observed among the abovementioned studies should be considered with caution due to a lack of standardization in the methods used, areas sampled, and sampling effort deployed.

Aboveground and subterranean communities – just how similar are they? The majority of studies employing subterranean sampling techniques have often sampled aboveground ant communities using conventional surface sampling techniques. Some studies have also specifically compared ant assemblages from above and below the ground surface (e.g., SILVA & SILVESTRE 2004, PACHECO & VASCONCELOS 2012, YEO & al. 2017). When subterranean sampling techniques are paired with protocols using surface sampling techniques, the proportion of total species collected in subterranean samples varies substantially (17 - 91%) (Tab. 3). Perhaps of greater interest, the proportion of total species unique to subterranean samples ranges from as low as zero to almost half of all collected species (Tab. 3). For example, in a tropical Thai forest all ant species found in soil samples were also collected by surface baiting, hand collection or leaf litter extraction (WATANSIT & NHU-EARD 2011). However, in a tropical Brazilian cocoa plantation and a subtropical Brazilian forest as many as 55 of 124 and 32 of 113 species, respectively (DELABIE & FOWLER 1995, SILVA & SILVESTRE 2004), were unique to soil samples and not collected in leaf litter extractions (Tab. 3). Recently, YEO & al. (2017) also found that subterranean ant assemblages collected via DS were distinct from aboveground ant assemblages collected by leaf litter extractions and pitfall traps, on the basis of significantly lower species richness and species evenness in the former. On the whole, therefore, inconsistent findings among studies that compare subterranean and aboveground ant assemblages are likely attributed to the large variance in sampling location, protocol, and effort. Current data suggests that use a greater variety of surface sampling methods (Tab. 3) reduces the likelihood of collecting species which are unique to subterranean samples, but this can only be confirmed in a future controlled study.

Rarity and stratum-specificity of subterranean ants: The main biological properties commonly associated with the rarity of a species include small population size, restricted geographical distribution or high habitat specificity. On the other hand, the perceived rarity of a species may also be influenced by non-biological criteria, such as a prolonged absence of collection records (e.g., over several decades), the discovery of the species outside its normal geographical range, as well as the inability of experts to collect representative samples of the species (RABINOWITZ 1981, ESPADALER & LÓPEZ-SORIA 1991). Both opportunistic and systematic subterranean sampling efforts have uncovered many ant species that are traditionally considered to be rare (see sections on "notable species collected" by the three subterranean sampling methods above). Yet, the "rarity" of many species is more likely an artefact of historic collections using conventional methods (which under-

Tab. 3: A comparison of ant species diversity in surface and subterranean collections for studies that incorporated subterranean sampling techniques into a wider sampling protocol. The studies listed here sampled ants from multiple strata including the subterranean habitat, ground surface and leaf litter layer, understory vegetation and canopy. For this comparison, collections from canopy samples have been excluded. The techniques employed for subterranean sampling include subterranean baiting techniques such as Subterranean Baited Containers (SBC) and Subterranean Pitfall Traps (SPT); Soil Sampling techniques such as the retrieval of ants from collected soil samples either manually (SSm), with a Winkler (SSw) or Berlese extraction (SSb); and Direct Sampling (DS) of ants while excavating soil in situ. The techniques used to sample ants from surface strata include the usage of baiting stations or containers on the ground surface (B); setting pitfall traps flushed with the ground surface (PT); leaf litter sampling techniques such as the retrieval of ants from leaf litter samples either manually (LLm), with a Winkler (LLw) or Berlese extraction (LLb); and direct sampling in surface strata by searching for ants on the ground surface (S) or from beating understory vegetation (V).

