

Hunting for Wasps In-between: the Use of the Winkler Extractor to Sample Leaf Litter Hymenoptera

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Abstract

Soil ecosystems are considered biodiversity hotspots. Most of terrestrial organisms depend almost exclusively on soils at least during some part of their life cycle. Although there is an intensive effort to understand the dynamics involving communities of soil animals, most groups are unappreciated in faunal inventories, such as some parasitic Hymenoptera with soil or litter hosts (SLH Hymenoptera), which are considered one of the largest and most diverse groups of soil- or litter-dwelling animals. Contrasting with flight interception traps, techniques that sample soil and litter specimens normally present low costs and easy transportation, being easily replicated within a site. Among them, the Winkler extractor is a useful technique to sample cryptic species associated with leaf litter. The aim of the present work was to describe the SLH Hymenoptera fauna collected in a pool of samples, using a technique to sample leaf litter arthropods. In this work, we report a total of 600 specimens belonging to Hymenoptera (excluding ants), belonging to 15 families distributed within 24 subfamilies, in samples of leaf litter obtained using the Winkler extractor. We observed that this technique is promising for sampling SLH Hymenoptera and should be considered as a complement for inventories of the Hymenoptera fauna.

Introduction

Soil ecosystems are considered biodiversity hotspots (Ghilarov 1977, Giller 1996). Most of terrestrial organisms depend almost exclusively on soils at least during some part of their life cycle (Krell *et al* 2005).

The last three decades have seen an exponential increase in the interest by ecologists to study the soil fauna (Schaefer 1990, Kampichler 1999, Mäder *et al* 2002, Petersen 2002, Xin *et al* 2012, García-Palacios *et al* 2013). As a result, some animal groups, especially decomposers, have been intensively studied (Schaefer & Schauer mann 1990, Scheu & Schaefer 1998, Hövemeyer 1999a, b, Filser 2002). According to Ulrich (2004), these efforts have yielded a better understanding of the structure of soil fauna communities, succession and decomposition processes, food webs, and energy turnover. Nevertheless, our knowledge of many animal groups, associated with the soil, is still scarce and we are far from solving the so-called enigma of soil animal species diversity

(Anderson 1975, Nielsen *et al* 2010). According to Krell *et al* (2005), however, to access information of the soil fauna, we must extract them from the soil, due to our inability to perceive this ecosystem as a whole.

Although there is an intensive effort to understand the dynamics involving communities of soil animals, most groups are unappreciated in faunal inventories. In particular, the parasitic Hymenoptera with soil or litter hosts (SLH Hymenoptera), which are considered as one of the largest and most diverse groups of soil- or litter-dwelling animals (Ulrich 2004), has been neglected during sampling of soil invertebrates. The SLH Hymenoptera, together with Diptera and probably Coleoptera, are one of the richest species groups of animals associated with the litter and soil subsystem (Ulrich 2004).

As pointed out by LaSalle and Gauld (1993) and Grissell (2000), the parasitoids probably are one of the most diverse groups within Hymenoptera, however, poorly studied. Shaw and Hochberg (2001) provide an excellent contrast on how

the diversity of this group can compare to better studied insect groups in particular regions.

Parasitoids are particularly sensitive to environmental changes, due to their high trophic position and, generally, with a high degree of host specificity (Siemann 1998, Tschardt et al 1998), performing essential ecosystem services in the regulation of other insect populations (Hassell 2000, Price 1980, Strong et al 1984). Besides, they are a valuable resource for use in biological control (Jervis 2005), possess biologies likely to make them at risk from population decline and extinction (Hochberg 2000, Shaw & Hochberg 2001), and should serve as a sensitive indicator of environmental threats (Fraser et al 2008). However, although their importance is evident, we have only basic knowledge of how parasitoid assemblages are structured, how they change along habitat gradients, and how environmental factors drive these changes (Hall et al 2015).

This lack of knowledge hampers conservation or monitoring work that specifically involves parasitic Hymenoptera outside an agricultural context (Fraser et al 2008). Although the scarce existing data suggest more drastic declines than is generally estimated for insects (Therion 1981, Shaw & Hochberg 2001, Shaw 2006b), only nine parasitoid Hymenoptera species have been included in the red list at a global scale, contrasting to the 633 species of aculeate Hymenoptera red listed globally (IUCN 2016). One alternative to species-level conservation is conservation of habitats, but absence of knowledge of the habitat preferences of parasitoid Hymenoptera species prevents their inclusion in assessment procedures (see Fraser et al 2007). Fraser et al (2008) states that there are three important short-term goals in the conservation of parasitoid Hymenoptera: (i) basic inventorying of the distribution of species, (ii) to understand better the relationship between habitat characteristics and parasitoid abundance and diversity (see Shaw 2006a, Fraser et al 2007), and (iii) to develop monitoring programmes that will allow us to assess better the conservation status of this group. All three goals depend on an understanding of efficient and informative field sampling procedures.

