



Accessing the subterranean ant fauna (Hymenoptera: Formicidae) in native and modified subtropical landscapes in the Neotropics

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Abstract: Several studies have characterized and delimited subterranean ant assemblages. Soil extraction, one of the methods employed to access this fauna, employs the removal of monoliths. One of the most widely used methods for the extraction of soil sampling is called TSBF (developed by the Tropical Soil Biology and Fertility Programme). This method provides relevant data about the species associated with the soil. In the present study we characterized assemblages of subterranean ants using the TSBF method in different subtropical areas of the Neotropics. We considered two sampling designs in different localities. The first design resulted in 315 TSBF samples obtained from layers at distinct depths. The second design resulted in 270 TSBF samples and 270 epigeic pitfall trap samples. This material was used to delimit the species that occur exclusively in the subterranean stratum (TSBF) and that are not found on the soil surface. A total of 281 species were recorded. Of these, 57 can be considered subterranean, based on their occurrence in subterranean strata. We also verified that the highest occurrence of ants was in the first 10 cm of soil depth. Due to the importance of using methods that efficiently extract the subterranean ant fauna in studies, we suggest the TSBF method should be used to sample ants or to associate this method with epigeic pitfall traps to delimit strictly subterranean assemblages in specific community stratification studies.

Keywords: Soil, hypogaeic, Brazil, monoliths, pitfall.

Avaliação da mirmecofauna subterrânea (Hymenoptera: Formicidae) em paisagens nativas e modificadas na região subtropical do Neotrópico

Resumo: Vários estudos têm caracterizado e delimitado assembleias de formigas subterrâneas. A extração de solo, um dos métodos utilizados para amostragem dessa fauna, implica na remoção de monólitos. Um dos métodos mais utilizados na extração de monólitos é chamado TSBF (desenvolvido pelo Programa *Tropical Soil Biology and Fertility*). Esse método fornece dados extremamente relevantes sobre as espécies associadas ao solo. No presente estudo, nós caracterizamos as assembleias de formigas subterrâneas usando o método TSBF em diferentes áreas subtropicais do Neotrópico. Utilizamos dois delineamentos amostrais distintos. O primeiro delineamento resultou em 315 amostras de TSBF obtidas a partir da estratificação do solo. O segundo resultou em 270 amostras de TSBF mais 270 provenientes de armadilhas pitfall epigeicas. Esse material foi utilizado para delimitar espécies que ocorrem exclusivamente no estrato subterrâneo (TSBF) e não são encontradas na superfície do solo. Registramos um total de 281 espécies. Destas, 57 podem ser consideradas subterrâneas (baseado na ocorrência no respectivo estrato). Também verificamos que a maior ocorrência de formigas se deu nos primeiros 10 cm de profundidade. Devido à importância do uso de métodos que colem eficientemente formigas subterrâneas, sugerimos o uso do método TSBF em estudos de estratificação de comunidades ou sua associação a armadilhas do tipo pitfall epigeica para delimitar assembleias estritamente subterrâneas.

Palavras-chave: Solo, hipogéicas, Brasil, monólitos, pitfall.

Introduction

Ants are conspicuous organisms present in most terrestrial environments (Folgarait 1998), from the arboreal to the subterranean strata (Lucky *et al.* 2013, Jacquemin *et al.* 2016). However, little is known about underground ant assemblages (Ryder Wilkie *et al.* 2007, Andersen & Brault 2010, Jacquemin *et al.* 2012), mainly because there are few efficient and specific methods for collecting this important fauna (Wong & Guénard 2017). Underground ants are the last biodiversity frontier to be explored in Formicidae (Rider Wilkie *et al.* 2007, Andersen & Brault 2010), and are also important for the understanding of the origin and evolution of Formicidae (Lucky *et al.* 2013). In addition, subterranean ants are considered bioturbators *sensu strictu* (Bottinelli *et al.* 2015), and because they respond to drastically changes in soil density, they can serve as bioindicators (Schmidt *et al.* 2013).

Aiming to develop an efficient sampling method for subterranean ants, researchers have been testing useful sampling techniques that can be replicated. Wong & Guénard (2017) compiled the published research on the collecting methods of subterranean ants and classified them into three types: (1) subterranean baits, (2) soil sampling, and (3) direct sampling. Most of those publications (65%) described the use of baits or other attractive items (Wong & Guénard 2017), which favor the capture of predatory, generalist and mass-recruiting species (Longino 2000, Bestelmeyer *et al.* 2000, Schmidt & Solar 2010, Wong & Guénard 2017).

Amongst the methods classified as “soil sampling” is the extraction of monoliths (Delabie & Fowler 1995, Fowler *et al.* 2000, Andersen & Brault 2010, Wong & Guénard 2017). The use of monoliths is promising because it enables us to determine the vertical stratification of species in the soil, among other advantages (see: Jacquemin *et al.* 2012, Jacquemin *et al.* 2016, Wong & Guénard 2017). A widely used technique of monolith extraction in soil macrofauna studies was proposed by the Tropical Soil Biology and Fertility Program (TSBF) (Anderson & Ingram 1993) and was standardized by the International Organization for Standardization (ISO) in the ISO norm 23611-5:2011. This technique, known as TSBF method, generates a substantial volume of ant specimens. It consists of the removal of soil monoliths that are 25 x 25 cm and are taken 20 or 30 cm deep in the soil, followed by hand sorting extraction of the associated fauna (Bartz *et al.* 2014a, Rosa *et al.* 2015, ISO 2018).

Despite the massive volume of material that has been sampled using the TSBF method, published information on the ecological dynamics of the ants collected using it is insufficient. This is a function of the fact that Pedobiology studies (which employ the TSBF method with high frequencies) traditionally identify the collected material at high taxonomic levels (like Order, Family and Genera) and therefore do not give information on community composition and species ecology (see: Lourente *et al.* 2007, Klenk *et al.* 2009, Correia 2010, Lima *et al.* 2010, Rousseau *et al.* 2010, Zagatto 2014, Benazzi *et al.* 2013, Pereira *et al.* 2012, Gutiérrez *et al.* 2017).

The present work characterizes subterranean ant assemblages in three subtropical regions of the Neotropics using two sampling methods: TSBF and epigeaic pitfall traps. *In situ* extraction methods, like monoliths, can help to detect ecological, behavioral and morphological patterns, since the specimens collected using these methods are not attracted from other strata (from the surface, for example). Furthermore,

TSBF is widely disseminated in studies that evaluate the impact of land use systems on the underground soil (micro, meso and macro fauna) (Lavelle *et al.* 1997, Bartz *et al.* 2014a, Rosa *et al.* 2015). In order to improve the scientific contribution of this study, we characterize the ant assemblages and compare the communities at the species level.

Material and Methods

1. Study area

The specimens came from three localities in the Southern Region of Brazil: (1) Ponta Grossa, *Campos Gerais* region, state of Paraná; (2) Western and (3) Plateau, regions of the state of Santa Catarina. In each region of Santa Catarina, the samples were collected from three municipalities: Xanxerê, Chapecó and São Miguel do Oeste in the West and Lages, Otacílio Costa and Campo Belo do Sul in the Plateau (Figure 1).

Ponta Grossa is in the Second Plateau of Paraná, about 120 kilometers from the state capital, Curitiba. According to Maack (2012), the region is characterized by open grasslands with *Araucaria angustifolia* (Bertol.) Kuntze 1898 patches, within the Atlantic Forest domain. Climate is type Cfb (climatic classification of Köppen-Geiger) -subtropical humid, without defined dry periods throughout the year and with the average temperature of the warmest month lower than 22 °C. The sampled areas have mean elevation of 875 m and total annual rainfall between 1.300 and 1.800 mm throughout the year (IAPAR, 1978). Seven land use systems (LUSs) were sampled: (1) Integrated Crop-livestock (ICL); (2) Integrated Crop-livestock-forestry (ICLF); (3) Grazed native field (GN); (4) *Eucalyptus* sp. plantation (EP); (5) no-tillage (NT); (6) *Campos Gerais* (CG), and (7) Mixed Ombrophilous forest (F). The native areas, CG and F, were at Vila Velha State Park (25°14'17" S 50°0'39" W). The land use systems ICL, ICLF, and GN were in the Model Farm of the *Instituto Agrônomo do Paraná* (IAPAR) (25° 5' 11" S 50° 9' 38" W) and the EU and NT systems were at the Experimental Field of the *Empresa Brasileira de Pesquisas Agropecuárias* (Embrapa) (25°08'17" S 50°04'47" W).