Study	**Subterranean technique**	**Surface technique(s)**	**Total species**	**Species in subterranean samples**		**Species unique to subterranean samples**	
				No.	**% of total spp.**	**No.**	**% of total spp.**
DELABIE & FOWLER (1995)	SSm	LLb	124	113	91.1	55	44.4
SILVA & SILVESTRE (2004)	SSw	LLw	113	71	62.8	32	28.3
SCHMIDT & DIEHL (2008)	SBC	B, HC	35	13	37.1	7	20.0
LINDSEY & SKINNER (2001)	DS	PT	43	29	67.4	7	16.3
FOWLER & DELABIE (1995)	SBC	B	74	19	25.7	11	14.9
LYNCH & al. (1988)	SSb	LLb	22	16	72.7	3	13.6
YEO & al. (2017)	DS	LLw, PT	138	96	69.6	17	12.3
VASCONCELOS & DELABIE (2000)	SSm	LLm, PT	227	106	46.7	20	8.8
LINDSEY & SKINNER (2001)	SSm	PT	37	14	37.8	3	8.1
SOUZA & al. (2010)	SBC	B, PT	45	14	31.1	3	6.7
JACQUEMIN & al. (2016)	SSm	LLw, PT	161	62	38.5	10	6.2
OSUNKOYA & al. (2011)	SPT	PT	100	36	36.0	6	6.0
PACHECO & VASCONCELOS (2012)	SPT	PT	232	75	32.3	12	5.2
GARCÍA-MARTÍNEZ & al. (2016)	SPT	B, PT, LLw	75	19	25.3	3	4.0
FISHER & ROBERTSON (2002)	DS	LLw, PT, V	59	21	35.6	2	3.4
RYDER WILKIE & al. (2010)	SBC	B, HC, LLw, PT	270	48	17.8	8	3.0
LUBERTAZZI & TSCHINKEL (2003)	SBC	B, PT	71	20	28.2	0	0.0
WATANASIT & NHU-EARD (2011)	SSm	B, HC, LLm	87	40	46.0	0	0.0
ANDERSEN & al. (2012)	SPT	HC, LLw, PT	82	25	30.5	0	0.0

sample subterranean ants), as opposed to a consequence of the biological properties of individual species. Evidence substantiating this hypothesis is the occasionally high number of species unique to subterranean collections when multiple habitat strata are sampled (Tab. 3), the unmatched occurrence rates of some species in subterranean collections (e.g., DELABIE & FOWLER 1995, BERGHOFF & al. 2003), as well as multiple accounts of "rare" ants being collected by subterranean methods in surprisingly high densities, unexpected habitats, or areas outside their reported geographic range (e.g., LÓPEZ & al. 1994, BRANDÃO & al. 2008, SCHMIDT & al. 2014). In future studies, the widespread use of subterranean sampling methods will help to distinguish ant species which have truly low occurrences in nature from those which are under-represented in the majority of studies employing conventional sampling methods such as leaf litter extractions and surface pitfall traps.

Ecological and environmental patterns of subterranean ant diversity: While a number of studies have used subterranean sampling methods to investigate specific relationships between environmental factors and subterranean ant diversity, inconsistencies in study aims and the variables measured preclude any broad relationships from being inferred with certainty. Nevertheless, a summary of findings in three main areas is provided below.

Spatial and temporal patterns: Species composition of subterranean ant communities from soil samples (SSm) is reported to vary substantially across spatial scales, with high species turnover observed at both regional (> 2 km) and limited scales (~ 1 m) (JACQUEMIN & al. 2012, 2016). A high species turnover across limited spatial scales may arise from varying distribution patterns of subterranean nest sites, which, in turn, relate to the unique ecology of individual species. For example, using DS to discover and excavate subterranean ant nests, MASUKO (2010) classified the distribution patterns of Japanese subterranean nesting species into three distinct groups: random, over-dispersed, and weakly clumped. The diversity of subterranean ants is also known to display temporal variation in both tropical and temperate climates. For example, subterranean ant diversity in a tropical Ecuadorian forest varied with the wet-dry cycle, with higher species richness and abundance observed in soil samples (SSm) during the dry season – possibly owing to the migration of drought-sensitive epigaeic species downwards into the soil during these periods (JACQUEMIN & al. 2016). Similarly, in a temperate forest of Maryland USA, the species richness of ants collected in soil samples (SSb) almost doubled throughout May to August (i.e., late spring to summer) as compared to all other times of year (LYNCH & al. 1988). With a single study recording the differences in diurnal versus nocturnal subterranean ant activity, and based on a rather limited sampling effort of SBCs (N = 20) (EGUCHI & BUI 2009), the overall knowledge on circadian patterns of subterranean ant communities is extremely limited. To this end, more extensive sampling with either subterranean baiting (i.e., by staggering baiting periods) or soil sampling (i.e., by collecting soil samples at different times of day) may elucidate possible circadian rhythms of subterranean ants in future work.