Among a variety of techniques to sample Hymenoptera (such as Moerick traps and light traps; see Sarmiento 2006), Malaise traps (Townes 1962) are a widely used sampling method for many parasitoid Hymenoptera usually resulting in numerically large samples (e.g., Sääksjärvi et al 2004, 2006, Wells & Decker 2006, Fraser et al 2007). As a flight interception trap that can be left for long periods unattended in the field, Malaise traps are both time and cost effective (Fraser et al 2008). They are likely to be the method of choice in many sampling or monitoring schemes, although this will vary depending on the taxa targeted (e.g., see Noyes 1982, 1989). However, because of their relative bulk and cost (per

trap), Malaise traps are commonly used singly or with low replication within a site (e.g., Owen & Owen 1974; Fraser et al 2007).

Contrasting with flight interception traps, techniques that sample soil and litter specimens normally present low costs and easy transportation, being easily replicated within a site. Among them, the Winkler extractor is a useful technique to sample cryptic species associated with leaf litter (Fisher 1998). It is normally applied in inventories of soil invertebrates, especially centipeds, isopods, mites, and apterous insects, such as ants, resulting in large samples containing numerous species. As a powerful technique to unravel community structures in soil and litter environments, the Winkler extractor could stretch the boundaries of Hymenoptera knowledge deep within tropical forests, especially when trying to understand the dynamics involving SLH Hymenoptera species.

To date, only a few studies have dealt with SLH Hymenoptera species (Thiede 1977, Garbarczyk 1981, Funke 1983, Hilpert 1989, Ulrich 2004). In this paper, we provide an inventory of the SLH Hymenoptera collected with the main sampling technique for leaf litter arthropods, the Winkler extractor. We also observe the proportion of winged, apterous, and brachypterous specimens in the total pool of specimens.

Methods

Leaf litter samples from different phytophysiognomies and 14 localities were studied (13 in Brazil and one in Ecuador; Table 1 and Fig 1). Localities were chosen based on samples available from previous ant inventories. Therefore, there was no standard sampling effort among the localities studied. For each locality, we studied a pool of samples representing the sum of a variable number of sampling sites. The protocol for sampling leaf litter Hymenoptera was adapted from Agosti and Alonso (2000) and Bestelmeyer et al (2000) using mini-Winkler extractors. Samples of 1 m² of leaf litter were collected and vigorously sifted before being submitted to Winkler extractors for 48 h. After that, the hymenopterans were sorted out and transferred to vials with 70% ethanol.

Vouchers were deposited at the *Coleção Entomológica Padre Jesus Santiago Moure*, Universidade Federal do Paraná, Curitiba, Paraná, Brazil (DZUP), and the Hymenoptera Collection of *Museu de Zoologia da Universidade de São Paulo*, São Paulo, Brazil (MZSP).

Although the identification of specimens to the lowest possible taxonomic rank would better describe the efficiency of the Winkler extractor as a sampling technique for leaf litter Hymenoptera, specimens were identified as subfamilies, due to the unavailability of identification keys to genera and/or species for the majority of Neotropical groups. We used identification

Table 1 List of localities where the leaf litter samples were obtained.