The western region of Santa Catarina is characterized by humid subtropical Cfa climate, with no defined dry season, and the average temperature during the warmest month is above 22 °C (Bartz *et al.* 2014a). The native forest in the Western region of the state is a transition between mixed ombrophilous and semi-deciduous forest (Bartz *et al.* 2014b). In the Plateau region the climate is classified as Cfb (as described for Ponta Grossa), and native grasslands with mixed ombrophilous forest patches predominate (Rosa 2013). The municipalities of each region of Santa Catarina were chosen based on their geographic characteristics, soil type, LUSs, and management history. Five LUSs were sampled in each of the three municipalities: (1) Native forest (FN); (2) *Eucalyptus* sp. plantation (EP); (3) Pasture (PA); (4) No-tillage (NT) and (5) Integrated Crop-livestock integration (ICL) (for details on the location and characterization of the areas see: Rosa 2013, Bartz *et al.* 2014a, b, Rosa *et al.* 2015).

All areas were part of a study of soil quality in different land use systems developed by the Embrapa Forestry (Colombo, PR), and Santa Catarina SisBiota Project, led by the *Universidade Estadual de Santa Catarina* (UDESC) at Chapecó. In these projects, soil fauna was evaluated as an indicator of soil quality in different LUS.

Subterranean ants of southern Brazil

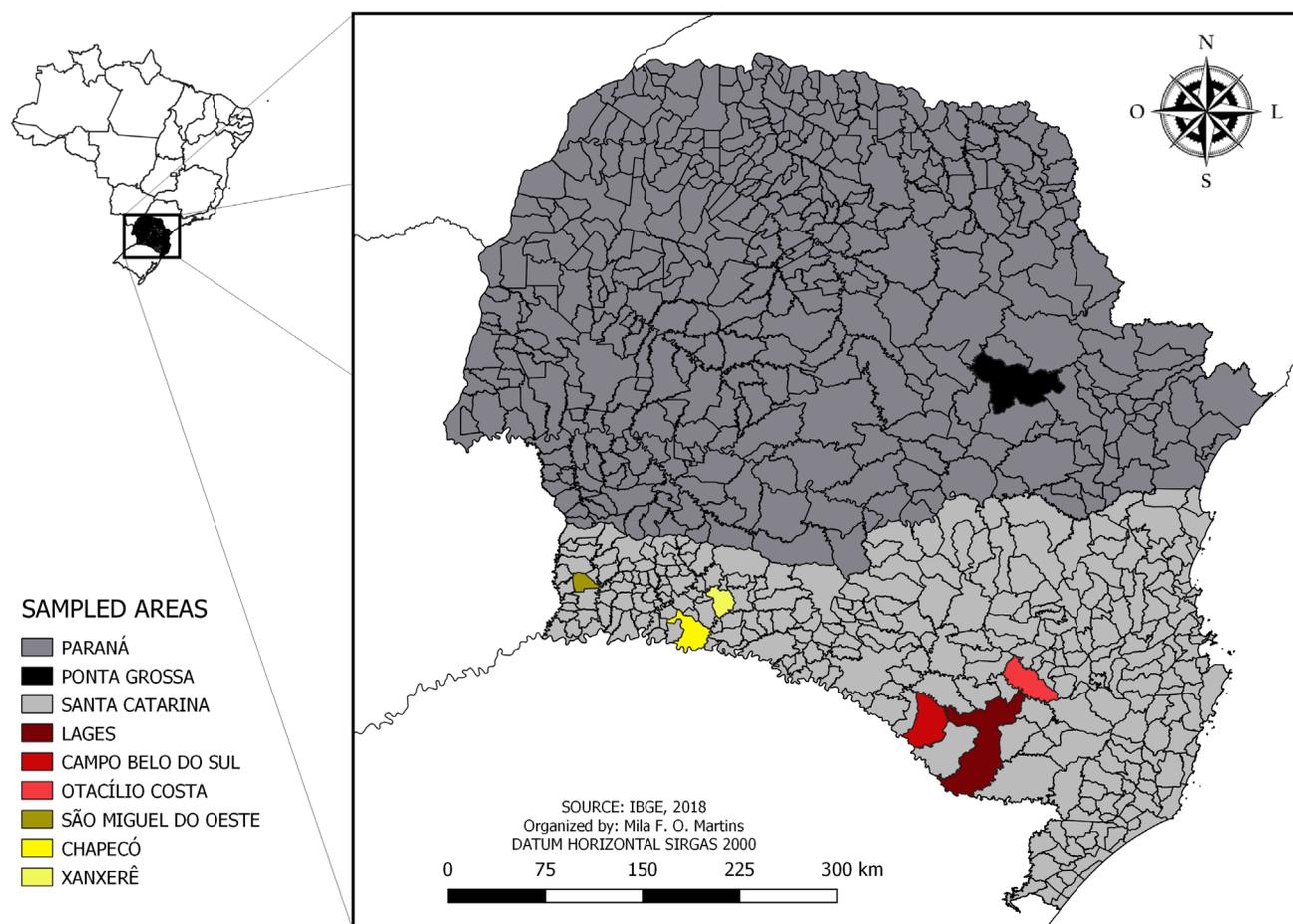


Figure 1. Map of the sampled areas.

2. Sampling design

The Tropical Soil Biology and Fertility Program organizes measures to monitor soil quality through the removal of monoliths in places where financial and technological resources are limited. The soil monoliths were 25 x 25 cm, and the depth at which they were collected was variable according to the extent of horizon A of the soil (Anderson & Ingram 1993, ISO 2018). This method can be applied to any soil type. The area for the removal of monoliths is delimited by a metallic quadrangular structure, followed by the excavation and removal of soil layers. Each layer is packed separately, and the associated fauna can be extracted either in the field or in the laboratory, with or without assistance of a stereomicroscope (Anderson & Ingram 1993). In the present study, all extractions were performed in the laboratory, without stereomicroscope. Our extractions, in Ponta Grossa, were made in layers up to 20 cm, delimited in three strata: 0 - litter; I - 0 to 10 cm and II - 10 to 20 cm. In Santa Catarina, extractions were not stratified.

We used material from two different sample designs, according to the research group involved in the sampling. In Paraná, samples were collected on four different dates. In the areas of ICLF, ICL, NF, NT, and EU, samples were taken in October and November 2012 and in April and May 2013. In the Vila Velha State Park, samples were taken in September 2013 and in January 2014. All areas of Santa Catarina (West and Plateau) were sampled in the course of two seasons: winter (July and August 2011) and summer (December 2011 and January 2012).

In Paraná, for each area (seven LUSs) three plots of 50 x 100 m (0.5 hectares) were delimited per LUS, within which five monoliths were extracted, separated by at least 200 m. These samples were distributed in a central transect, 20 meters from the edge of the plot. A zigzag pattern was drawn with samples always at least 20 m apart and following the slope of the plot (Zagatto, 2014) (Figure 2). A total of 105 monoliths (seven LUSs x three plots x five monoliths) were collected, divided into the depth layers mentioned above (0, I and II), totalling 315 sample units per season of the year (630 total samples).