Biotic and abiotic drivers: Preliminary findings from few available studies suggest that biotic properties of aboveground vegetation may exert little influence on subterranean ant communities. For example, the infestation of an invasive creeper in Australia had limited impacts on subterranean ant diversity sampled through SPTs (OSUNKOYA & al. 2011). Similarly, the diversity of ants collected in SBCs did not vary significantly across a gradient of herbaceous ground cover in a pine forest in Florida (cf. contrasting patterns displayed by ant assemblages of the ground surface and vegetation) (LUBERTAZZI & TSCHINKEL 2003). Studies on the associations between subterranean ants and the abiotic properties of their soil environments report equivocal results. While physicochemical properties of soil (e.g., organic matter content, pH, electrical conductivity, degree of humification, and soil texture) were relatively poor predictors of ants collected by SSm (JACQUEMIN & al. 2012), the degree of soil compaction has recently shown to affect the composition of subterranean ant assemblages, with small-sized species occurring more frequently in compacted soil than larger-sized species (SCHMIDT & al. 2016). However, the latter findings were based on a limited sampling effort using SPTs (N = 20) and a separate study found no effects of soil compaction on subterranean ant assemblages (GARCÍA-MARTÍNEZ & al. 2016). As soil compaction impacts the diversity of other arthropod populations (EATON & al. 2004), compacted soils may also indirectly affect subterranean ants by affecting prey availability (e.g., oribatid mites in BATTIGELLI & al. 2004). Future experiments modifying soil compaction as well as moisture and temperature will further elucidate how these factors influence subterranean ant diversity and composition.

Habitat modification and land use change: Like other ecological communities (e.g., LIU & al. 2016), subterranean ants are affected by extensive habitat modification and land use change. For example, significant differences in the richness and composition of ant species in soil samples (SSm) were observed between heterogenous and homogenous rubber plantations in Thailand (WATANASIT & NHU-EARD 2011), and in Brazil, the composition of subterranean ant communities collected by SBCs in agricultural croplands was shown to be distinct from those in adjacent secondary forests and forestry plantations (SCHMIDT & DIEHL 2008). However, while the effect of land use change on the ecology of surface ant communities has been explored, such as in their contributions to ecosystem function and interspecific interactions (e.g., BESTELMEYER & WIENS 1996, CRIST 2009, GRAY & al. 2015), these relationships are unknown for subterranean ants and certainly deserve investigation.