| Country | State/province | Locality | Coordinates | Ecosystem | |
|---------|--------------------|--|----------------------------------|--------------------------------|---------------------------|
| Brazil | Bahia | Milagres | 12°54'30.0"S 39°51'18.0"W | Brazilian steppe | |
| | | Uruçuca/Fazenda Barra do Tijuípe | 14°24'43"S 39°01'33"W | Dense Ombrophilous Forest | |
| | Mato Grosso do Sul | Porto Murtinho/Estrada Pirizal | 21°37'19.9"S 57°49'07.0"W | Savannah and Pantanal wetlands | |
| | Minas Gerais | Conceição do Mato Dentro/S. Serpentina | 19°6'26.86"S 43°17'55.46"W | Brazilian steppe | |
| | Paraná | Tunas/Parque das Lauráceas | 24°48'45.90"S 48°41'9.54"W | Dense Ombrophilous Forest | |
| | Rio Grande do Sul | São Francisco de Paula/Centro de Pesquisa e Conservação da Natureza Pró-Mata | 29°27'–29°35'S 50°08'–50°15'W | Mixed Ombrophilous Forest | |
| | Santa Catarina | São Bonifácio | 27°49'06"S 48°54'41"W | Dense Ombrophilous Forest | |
| | São Paulo | Cunha/Parque Estadual da Serra do Mar | | 23°15'03"S 45°00'26"W | Dense Ombrophilous Forest |
| | | | Iguape/Juréia-Itatins | 24°32'59"S 47°15'26"W | Dense Ombrophilous Forest |
| | | Matão/Fazenda Cambuhy/Mata da Virgínia | 21°41'31.5"S 48°31'45.5"W | Savannah | |
| | | Salesópolis/Estação Biológica Boracéia | 23°37'51"S 45°52'11"W | Dense Ombrophilous Forest | |
| | | Santo Antônio do Pinhal | 22°49'31"S 45°39'48"W | Mixed Ombrophilous Forest | |
| | | Tapiraí | 24°01'55"S 47°27'56"W | Dense Ombrophilous Forest | |
| | | Ecuador | Francisco Orellana | Reserva Nacional Yasuni | 0°46'12"S 76°06'00"W |

keys available in Fernández and Sharkey (2006) and Melo *et al* (2012), for both family and subfamily identification. Classification followed Aguiar *et al* 2013, which reflects recent phylogenetic arrangements in the order.

Coordinates of localities were obtained from specimens' labels, using Google Earth® application; distribution maps were generated by the software QuantumGis 2.6.1 (Quantum GIS Development Team 2015) and edited in Adobe Illustrator CS5.

Results and Discussion

A total of 600 specimens belonging to Hymenoptera (excluding ants) were found in the samples. A total of 15 families were collected, in 24 subfamilies. Ceraphronidae was the most abundant family (211 specimens; 35.2%), followed by Diapriidae (153 specimens; 25.5%), Platygastridae (118 specimens; 19.7%), and Bethyidae (67 specimens; 11.2%) (Fig 2). Although the most abundant group was Ceraphronidae, due to the absence of a subfamily rank for this family, the most abundant subfamily was Diapriinae (152

specimens; 39.5%), followed by Scelioninae (98 specimens; 25.5%) and Pristocerinae (67 specimens; 17.4%) (Fig 3).

Contrary to previous hypotheses, we found that most specimens collected were winged, comprising 74% of the specimens' total, with only a small percentage of apterous or brachypterous specimens (17 and 9%, respectively). Apterous specimens belonged to the families Bethyidae (67), Diapriidae (29), Platygastridae (4), and Mutillidae (2), while brachypterous specimens belonged to the families Ceraphronidae (41), Platygastridae (13), and Mymaridae (1).

These results differ expressively from those found in studies carried outside the Neotropical region (Ulrich 2004, Smith *et al* 2008, Hall *et al* 2015), since only Diapriidae was prevalent in inventories. However, when surveying in watermelon plantations in a semiarid formation in Rio Grande do Norte, Brazil, Costa *et al* (2016) obtained a composition of SLH Hymenoptera similar than that presented here. This probably indicates that the constraints for the parasitoid fauna differ between geographical regions but are not restricted to environmental factors. Besides, environmental factors that might structure SLH Hymenoptera communities, the availability, density,