In Santa Catarina, one area of each LUS (NF, EU, PA, ICL, and NT) was evaluated in each of the three municipalities per region (West and Plateau). Sampling grids, with nine points mounted in three lines of three points each, were sampled 20 m from the border and 30 m apart in each LUS, totaling one hectare. At each point a monolith was extracted and an epigeic pitfall trap was installed (one meter distant from the monolith) (Figure 3). Each pitfall trap consisted of a 200 ml capacity recipient, half filled with water and detergent, buried with the opening at ground level and left in the field for 72 hours. A total of 270 samples of TSBF and 270 of epigeic pitfalls (three municipalities x five LUSs x nine monoliths x two regions) were obtained per season (1080 total samples).

The ants from all areas were conditioned in 80% alcohol and sent to the *Laboratório de Sistemática e Biologia de Formigas* of the *Universidade Federal do Paraná* (UFPR). The material was processed

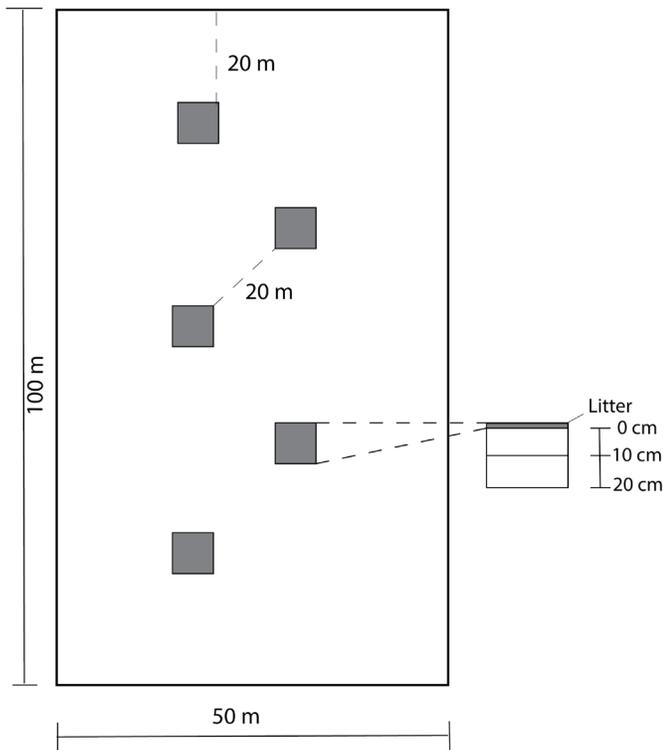


Figure 2. Schematic drawing of the sampling design in the state of Paraná. Adapted from Zagatto (2014).

and identified to the specific level whenever possible. The identifications were generally based on the Guide for the Ant Genera of Brazil (Baccaro *et al.*, 2015). In addition, UFPR specialists (Alexandre C. Ferreira, Gabriela P. Camacho, John E. Latke, Mayron Escárrega, Rodrigo M. Feitosa, and Thiago S. R. da Silva) were consulted to confirm species identifications. Finally, whenever necessary, we performed comparisons with the material deposited in the *Coleção Entomológica Padre Jesus Santiago Moure* at UFPR (DZUP), where voucher material was deposited. The species that were not possible to name were identified as morphospecies.

3. Data analysis

Due to differences in the sampling design, the data were organized in two separate matrices that indicate presence and absence: one matrix with data from Paraná and the other with data from Santa Catarina. Since samples were taken at different seasons to increase the likelihood of finding different species, each species in a sampling unit was considered only once, regardless of the season when it was collected, as suggested by Brandão *et al.* (2011). The observed richness (Sobs) obtained by each method and stratum were compared with the richness estimated by Jackknife1 and Bootstrap. These estimators were chosen because they provide a satisfactory balance in the analyses, since they separately consider the effect of species occurring in a single sample (Jackknife1) and of all species in the samples, without overweighting the presence of rare species (Bootstrap). To determine whether a species is epigeaic or hypogaeic, we used the exclusivity criterion: species collected only by the TSBF method or those present only in strata I and II were considered subterranean; conversely, the species collected only by pitfall traps or

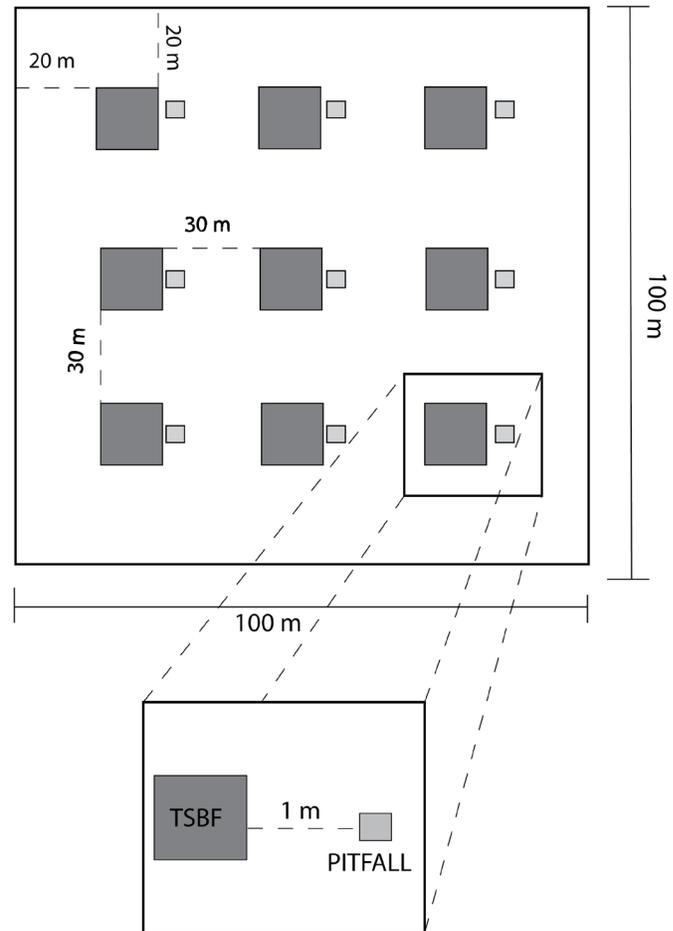


Figure 3. Schematic drawing of the sampling design in the state of Santa Catarina. Adapted from Bartz *et al.* (2014), and Rosa *et al.* (2015).

those present only in stratum 0 of the TSBF method were considered epigeaic. We used the samples from Paraná to evaluate the sampling efficiency of the TSBF method, since only these samples were stratified (layer separation by depth). The stratification allowed us to evaluate the presence of ants in the different strata, providing data not only on ant richness but also on aspects related to their biology (stratum of occurrence).

A generalized linear mixed model (GLMM) was used to verify if the presence of ants in strata was homogeneous (providing an equal probability of collection success between the layers). The model was elaborated from the proportion of ant occurrence in the plots (ranging from 0 to 5, in a half-hectare), with the strata as fixed variables and the LUSs as random variables. The model was compared by maximum likelihood and the data were evaluated for overdispersion, assuming a binomial distribution.

The analyses and graphs were performed in the R environment (version 3.1.3, R-CORE-TEAM 2016) with the *vegan*, *car*, *MASS*, *iNEXT* and *lme4* packages. The map of the localities was generated using Program QGIS v. 2.16.3 and the coordinates provided in Zagatto (2014) and Rosa (2013) and the shape files obtained from the Brazilian Institute of Geography and Statistics data base - IBGE (2016). The figures were drawn in Adobe Illustrator (version CC 2018).

Results

1. Richness and species frequency

In total, 281 ant species were collected (considering all counties sampled and the different methods – epigaeic pitfall trap and TSBF). These species were distributed in 49 genera and 10 subfamilies (Table 1). The subfamilies with the greatest number of species were Myrmicinae, with 175 (61.4% of the species collected); Formicinae, 39 (13.7%); and Ponerinae, with 32 species (11.2%). The richest genera were *Pheidole*, with 98 species (34%), *Solenopsis* and *Hypoponera* with 22 species (7.7%) each.