Foraging ecology of subterranean ants: Thus far, studies on the foraging ecology of subterranean ants have been achieved with the usage of subterranean baiting, specifically SBCs (Tab. 2), which allow for bait manipulation and facilitate the observation of continuous foraging activities by subterranean ants. The majority of studies have investigated the foraging ecology of the subterranean Dorylinae, with some monitoring and mapping the foraging patterns of individual hypogaeic species (e.g., *Dorylus laevigatus* SMITH, 1857 in BERGHOFF & al. 2002 and *Acanthostichus quadratus* EMERY, 1895 in MORINI & al. 2004), and others examining the effect of environmental gradients on foraging by subterranean Dorylinae communities (O'DONNELL & KUMAR 2006, KUMAR & O'DONNELL 2009). In spite of their differing focal species and research aims, all abovementioned studies report that subterranean Dorylinae have unique foraging ecologies which are distinct from those of their surface-dwelling counterparts. For example, BERGHOFF & al. (2002) observed the hypogaeic species *D. laevigatus* occupying the same foraging site for several months, contrasting with epigaeic army ant species which are characterized by a very localized and temporary presence at foraging sites. The stable subterranean trunk trail system used by *D. laevigatus* also contrasts with the short-lived swarm raids of epigaeic doryline species (BERGHOFF & al. 2002, KRONAUER 2009). In Costa Rican cloud forests, foraging rates of subterranean Dorylinae were neither affected by habitat type nor by day-to-day weather variation within sites, but foraging rates displayed an increase with elevation. These findings contrast patterns observed in surface Dorylinae communities (O'DONNELL & KUMAR 2006, KUMAR & O'DONNELL 2009). Collectively, the findings from a variety of studies using SBCs to study subterranean Dorylinae highlight their unique foraging ecology, which remains understudied for the majority of species (KRONAUER 2009). Subterranean baiting is an effective method for studying the foraging ecology of subterranean Dorylinae communities because these ants tend to exist in large populations that aggressively recruit to subterranean bait in extensive foraging raids (WEISFLOGG & al. 2000, BERGHOFF & al. 2002). This method has also been successfully applied to investigate other subterranean ant communities, although such studies are less common. For example, YAMAGUCHI & HASEGAWA (1996) used SBCs with a variety of live baits to study trophic preferences and predation rates of subterranean ant communities in Japan, with earthworms and woodlice corresponding to the highest predation rates by subterranean ants. When bait was controlled for, species of the genera *Tetramorium* (MAYR, 1855), *Solenopsis* (WESTWOOD, 1840), *Formica* (LINNAEUS, 1758) and *Ponera* (LATREILLE, 1804) displayed a vertical stratification of predation in the 50 cm-deep soil layer (YAMAGUCHI & HASEGAWA 1996). As a caveat, the potential for subterranean baiting to collect or observe specific ants may be severely limited if a non-target dominant species recruits in large numbers – this effect was observed when *D. laevigatus* raided SPTs in Singapore in densities of over 500 individuals per device, excluding almost all other species from the collections (M.K.L. Wong, unpubl.).

Discussion

In spite of their recognized importance to multiple fields across myrmecology, the majority of the world's subterranean ant faunas remain understudied. While conventional ant sampling methods potentially under-sample the ants found below ground (Tab. 3), studies that systematically employ techniques for specifically collecting ants from the subterranean environment are few in number and geographically scattered (Fig. 2). Collectively, the findings of the studies in this review indicate that subterranean ant communities are often distinct and diverse in composition, and may also exhibit unique ecological relationships and life histories. A variety of novel and significant taxonomic accounts have also been realised for hypogaeic species collected with these techniques. Therefore, there are strong grounds for increased sampling and investigation into the diversity and ecology of subterranean ant communities. However, a key obstacle to future work on subterranean ants is the substantial variation in the sampling techniques used to date, which precludes meaningful comparisons across studies and the inference of broad relationships about this important ant community. In this review, we identified at least seven different subterranean sampling techniques (Tab. 1). Furthermore, there is marked variation among the technical specifications of individual techniques; for instance, the designs and sizes of devices used in subterranean baiting (Fig. 1). Below we discuss two methodological approaches that should be explored simultaneously in order to rapidly advance knowledge on subterranean ant communities.

Assessing subterranean ant diversity – method selection and familiar considerations: A standard sampling technique and protocol for assessing subterranean ant diversity is urgently needed to address the current dearth of basic information on the composition and richness of this group of ants. Establishing an approach for systematically assessing the diversity of subterranean ant communities will promote consistency among studies, and should in turn allow for cross-study comparisons to reveal important patterns of diversity that relate to macroecology and biogeography – such as patterns across continents or along elevation gradients. In assessments of ant diversity, the selected method(s) should be readily standardized for effective usage across many different habitats, so as to facilitate the comparison of species assemblages among multiple sites and regions (STEINER & al. 2005, GOTELLI & al. 2011).