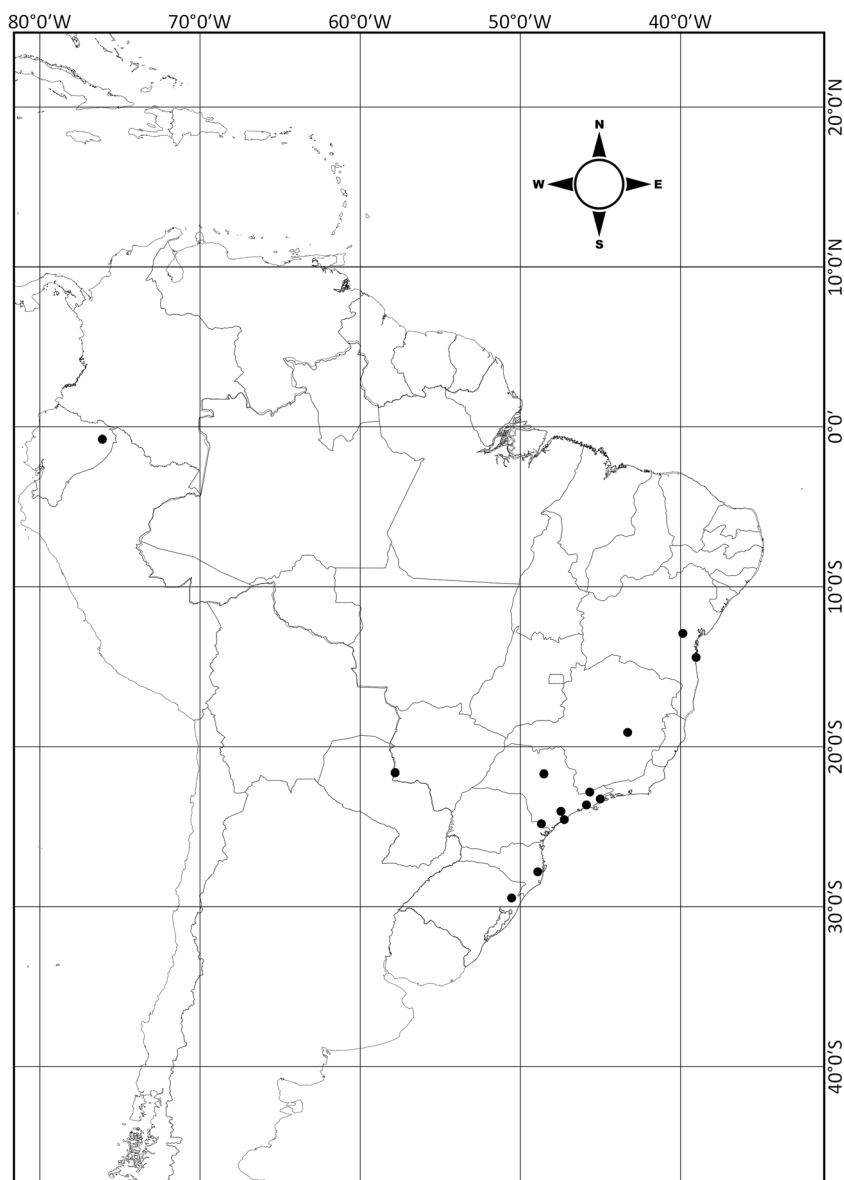


Fig 1 Map illustrating the sampled areas.

and assemblage composition of their hosts probably play an important role in modeling parasitoid assemblages (Hall *et al* 2015).

Ceraphronids are a group of hymenopteran insects that have a vast array of hosts, contemplating insects from the orders Diptera, Hymenoptera, Thysanoptera, Hemiptera, and Neuroptera (Masner 2006a). Although intensively collected in inventories in the Neotropical region (e.g., Fernandes *et al* 2014, Lara *et al* 2015, and Costa *et al* 2016), this family has scarce records and studies about its biology. Therefore, infer biological processes that model the observed patterns of prevalence within this family, in the present study, would prove an unfruitful task. Robust studies on the specific behaviors and biologies in representatives of Ceraphronidae would bring a much

needed framework upon which works on diversity and ecology would be built on.

Specimens belonging to the family Diapriidae are commonly primary endoparasitoids of dipterans and the adult forms can be normally found in humid habitats, such as forests and swamps, in the soil or near water streams (Masner 2006b). However, the knowledge about the biology of this family remains fragmented (but see Masner 1995). The most frequent subfamily is Diapriinae, with high species richness and biological diversity (Masner & García 2002). They are primarily cenobiontic endoparasitoids of Diptera, with few records in Coleoptera and many genera associated with Formicidae (Hymenoptera) (Masner 2006b).