In Santa Catarina, 238 species were collected by both methods (epigaeic pitfall and TSBF). Pitfalls collected 202 species and generated 1,585 records of occurrence, while the TSBF collected 149 species, generating 769 records (Table 2). From the total, 89 species were collected only in pitfall (51.7% of them composed by the genus *Pheidole*) and 36 only in the TSBF (Table 1). The three most frequent species in the pitfalls were *Pachycondyla striata* Smith, 1858, *Pheidole subarmata* Mayr, 1884 and *Linepithema micans* (Forel, 1908), with 109, 97 and 71 occurrences, respectively. In TSBF the species with the greatest number of records were *Hypoponera* sp. 1, *Hypoponera* sp. 8 and *Pheidole subarmata*, with 60, 57 and 42 records, respectively.

Table 1. Species list by region (Campos Gerais, West and Plateau) and municipality. PG - Ponta Grossa, SMO - São Miguel do Oeste, CHP - Chapecó, XAN - Xanxerê, LGS - Lages, OTC - Otacílio Costa, and CBS - Campo Belo do Sul). The species sampled exclusively by a method (in Santa Catarina) are indicated by symbols: ♦ - TSBF, ♣ - Pitfall; and the exclusive species per stratum are indicated with superscript numbers: ⁰ - litter, ¹ - stratum I (0-10 cm), and ^{II} - stratum II (10-20 cm).

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
Amblyoponinae							
<i>Fulakora armigera</i> (Mayr, 1887)♦						X	
<i>Fulakora elongata</i> (Santschi, 1912)♦ ¹	X	X			X	X	X
Dolichoderinae							
<i>Dorymyrmex brunneus</i> Forel, 1908	X	X		X			
<i>Dorymyrmex</i> sp. 1♣			X				X
<i>Dorymyrmex</i> sp. 2♣							X
<i>Linepithema gallardoii</i> (Brèthes, 1914)♣		X					X
<i>Linepithema humile</i> (Mayr, 1868)♣	X					X	
<i>Linepithema iniquum</i> (Mayr, 1870)		X				X	
<i>Linepithema leucomelas</i> (Emery, 1894)♣					X		
<i>Linepithema micans</i> (Forel, 1908)	X	X	X	X	X	X	X
<i>Linepithema pulex</i> Wild, 2007 ¹	X						
Dorylinae							
<i>Labidus coecus</i> (Latreille, 1802)		X				X	
<i>Labidus praedator</i> (Smith, 1858)♣						X	X
<i>Neivamyrmex</i> sp. 1♦ ^{II}	X	X	X				
<i>Neocerapachys splendens</i> (Borgmeier, 1957)♦	X					X	
<i>Sphinctomyrmex stali</i> Mayr, 1866♦		X					
Ectatomminae							
<i>Ectatomma edentatum</i> Roger, 1863♣			X		X		
<i>Ectatomma permagnum</i> Forel, 1908 ¹	X						
<i>Gnamptogenys rastrata</i> (Mayr, 1866)♣					X		
<i>Gnamptogenys regularis</i> Mayr, 1870	X						
<i>Gnamptogenys</i> nr. <i>striatula</i>	X		X	X	X		X
<i>Gnamptogenys</i> sp. n. A♦						X	
<i>Gnamptogenys striatula</i> Mayr, 1884 ¹	X		X	X	X	X	X
<i>Gnamptogenys sulcata</i> (Smith, 1858)♣					X		
<i>Typhlomyrmex major</i> Santschi, 1923♦	X	X	X	X	X	X	X
<i>Typhlomyrmex pusillus</i> Emery, 1894 ¹	X		X		X	X	X

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
Formicinae							
<i>Acropyga decedens</i> (Mayr, 1887)♦					X		
<i>Acropyga goeldii</i> Forel, 1893	X			X	X	X	
<i>Brachymyrmex coactus</i> Mayr, 1887 ^I	X						
<i>Brachymyrmex pilipes</i> Mayr, 1887♣	X		X				
<i>Brachymyrmex pilipes</i> gr. sp. 1♦		X					
<i>Brachymyrmex</i> sp. 1	X	X	X	X	X	X	
<i>Brachymyrmex</i> sp. 2	X	X	X	X	X	X	X
<i>Brachymyrmex</i> sp. 3	X	X	X	X	X	X	X
<i>Brachymyrmex</i> sp. 4	X						
<i>Brachymyrmex</i> sp. 5	X	X	X	X		X	
<i>Brachymyrmex</i> sp. 6	X	X		X		X	
<i>Brachymyrmex</i> sp. 7	X		X	X	X	X	X
<i>Brachymyrmex</i> sp. 8♣					X		
<i>Brachymyrmex</i> sp. 9		X				X	
<i>Brachymyrmex</i> sp. 10♣		X		X		X	
<i>Camponotus balzani</i> Emery, 1894					X	X	X
<i>Camponotus blandus</i> (Smith, 1858)					X	X	X
<i>Camponotus brasiliensis</i> Mayr, 1862 ^I	X						
<i>Camponotus crassus</i> Mayr, 1862	X	X	X			X	X
<i>Camponotus melanoticus</i> Emery, 1894♣ ^I	X	X	X		X		X
<i>Camponotus novogranadensis</i> Mayr, 1870	X		X	X			
<i>Camponotus</i> nr. <i>cingulatus</i> ♣					X		X
<i>Camponotus punctulatus</i> Mayr, 1868					X	X	X
<i>Camponotus renggeri</i> Emery, 1894 ^{II}	X		X	X			X
<i>Camponotus rufipes</i> (Fabricius, 1775)		X	X	X		X	X
<i>Camponotus sexguttatus</i> (Fabricius, 1793) ^{II}	X						
<i>Camponotus zenon</i> Forel, 1912♣			X	X			X
<i>Camponotus</i> sp. 1 ^I	X						
<i>Camponotus</i> sp. 2	X						
<i>Camponotus</i> sp. 4♣							X
<i>Camponotus</i> sp. 5♣							X
<i>Camponotus</i> sp. 6♣				X			
<i>Myrmelachista catharinae</i> Mayr, 1887♣				X			
<i>Myrmelachista nodigera</i> Mayr, 1887♣						X	
<i>Myrmelachista gallicola</i> Mayr, 1887 ^I	X						
<i>Nylanderia fulva</i> (Mayr, 1862) ^I	X	X	X	X			
<i>Nylanderia</i> sp. 1	X	X	X	X		X	
<i>Nylanderia</i> sp. 2			X	X	X		
<i>Nylanderia</i> sp. 3♦		X					