Individual subterranean sampling methods are subject to unique sampling biases, and as such they differ substantially in their specific advantages and limitations for use in diversity assessments. Notably, many aspects relating to the suitability of subterranean sampling methods for diversity assessments are also relevant to sampling methods for ants of other habitat strata (e.g., ground surface and canopy ants) (see GOTELLI & al. 2011). For example, direct sampling in soil for subterranean ants, as well as aboveground for litter and canopy ants, may be too taxing on time and labour to accommodate the repetitive and rapid nature of diversity assessments. Moreover, these "active sampling methods" (BESTELMEYER & al. 2000) are generally unsuitable for comparative assessments because their findings are heavily dependent on the relative skills of personnel undertaking the sampling (GOTELLI & al. 2011).

Similarly, despite their potential sampling efficiency (i.e., when small devices are used), subterranean baiting and comparable methods used in aboveground sampling may not be ideal for diversity assessments because they do not capture "random" samples of ants. In SBCs, SPTs and the extensively utilised surface pitfall traps alike, the container's overall size, as well as the dimensions and positions of entrances will disproportionately influence the collection of different ant species according to specific body sizes and foraging patterns (ABENSPERG-TRAUN & DION 1995, GOTELLI & al. 2011). Furthermore, both surface and subterranean bait are susceptible to biased community composition and abundance, as they may be overrun by generalist species while simultaneously excluding trophic specialists. Although pitfall trapping without the usage of bait is a well-established and relatively effective method for assessing ant diversity on the ground surface (but see limitations discussed in GOTELLI & al. 2011), we know of only one example where unbaited subterranean pitfall traps performed effectively in a standard sampling protocol (PAOLUCCI & al. 2016). Thus far, it appears that the majority of ant studies have avoided the usage of unbaited collection devices in subterranean sampling, and preliminary sampling with unbaited SPTs for a 72-hour period also proved unsuccessful (Fig. 1d; M.K.L. Wong, unpubl.). This low success may be due to the lower activity levels of hypogaeic species, as well as the limited number of entrances to most collection containers (Fig. 1), which together account for a low probability that ants will randomly stumble into the SPTs in the absence of attractants. On this note, there are several examples from non-myrmecological literature of SPTs constructed with porous wire mesh (as opposed to solid containers), which maximise the surface area for subterranean fauna to enter the collection devices (e.g., OWEN 1995, HERIBERTO & PEDRO 2010); these may be trialled for collecting subterranean ants.

In comparison to direct sampling by hand and subterranean baiting with SBCs or SPTs, soil sampling methods – which are relatively similar to litter sampling at the ground surface – are likely to provide less biased measurements of ant diversity (BESTELMEYER & al. 2000). As with litter sampling, variation arising from human error in soil sampling should be further minimized through the employment of a consistent extraction technique such as a Winkler extractor to retrieve specimens from the soil samples. Using SSw therefore lowers the risk of escapees and missed specimens that are likely to occur in SSm where the ants are collected by hand after sieving. This is especially important when sampling the subterranean stratum, since many hypogaeic species are small in size and pale in colour (Box 1), and can be missed even by a well-trained eye. Leaf litter sampling with Winkler extraction has repeatedly demonstrated the highest sampling efficiency among available ground surface sampling methods (ANDERSEN 1991, FISHER 1999, BESTELMEYER & al. 2000, DELABIE & al. 2000). However, in subterranean sampling it remains to be examined whether SSw is more efficient than direct sampling