Platygastridae are cenobiontic endoparasitoids of Cecidomyiidae (Diptera) (Masner 1995), with few members

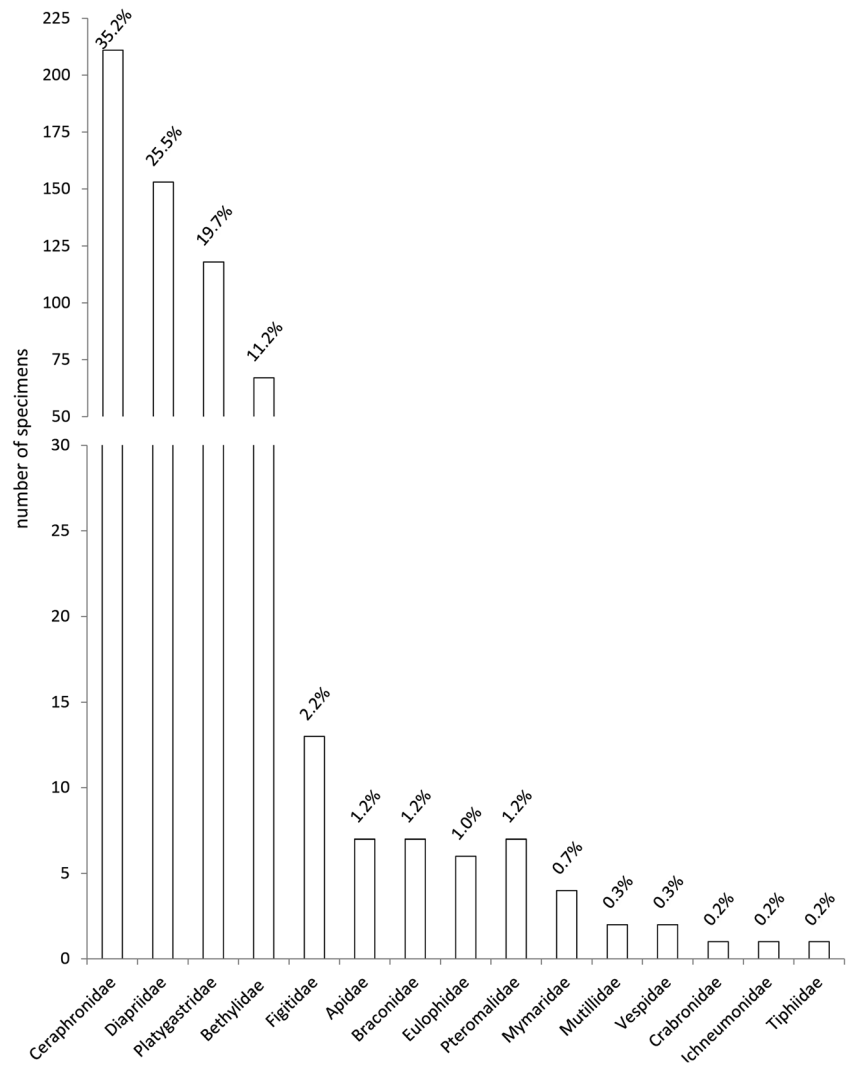


Fig 2 Number and percentage of specimens per family found in a pool of samples.

with idiobiontic life style (Masner & Arias-Penna 2006a). According to Masner and Arias-Penna (2006b, c), adult forms of Platygasteridae can be found mainly in open areas, forests, both soil (mainly Scelioninae) and canopy (mainly Platygasterinae). This reflects the high prevalence of Scelioninae in the samples studied in the present work, although representatives of Platygasterinae were also collected. Contrasting with other members of the family, representatives of Scelioninae are parasitoids of eggs from other insects, such as Orthoptera (Masner 1976) and Hemiptera (Masner 1972, 1976, 1983), and from spiders (Austin 1985, Masner & Dessart 1972, Masner 1976).

Bethyidae are, mainly, gregarious ectoparasitoids of immature stages of Lepidoptera and Coleoptera (Vargas-Rojas & Terayama 2006). According to Vargas-Rojas and Terayama (2006), bethylids originally evolved towards exploitation of free-living hosts with moderate and large sizes, and, posteriorly, towards exploitation of small-sized hosts. On the other hand, some groups

developed the ability to explore small larvae housed in sheltered conditions. Although the most speciose sub-family within Bethyidae is Epyrinae (Vargas-Rojas & Terayama 2006), the most frequent one in the samples studied here was *Pristocerinae*, probably because all species known have an evident sexual dimorphism, with winged males and apterous females (Vargas-Rojas & Terayama 2006). They are parasitoids of immature stages of Coleoptera and adult females are frequently collected in leaf litter samples (Vargas-Rojas & Terayama 2006).

The composition found in samples of leaf litter and soil obtained in tropical forests differ considerably from those in other formation (especially in temperate regions). Some works tried to answer these question, although for restricted groups (such as ichneumonids; Timms *et al* 2016).