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
Heteroponerinae							
<i>Heteroponera dentinodis</i> (Mayr, 1887)					X	X	X
<i>Heteroponera mayri</i> Kempf, 1962		X		X			X
<i>Heteroponera microps</i> Borgmeier, 1957♣		X					
Myrmicinae							
<i>Acanthognathus ocellatus</i> Mayr, 1887♦			X				
<i>Acromyrmex aspersus</i> (Smith, 1858)♣			X				
<i>Acromyrmex crassispinus</i> (Forel, 1909) ¹	X	X	X	X	X		X
<i>Acromyrmex landolti</i> (Forel, 1885)♣					X		
<i>Acromyrmex lundii</i> (Guérin-Méneville, 1838)♣					X		X
<i>Acromyrmex subterraneus</i> (Forel, 1893)♣			X	X			X
<i>Apterostigma</i> sp. 1			X				
<i>Apterostigma</i> sp. 2♣			X	X			
<i>Atta sexdens</i> (Linnaeus, 1758)		X	X	X	X	X	
<i>Carebara</i> sp. 1♦			X				
<i>Carebara</i> sp. 2♣						X	X
<i>Carebara brasiliana</i> Fernández, 2004♣			X				
<i>Crematogaster ampla</i> Forel, 1912♣						X	X
<i>Crematogaster bruchi</i> Forel, 1912	X		X				
<i>Crematogaster corticicola</i> Mayr, 1887♣	X			X			
<i>Crematogaster quadriformis</i> Roger, 1863			X		X		
<i>Crematogaster</i> sp. 1				X			
<i>Crematogaster</i> sp. 2					X		X
<i>Cyphomyrmex rimosus</i> (Spinola, 1851)		X	X		X	X	X
<i>Cyphomyrmex</i> sp. 1	X						
<i>Cyphomyrmex</i> sp. 2		X	X			X	X
<i>Cyphomyrmex</i> sp. 3♣						X	
<i>Hylomyrma balzani</i> (Emery, 1894)		X	X		X	X	X
<i>Hylomyrma reitteri</i> (Mayr, 1887) ¹	X	X	X				
<i>Megalomyrmex pusillus</i> Forel, 1912							X
<i>Mycetophylax asper</i> (Mayr, 1887)♣				X			
<i>Mycetophylax lectus</i> (Forel, 1911)		X	X	X			X
<i>Mycetophylax olitor</i> Forel, 1893		X					X
<i>Mycetophylax plaumanni</i> Kempf, 1962		X		X			
<i>Mycocepurus goeldii</i> (Forel, 1893)	X		X	X			
<i>Mycocepurus smithii</i> (Forel, 1893)		X	X				
<i>Ochetomyrmex semipolitus</i> Mayr, 1878♣					X		
<i>Octostruma rugifera</i> (Mayr, 1887)			X		X	X	X
<i>Octostruma stenognatha</i> (Brown & Kempf, 1960)♣		X				X	X
<i>Oxyepoecus plaumanni</i> Kempf, 1974 ¹	X						
<i>Oxyepoecus reticulatus</i> Kempf, 1974	X	X	X		X	X	
<i>Oxyepoecus rosai</i> Albuquerque & Brandão, 2009		X	X			X	

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
<i>Pheidole aberrans</i> Mayr, 1868	X	X	X	X	X	X	X
<i>Pheidole acutidens</i> (Santschi, 1922) [♣]						X	
<i>Pheidole breviseta</i> Santschi, 1919		X	X	X	X	X	X
<i>Pheidole cavifrons</i> Emery, 1906	X				X	X	X
<i>Pheidole fallax</i> gr. sp. 1 ⁰	X						
<i>Pheidole gertrudae</i> Forel, 1886 ⁰	X	X	X	X			
<i>Pheidole heyeri</i> Forel, 1899		X	X	X	X	X	X
<i>Pheidole oxyops</i> Forel, 1908	x						
<i>Pheidole</i> nr. <i>rosula</i>					X	X	X
<i>Pheidole radoszkowskii</i> Mayr, 1884 ¹	X						
<i>Pheidole rosae</i> Forel, 1901	X						
<i>Pheidole subarmata</i> Mayr, 1884	X	X	X	X	X	X	X
<i>Pheidole transversostriata</i> Mayr, 1887		X					
<i>Pheidole triconstricta</i> Forel, 1886		X	X	X			
<i>Pheidole vafra</i> Santschi, 1923	X						
<i>Pheidole</i> sp. n. A	X						
<i>Pheidole</i> sp. n. I ¹	X						
<i>Pheidole</i> sp. n. N ¹	X						
<i>Pheidole</i> sp. 1	X						
<i>Pheidole</i> sp. 2 ^{♦1}	X		X				
<i>Pheidole</i> sp. 3 ¹	X						
<i>Pheidole</i> sp. 4	X						
<i>Pheidole</i> sp. 5 ¹	X						
<i>Pheidole</i> sp. 6	X						
<i>Pheidole</i> sp. 7	X						
<i>Pheidole</i> sp. 8	X						
<i>Pheidole</i> sp. 9	X						
<i>Pheidole</i> sp. 10	X						
<i>Pheidole</i> sp. 11		X			X	X	X
<i>Pheidole</i> sp. 12		X					X
<i>Pheidole</i> sp. 13					X	X	
<i>Pheidole</i> sp. 14 [♣]		X			X	X	X
<i>Pheidole</i> sp. 15 [♣]		X	X		X		X
<i>Pheidole</i> sp. 16 [♣]		X	X	X		X	X
<i>Pheidole</i> sp. 17 [♣]		X		X	X		
<i>Pheidole</i> sp. 18					X	X	
<i>Pheidole</i> sp. 19		X	X	X	X	X	X
<i>Pheidole</i> sp. 20			X	X	X	X	X
<i>Pheidole</i> sp. 21		X			X	X	
<i>Pheidole</i> sp. 22 [♦]						X	
<i>Pheidole</i> sp. 23 [♣]					X		
<i>Pheidole</i> sp. 24 [♣]		X		X		X	
<i>Pheidole</i> sp. 25 [♣]		X					

Subterranean ants of southern Brazil

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
<i>Pheidole</i> sp. 26		X	X		X		X
<i>Pheidole</i> sp. 27		X					
<i>Pheidole</i> sp. 28♣				X			
<i>Pheidole</i> sp. 29♣		X	X			X	X
<i>Pheidole</i> sp. 30			X	X	X		X
<i>Pheidole</i> sp. 31		X	X	X			
<i>Pheidole</i> sp. 32♦					X		
<i>Pheidole</i> sp. 33				X		X	
<i>Pheidole</i> sp. 34			X		X	X	
<i>Pheidole</i> sp. 35♣					X	X	X
<i>Pheidole</i> sp. 36♣			X				
<i>Pheidole</i> sp. 37♣				X	X		X
<i>Pheidole</i> sp. 38♣			X	X			
<i>Pheidole</i> sp. 39♣		X					
<i>Pheidole</i> sp. 40♣		X	X	X	X		
<i>Pheidole</i> sp. 41			X	X	X		X
<i>Pheidole</i> sp. 42♣		X	X				
<i>Pheidole</i> sp. 43♣					X	X	X
<i>Pheidole</i> sp. 44♣		X	X				
<i>Pheidole</i> sp. 45♣		X	X	X	X	X	X
<i>Pheidole</i> sp. 46♣			X	X	X		X
<i>Pheidole</i> sp. 47♣		X	X	X			
<i>Pheidole</i> sp. 48♣			X	X	X		
<i>Pheidole</i> sp. 49♣		X	X	X			
<i>Pheidole</i> sp. 50			X			X	
<i>Pheidole</i> sp. 51			X			X	
<i>Pheidole</i> sp. 52						X	X
<i>Pheidole</i> sp. 53♣				X			
<i>Pheidole</i> sp. 54♣		X					
<i>Pheidole</i> sp. 55♣		X	X				
<i>Pheidole</i> sp. 56♣				X		X	X
<i>Pheidole</i> sp. 57					X		X
<i>Pheidole</i> sp. 58♦					X		
<i>Pheidole</i> sp. 59♣					X		
<i>Pheidole</i> sp. 60♣					X		
<i>Pheidole</i> sp. 61					X	X	
<i>Pheidole</i> sp. 62		X	X	X			
<i>Pheidole</i> sp. 63♣				X			
<i>Pheidole</i> sp. 64♣				X			
<i>Pheidole</i> sp. 65♣				X			
<i>Pheidole</i> sp. 66♣							X
<i>Pheidole</i> sp. 67♣		X					
<i>Pheidole</i> sp. 68				X	X		X