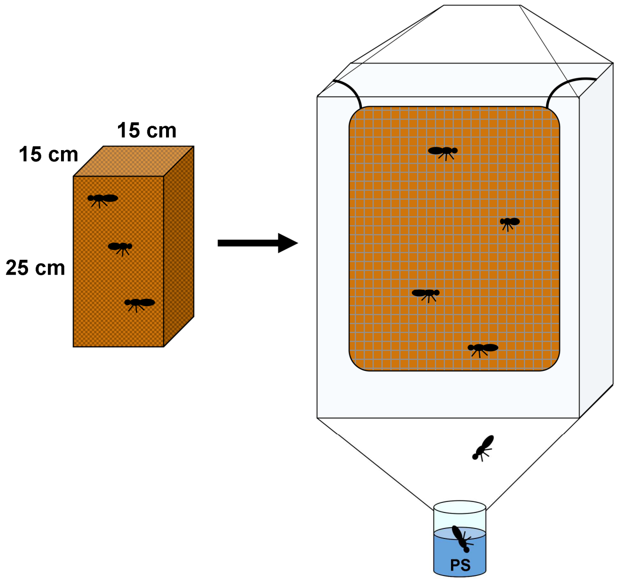


Fig. 4: Soil sampling with Winkler extraction (SSw), a proposed sampling technique for subterranean diversity assessments. A soil sample of the dimensions indicated is excavated and processed with a Winkler extractor column to retrieve subterranean ants, which are collected in preservation solution (PS).

and subterranean baiting, since no studies have adopted more than one method in a single protocol. In addition, to investigate or account for temporal variation in subterranean ant activity, soil sampling can be performed at different times of day or year – this is akin to the recommendations for litter sampling, which is subject to similar limitations (see BESTELMEYER & al. 2000).

Based on the results of previous soil sampling efforts (Tab. S1), we find that collecting a soil sample of dimensions 15 cm × 15 cm and to a depth of 25 cm should suffice for a representative sample of the subterranean ant community. In particular, the sampling depth of 25 cm is chosen because it incorporates the agreed range of peak subterranean diversity and maximum number of hypogaeic species among the studies presently reviewed (i.e., 10 - 20 cm). Therefore, we suggest that an SSw with the above specifications (illustrated in Fig. 4) may be an effective standardized sampling technique for future assessments on the diversity of subterranean ant communities. The Winkler extraction is preferred over the Berlese extraction for its accessibility (i.e., electricity not required) and sampling efficiency (see comparison in BESTELMEYER 2000), which can be substantially increased by practicing regular Winkler shuffling (GUÉNARD & LUCKY 2011). In keeping with previous studies, researchers targeting hypogaeic species should distinguish these from epigaeic species in subterranean samples on the basis of their cryptobiotic morphology (Box 1) and relative abundance, since the collection of epigaeic species nesting in soil cannot be completely avoided regardless of sampling technique used.

Exploring other methods for new areas of study: As techniques that are well suited for rapid diversity assessments like soil sampling may not reveal other poorly known aspects of subterranean ants such as their activity patterns, foraging ecology, and interspecific interactions, we also propose that investigators should not dismiss alternative subterranean sampling techniques that may better address these specific research hypotheses. It is clear that subterranean baiting techniques are better suited than others for such research as they can obtain meaningful sets of data (e.g., trophic preferences, predation rates, activity patterns, etc.) and their designs lend well to in situ experimental setups (e.g., separating or excluding collections by depth, long-term monitoring, etc.). Our survey of myrmecological literature suggests that the potential research applications of subterranean baiting have not been fully explored. For example, some areas not yet investigated with subterranean baiting include the study of short-term circadian activity patterns in subterranean ants, as well as long-term comparisons of foraging rates across the vertical soil profile. In addition to the current assortment of designs for SBCs and SPTs (Fig. 2), a brief survey of non-myrmecological literature reveals other potentially useful subterranean baiting and trapping devices (e.g., OWEN 1995, SCHLICK-STEINER & STEINER 2000, LÓPEZ & OROMÍ 2010, ORTUÑO & al. 2013, 2014) as well as alternative methods for collecting and tracking the movements of other subterranean fauna (e.g., FORSCHLER 1994, LAWRENCE & BOWERS 2002, BASTARDIE & al. 2005). In this spirit, we encourage myrmecologists to pursue novel approaches for subterranean sampling, and to consider modifying current techniques to overcome long-standing limitations of observing ant behaviour occurring underground (e.g., installing cameras in SBCs to observe interspecific interactions or to measure scavenging rates).

We anticipate that standardized diversity assessments complemented by the innovative exploration of new hypotheses about this final frontier will bring to light the cryptic biology of the enigmatic ants from the underworld.

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