To address such questions, however, a researcher should properly standardize the method applied, considering the limitations that the Winkler extractor possesses. A possibility

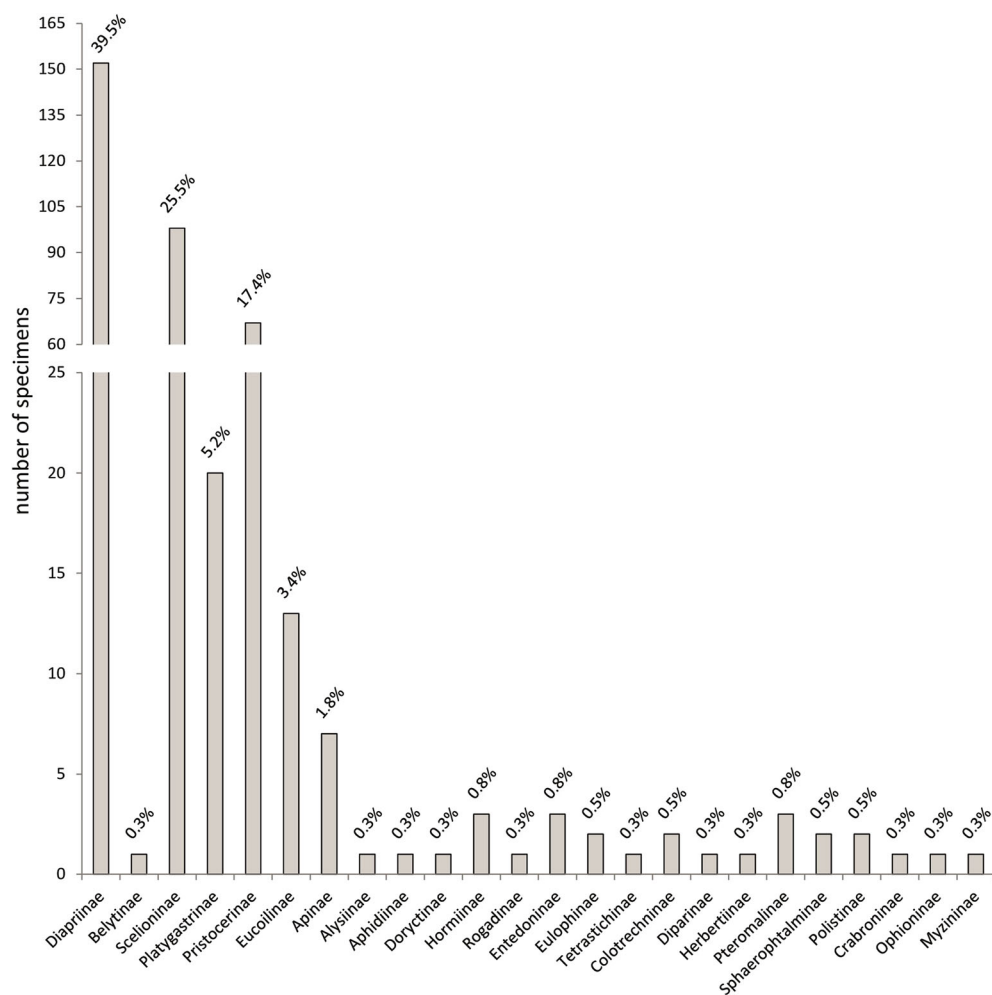


Fig 3 Number and percentage of specimens per subfamily found in a pool of samples.

would be use the technique in conjunction with Malaise traps, in order to obtain species more frequently found using flight interception traps, due to their active flying during adulthood. In addition, studies in different areas would provide a deeper comprehension on parasitoid community structuring in regional scales.

Contrary to believed, the prevalence of winged individuals, opposed to apterous/brachypterous, indicates a possible absence of morphological syndromes explaining the presence of parasitoid hymenopterans in litter assemblages. Most possibly, there might exist environmental constraints on parasitism behavior and host specificity, instead of anatomical modifications, which selects some groups of parasitoids based on their preferences and strategies used to reach optimal or suboptimal oviposition sites.

To trace those behaviors\host preferences, a more complete taxonomic base would be required, as well as broader knowledge on the biology of parasitoid species and their mode of actions. However, to address questions that explain which are the determinant factors that structure the soil and litter parasitoid communities, one must thoroughly sample

this fauna, applying specific techniques. The Winkler extractor is a promising technique for sampling SLH Hymenoptera and should be considered as a complementary technique in inventories of the Hymenoptera fauna.

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