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
<i>Pheidole</i> sp. 69♦						X	
<i>Pheidole</i> sp. 70♣					X		
<i>Pheidole</i> sp. 71♣		X					
<i>Pheidole</i> sp. 72♣					X	X	X
<i>Pheidole</i> sp. 73♣					X		X
<i>Pheidole</i> sp. 74♣						X	
<i>Pheidole</i> sp. 75♣					X		X
<i>Pheidole</i> sp. 76♣				X		X	X
<i>Pheidole</i> sp. 77♣				X	X	X	
<i>Pheidole</i> sp. 78♣							X
<i>Pheidole</i> sp. 79♣				X			
<i>Pheidole</i> sp. 80♣		X					
<i>Pogonomyrmex naegelli</i> Emery, 1878	X		X		X	X	
<i>Rogeria bruchi</i> Santschi, 1922♦					X		
<i>Solenopsis</i> gr. <i>geminata</i> sp. 1 ^I	X						
<i>Solenopsis</i> gr. <i>geminata</i> sp. 2		X	X	X	X	X	X
<i>Solenopsis</i> gr. <i>geminata</i> sp. 3♣			X	X			
<i>Solenopsis invicta</i> Buren, 1972	X	X	X	X	X	X	X
<i>Solenopsis</i> sp. 1♣	X	X		X			
<i>Solenopsis</i> sp. 2	X	X				X	
<i>Solenopsis</i> sp. 3 ^I	X			X		X	X
<i>Solenopsis</i> sp. 4	X						
<i>Solenopsis</i> sp. 5	X						
<i>Solenopsis</i> sp. 6 ^{II}	X						
<i>Solenopsis</i> sp. 7♣	X					X	
<i>Solenopsis</i> sp. 8 ^I	X						
<i>Solenopsis</i> sp. 9	X	X	X	X	X	X	X
<i>Solenopsis</i> sp. 10		X	X	X	X	X	X
<i>Solenopsis</i> sp. 11			X	X	X		X
<i>Solenopsis</i> sp. 12		X	X	X	X	X	X
<i>Solenopsis</i> sp. 13		X	X	X	X	X	X
<i>Solenopsis</i> sp. 14♣		X	X		X	X	
<i>Solenopsis</i> sp. 15		X	X	X	X	X	X
<i>Solenopsis</i> sp. 16		X					X
<i>Solenopsis</i> sp. 17					X	X	
<i>Solenopsis</i> sp. 18♦				X			
<i>Strumigenys appretiata</i> (Borgmeier, 1954)					X		X
<i>Strumigenys denticulata</i> Mayr, 1887♦			X				
<i>Strumigenys eggersi</i> Emery, 1890		X	X				
<i>Strumigenys louisianae</i> Roger, 1863 ^I	X						
<i>Strumigenys oglobini</i> Santschi, 1936♣		X					
<i>Strumigenys</i> nr. <i>epinotalis</i> ^I	X						

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
<i>Strumigenys</i> nr. <i>louisianae</i> sp. 1♣					X		
<i>Strumigenys</i> nr. <i>louisianae</i> sp. 2			X	X	X	X	
<i>Strumigenys</i> nr. <i>louisianae</i> sp. 3♣					X		
<i>Mycetomoellerius holmgreni</i> Wheeler, 1925					X	X	X
<i>Mycetomoellerius</i> sp. 1					X	X	X
<i>Mycetomoellerius</i> sp. 2♣					X		
<i>Tranopelta gilva</i> Mayr, 1866	X				X		
<i>Wasmannia affinis</i> Santschi, 1929	X	X	X		X	X	
<i>Wasmannia auropunctata</i> (Roger, 1863)		X	X	X	X	X	X
<i>Wasmannia sulcaticeps</i> Emery, 1894		X	X		X	X	
Ponerinae							
<i>Centromyrmex brachycola</i> (Roger, 1861)	X						
<i>Hypoponera foreli</i> (Mayr, 1887)	X	X	X	X	X	X	X
<i>Hypoponera opaciceps</i> (Mayr, 1887)		X	X	X	X	X	X
<i>Hypoponera</i> sp. 1	X	X	X	X	X	X	X
<i>Hypoponera</i> sp. 2	X	X	X	X			X
<i>Hypoponera</i> sp. 3	X						
<i>Hypoponera</i> sp. 4	X						
<i>Hypoponera</i> sp. 5♦	X		X		X	X	
<i>Hypoponera</i> sp. 6 I	X	X		X	X	X	X
<i>Hypoponera</i> sp. 7	X						
<i>Hypoponera</i> sp. 8	X	X	X	X	X	X	X
<i>Hypoponera</i> sp. 9 ⁰	X						
<i>Hypoponera</i> sp. 10 ^I	X				X	X	X
<i>Hypoponera</i> sp. 11	X		X	X	X	X	X
<i>Hypoponera</i> sp. 12 ^{II}	X						
<i>Hypoponera</i> sp. 13		X			X	X	X
<i>Hypoponera</i> sp. 14			X			X	
<i>Hypoponera</i> sp. 15						X	X
<i>Hypoponera</i> sp. 16♦		X	X		X	X	X
<i>Hypoponera</i> sp. 17♦			X	X			
<i>Hypoponera</i> sp. 18♦					X		
<i>Hypoponera</i> sp. 19♦						X	
<i>Hypoponera</i> sp. 21♦						X	
<i>Hypoponera</i> sp. 22♦				X			
<i>Hypoponera</i> sp. 23♦	X	X					
<i>Neoponera bucki</i> (Borgmeier, 1927)♣					X		X
<i>Odontomachus chelififer</i> (Latreille, 1802)		X	X	X			
<i>Pachycondyla harpax</i> (Fabricius, 1804)		X	X	X			X
<i>Pachycondyla striata</i> Smith, 1858		X	X	X	X	X	X
<i>Rasopone ferruginea</i> (Smith, 1858)♦				X			
<i>Simopelta curvata</i> (Mayr, 1887)♦		X	X				

Continuation Table 1.

Species	Paraná		Santa Catarina				
	Campos Gerais		West		Plateau		
	PG	SMO	CHP	XAN	LGS	OTC	CBS
Proceratiinae							
<i>Discothyrea sexarticulata</i> Borgmeier, 1954 ⁰	X						
<i>Proceratium brasiliense</i> Borgmeier, 1959 [◆]						X	
Pseudomyrmecinae							
<i>Pseudomyrmex flavidulus</i> (Smith, 1858) [◆]	X	X					
<i>Pseudomyrmex gracilis</i> (Fabricius, 1804) ^l	X						
<i>Pseudomyrmex longus</i> (Forel, 1912) ^l	X						
<i>Pseudomyrmex termitarius</i> (Smith, 1877) [◆]			X				
<i>Pseudomyrmex</i> sp. 1	X		X				
<i>Pseudomyrmex</i> sp. 2 [♣]							X
Total number of species	101	103	108	94	111	109	101

Table 2. Observed (Sobs) and estimated (estimators: Jackknife 1 – Jack1 and Bootstrap - Boot) richness and number of exclusive species by method (Pitfall and TSBF) in Santa Catarina.

Methods	Sobs	Jack1	Boot	Exclusive
Pitfall	202	260	227	89
TSBF	149	213	176	36
Total	238	294	323	-

In Paraná, the TSBF method collected 101 species (Table 1), with 44, 88 and 52 species at strata 0, I and II (Table 3), and 97, 301 and 121 occurrence records, respectively. Four out of the 101 species were collected exclusively in stratum 0, 30 only in stratum I and five in stratum II (Table 1). The most frequent species were *Hypoconera* sp. 1, *Brachymyrmex* sp. 5 and *Pheidole cavifrons* Emery, 1906, with 53, 25 and 16 occurrence records. Species occurrences in strata were as follows: in litter (stratum 0), the three most frequent species were *Hypoconera* sp. 1, with 12 records; *Brachymyrmex* sp. 1, and *Brachymyrmex* sp. 5, with seven records each. In stratum I (0-10 cm), the most frequent species were *Hypoconera* sp. 1, *Hypoconera* sp. 3 and *Brachymyrmex* sp. 3, with 29, 17 and 14 records, respectively. In stratum II (10-20 cm), the highest frequency was recorded for *Hypoconera* sp. 1, *Brachymyrmex* sp. 5 and *Pheidole cavifrons*, with 12, 8 and 7 records.

Table 3. Observed richness (Sobs) and estimated (estimators: Jackknife 1 – Jack1 E Bootstrap - Boot) and number of exclusive species by strata (0 - litter, I - 0-10 cm, and II- 10-20 cm).

Strata	Sobs	Jack1	Boot	Exclusive
0	44	66	54	4
I	88	125	106	30
II	53	77	64	5
Total	101	129	114	-

The collector curves constructed for each method demonstrate that a greater effort would be required to reach the asymptote (although this is apparently close), and that the number of accumulated species per sample unit was greater in pitfall than in TSBF (Figure 4a). Epigaeic pitfall collected 77% and TSBF 70% of the numbers estimated by Jackknife 1 and 89% and 84% of the numbers estimated by Bootstrap (Table 2).

The collector curves for strata performed with data from Paraná show that under the same sampling effort there is a greater addition of species in stratum I, in relation to the others (Figure 4b). The numbers of species observed approximate the Bootstrap estimated values (Table 3). The species collected in each stratum correspond to 66%, 70% and 69% of the species estimated by Jackknife 1 (for strata 0, I and II respectively) and 81%, 83% and 83% of the species estimated by Bootstrap. The total number of species corresponds to 78% and 88% of the estimate by Jackknife 1 and Bootstrap, respectively.

2. Subterranean ant assembly characterization

In the regions of Santa Catarina, we obtained 89 species exclusively in the epigaeic stratum and 36 species in the subterranean stratum. Seven species were recorded for the first time in the Plateau region of Santa Catarina and were collected only by TSBF: *Acropyga decedens* (Mayr, 1887), *Fulakora armigera* (Mayr, 1887), *Gnamptogenys reichenspergeri* (Santschi, 1929), *Neocerapachys splendens* (Borgmeier, 1957), *Proceratium brasiliense* Borgmeier, 1959, *Sphinctomyrmex stali* Mayr, 1866 and *Typhlomyrmex major* Santschi, 1923. No new records were found for the western region. Fifty-eight species were collected in Pitfall traps only once, while single species occurrences in TSBF samples totalled 64. These numbers correspond to different proportions ($\chi^2 = 7.05$, $p = 0.007$, $g1 = 1$), with 28.7% and 42.9%, respectively, of the total species collected by each method.

In Paraná, where the method utilised was exclusively TSBF, seven species were present only in stratum I: *Fulakora elongata* (Santschi, 1912), *Gnamptogenys reichenspergeri*, *Oxyepoecus plaumanni* Kempf 1974, *Pheidole radozkowskii* Mayr, 1884, *Pseudomyrmex gracilis*

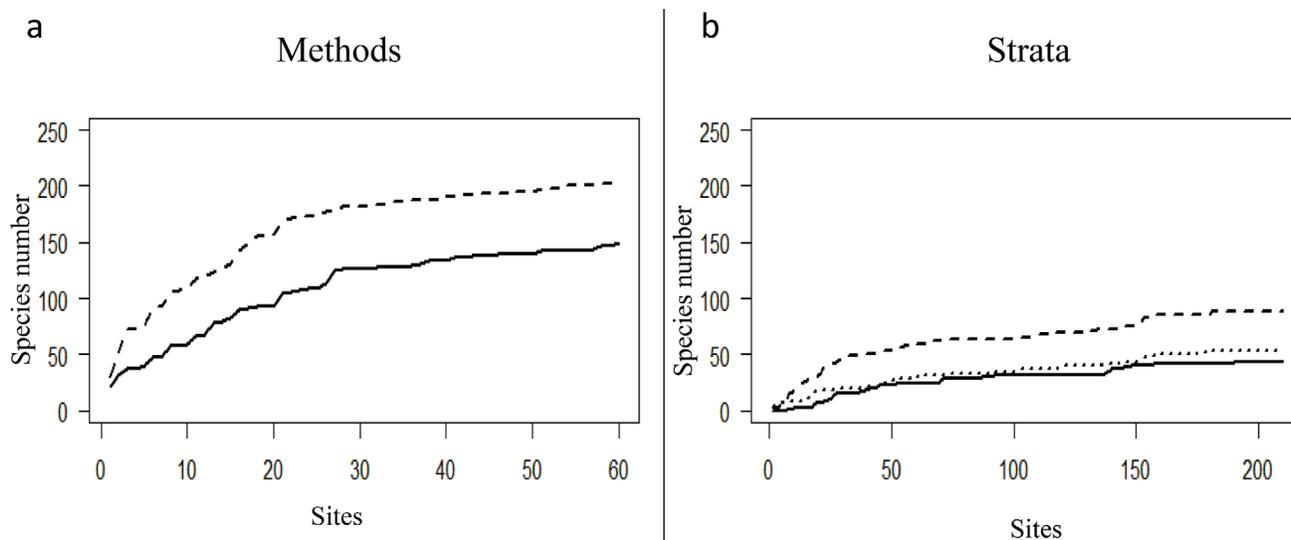


Figure 4. a - Collector's curves for both methods (Pitfall and TSBF). Dashed line: Pitfall; and continuous line: TSBF. b - Collector's curve for the strata. Continuous line: stratum 0; dashed line: stratum I; and dotted line: stratum II.

(Fabricius, 1804), *P. longus* (Forel, 1912) and *Tranopelta gilva* Mayr, 1866. In stratum II, *Neivamyrmex* sp.1 was the only species (with only one occurrence).

The number of species with only one record of occurrence in strata was 22 for stratum 0, 37 for stratum I and 24 for stratum II (Figure 5a, b, and c). These species correspond to 50%, 42.1% and 46.1% of the total species collected in each stratum. The graph of observed richness (Sobs) and frequency of occurrence per stratum (Figure 5d) shows that in stratum I there is a great number of species that occur more frequently. Stratum I had the highest number of occurrence frequencies, once this is the stratum with the highest number of occurrences of ants (GLMM, Binomial, $z = 5,239$, $df = 4$, $p < 0.05$), with a probability of occurrence of 86.10 % in the sampled areas.

Discussion

The present study delimited and characterized subterranean ant assemblages in the subtropical regions of the Neotropics. In these regions, only two previous studies collected subterranean ants: (1) Silva & Silvestre (2004) in Western Santa Catarina, and (2) Schmidt & Diehl (2008) in Rio Grande do Sul. This is the first survey that evaluates subterranean ant assemblages in Paraná and the Plateau region of Santa Catarina. Despite the different sampling efforts, the aforementioned publications presented 32 and seven species belonging to the subterranean stratum, representing only 28% and 20% of the total species observed by those authors, respectively. Considering the number of species with restricted occurrence in the subterranean stratum in all sampled localities, our results were similar (in percentage): 57 species, or 20.2% of the total collected (Table 1).

The biology of most subterranean species is unknown. TSBF samples provide data of occurrence and preference among strata, nesting, and species composition in the different soil layers. The manual extraction of ants in the stratified monoliths, characteristic of this

method, provides the opportunity for *in loco* observation of the collected organisms. Wong & Guénard (2017) emphasized the importance of the "observer bias" (represented by different levels of experience in active collections), in which a collector may underestimate the presence of subterranean ants (especially the small ones) in samples or even in the field. This error can be reduced if sorting is performed under a stereomicroscope.

The total number of species observed in each municipality of Santa Catarina is extremely close to that observed in other studies already conducted in those regions (see Silva & Silvestre 2004, Ulysséa *et al.* 2011, Lutinski & Morais 2013). However, the number of species collected in Paraná is considerably high when compared with the richness found by other studies employing different methods for sampling subterranean ants (see Table 1 and Wong & Guénard 2017). This could indicate a higher efficiency of TSBF in sampling subterranean ants in these areas. However, additional studies comparing methods for collecting hypogaeanic ants are indispensable to confirm this tendency. These comparisons should especially consider the most practical and widely employed method currently, the hypogaeanic pitfall traps (Schmidt & Solar, 2010).

When epigaeanic pitfall traps and TSBF are compared, it becomes clear that pitfall traps capture a greater number of species that have a high frequency of occurrence. However, when evaluating the number of infrequent species, TSBF is superior (42.7% of the species sampled). This may be explained by the fact that pitfall traps remained in the field for 72 hours, increasing the likelihood of species occurring more than once and increasing the number of species captured (see Lasmar *et al.* 2017). Another factor that can explain this difference is the higher ant activity in the ground surface due to nesting and foraging than in the hypogaeanic stratum (Jacquemin *et al.* 2016). In addition, in Santa Catarina, 36 species were collected exclusively by TSBF. This means that 15% of the species sampled there in this study would not have been registered without an association between the pitfall and a specific sampling method for the subterranean stratum.

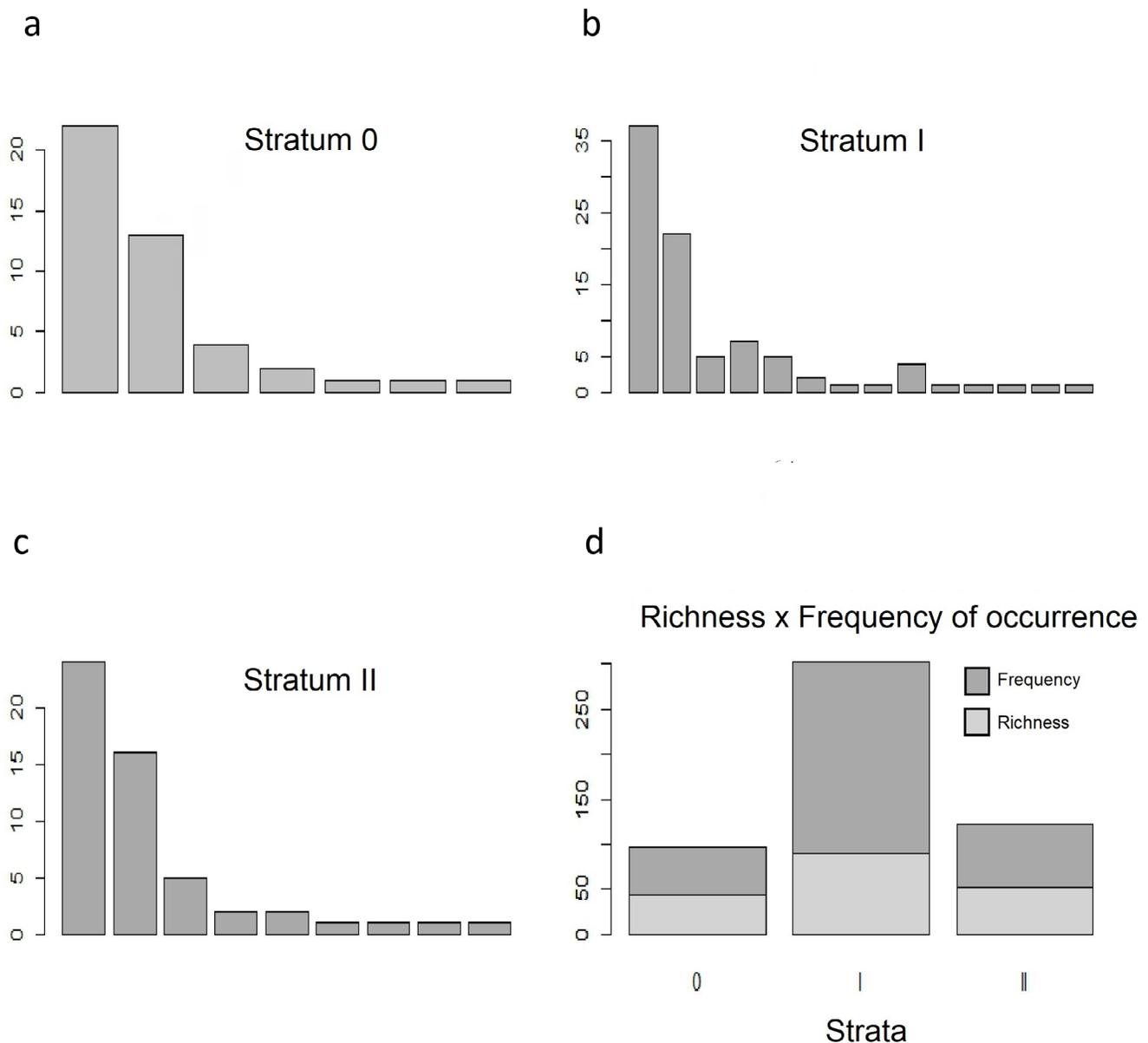


Figure 5. a to c - Fisher graphs for each stratum sampled in Paraná; d - Comparative graph of observed richness (Sobs) and frequency of occurrence between the strata.

The results obtained by the GLMM analysis of the Paraná data show that stratum I has more species than the other strata. A similar result was found by Andersen & Brault (2010) and Rider Wilkie *et al.* (2007), who also obtained a greater number of species in the topsoil and a great number of exclusive species (19 species) in this layer (up to 12,5 cm). These results indicate a tendency for greater richness and frequency of occurrence in this stratum, which consequently must be considered during the sampling design of projects focusing on subterranean ants or even in comprehensive inventories of the ant fauna.

Sampling methods that record assemblages in different vertical strata allow for a more efficient evaluation of the association between ants and micro habitats. This association was effective for the delimitation of species that occur in different strata also in Jacquemin *et al.* (2016) and in the present work. The criterion of exclusive delimitation of

occurrence used here proved to be efficient, as observed by Fowler *et al.* (2000). Most species identified by these authors belonged to a unique stratum (collected only in epigeaic traps or only in hypogaic traps). Considering the exclusivity of occurrence in the strata, and collection methods (pitfall and TSBF), 33% of the total species are epigeaic and 20% are hypogaic.

More than a quarter (28.5%) of the species identified in this study are subterranean and most can be considered infrequent. We thus consider the criterion “exclusivity” (stratum and/or method) a good approach for delimiting the subterranean habit, as already mentioned in literature (e.g. Fowler *et al.* 2000). However, the delimitation of subterranean ant species was possible only with the association of epigeaic and hypogaic sampling methods. In addition, we found that in stratified TSBF samples the 0-10 cm stratum has the greatest number of species

and highest frequency of occurrence. This fact, allied to the number of species obtained through sampling by the TSBF method, indicates that this is a useful method for sampling subterranean ants.

The localities sampled in this study are under intense pressure for urbanization and agricultural production. Thus, TSBF is a promising tool in diversity assessments (because it is a widely used method in soil quality studies, facilitating the acceptance by producers for the development of research in their areas), especially to access the impact of human activities on subterranean species. In addition, it was demonstrated that the TSBF captures rarely sampled species or species that are still undiscovered.

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Author Contributions

Mila Ferraz de Oliveira Martins: contributed substantially in the concept of the study, in data collection, data analysis and interpretation, and to prepare the manuscript.

Marcílio José Thomazini: contributed in the concept and design of the study; data collection, and to critical revision, adding intellectual content.

Dilmar Baretta: did a substantial contribution in the concept and design of the study, data collection, contributed to data analysis and interpretation and did a critical revision, adding intellectual content

Marcio Gonçalves da Rosa: contributed in the concept and design of the study, data collection, data analysis and interpretation, and did a critical revision, adding intellectual content

Maurício Rumenos Zagatto: contributed in the concept and design of the study, and data collection.

Alessandra Santos: contributed in the concept and design of the study, and data collection.

Herlon S. Nadolny: contributed in data collection

Guilherme B. X. Cardoso: contributed in data collection

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Marie L. C. Bartz: contributed in the concept and design of the study, and data collection.

Rodrigo M. Feitosa: contributed substantially in the concept of the study, in data collection, data interpretation, and to prepare the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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