Hymenoptera parasitoids in protected area of Atlantic Forest biomes and organic rice field: compared assemblages

Hymenoptera parasitoides en áreas protegidas del bioma bosque Atlántico y de arroz orgánico: ensamblajes comparados

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Abstract: One way to improve the sustainability of agricultural systems is to generate similar characteristics to those in natural ecosystems by maintaining energy flows and habitat diversity, thereby ensuring the presence of natural enemies and other beneficial organisms that can regulate pest populations and maintain crop productivity with fewer environmental impacts. The objectives of this study were to identify and compare the diversity of parasitoid assemblages in irrigated rice crops under organic management in a nearby protected area; to compare the efficiency of two kinds of parasitoid traps; and to compare temporal variation in parasitoid species at the two sites. The study was developed in the Banhado dos Pachecos Wildlife Refuge (BPWR) and in Viamão, RS organic rice fields (OR). Specimens were collected monthly from May 2011 to April 2012. Two Malaise and four Moericke traps were used in each area. In the BPWR area, Platygastridae, Ichneumonidae and Braconidae showed the highest abundance (30 %, 21 % and 11 %, respectively), and in the OR area, the dominant taxa were Platygastridae (26 %), Braconidae (18 %) and Encyrtidae (15 %). Malaise traps captured the largest number of parasitoid species (58 %). The richness estimators Chao 1, Jack 1 and Bootstrap, pointed to a richness of 229 to 122 species in the OR area and of 454 to 260 in the BPWR area. Parasitoid diversity was higher in the BPWR than in the OR. Parasitoid abundance was highest in the rice crop during months in which crops were growing at the site.

Key words: Agricultural ecosystems. Conservation biological control. Natural enemies.

Introduction

An ecosystem is a functional system of complementary relationships between living organisms and their surroundings, defined by boundaries within which they are able to maintain a dynamic and stable equilibrium (Gliessman 2001). Because the diversity of ecosystems makes them resistant to disturbance and outside interference, more diverse ecosystems have a greater capacity for recovering from disturbance and restoring equilibrium to their processes of cycling materials and energy flows (Cuddington 2001). The low diversity of conventional agricultural systems (e.g., monocultures treated with large amounts of synthetic fertilizers and pesticides) makes them biologically unstable and vulnerable to economically damaging pests and disease agents (Gliessman 2001). Alternative agricultural practices in harmony with existing ecological processes in agricultural ecosystems can thus help increase the sustainability of food production (Gliessman 2001; Altieri et al. 2003; Altieri 2012). One way to improve the sustainability of agricultural systems is to generate characteristics similar to those in natural ecosystems by maintaining energy flows and habitat diversity, thereby ensuring the presence of natural enemies and other beneficial organisms that can regulate pest populations and maintain crop productivity with fewer environmental impacts (Gliessman 2001). In natural systems, methods to estimate diversity allow for more efficient environmental conservation and monitoring. Even as, in both natural and anthropogenic systems, diversity is considered a synonym of environmental quality, since it responds to adverse impacts such as those caused by pollution or community imbalances. Some estimation methods are based on community structure, while others rely on dominance or evenness (Moreno 2001). No other feature of agricultural systems offers as many fundamental ecosystem services for protecting plants against insect pests as plant diversity (Altieri and Letourneau 1982; Altieri et al. 2003). For that reason, conserving native forests close to agricultural ecosystems helps maintain...
and increase biodiversity in the latter, thereby boosting ecological processes there. Brazil’s government mandated the conservation of native forests within rural properties, by the Brazilian Forest Code (12.651/12, with alterations in Law 12.727/12) (Presidência da República Federativa do Brasil 2012). These undisturbed patches of native forest within a property (known as Legal Reserves) ensure that economic activities carried out there use natural resources sustainably, help preserve and restore ecological processes, and help conserve biodiversity, wildlife, and the native flora.

Rice (Oryza sativa L.), an annual grass native to Asia, was domesticated roughly 10,000 years ago (Khush 1997; Heinrichs 1998; Bambaradeniya and Amarasinghe 2003). Some insect and other phytophagous species can become common enough in irrigated rice crops to be economically important pests, causing losses in productivity on the order of 10-35% (Martins et al. 2009). One method for controlling these pests without serious environmental impacts is biological control. Parasitoids are one of the most important biological control agents for pests in agricultural systems, due both to their natural occurrence and to their use in biological control programs (Bale et al. 2008). Given that most organisms regarded as pests of rice are known to be attacked by parasitoids, controlling them via conservation of parasitoid hymenopterans is an important tool. The diversity of such parasitoids in different agricultural systems depends on environmental and biological factors, as well as management practices (Chay-Hernandez et al. 2006). Rice plantations are surrounded by aquatic and terrestrial habitats that form a mosaic of dynamic environments harbouring large stores of biological diversity, which are maintained both by rapid colonization and by the organisms’ rapid reproductive and growth rates (Fritz et al. 2008). The fauna associated with these systems includes vertebrates and invertebrates that inhabit the vegetation, water, and soils of rice plantations (Hook 1994). This diversity provides a more complex environment around the cultivated areas, where ecological interactions can be monitored by using parasitoid richness as a biological indicator (Lockwood et al. 1996).

The objectives of this study were: 1) to identify and compare the diversity of parasitoid assemblages in an irrigated rice crop under organic management and in a nearby protected area (both located in an Environmental Protection Area [Área de Proteção Ambiental]); 2) to compare the efficiency of two kinds of parasitoid traps; and 3) to compare the temporal variation in parasitoid species at the two sites.

**Materials and methods**

**Study sites.** The study was carried out in the 133,000 ha Banhado Grande Environmental Protection Area. Banhado Grande APA includes portions of the Pampa and Atlantic Forest biomes and accounts for 2/3 of the watershed of the Gravataí River. The original vegetation is primarily wetlands and “restinga” forest, but today the area also harbours urban centres and agricultural lands, where rice crops predominate (SEMA 2011).

Collections were made at two study sites: one a well protected area of native vegetation and the other an organic rice plantation in the Aguas Claras district (30°08’8.60”S, 50°53’52.60”W) of the town of Viamão, in the state of Rio Grande do Sul, Brazil.

**Wildlife refuge.** The first study site, a well preserved area of native vegetation known as the Banhado dos Pachecos Wildlife Refuge (BPWR), comprises 2,560 ha and is designated as a Legal Reserve of the “Filhos de Sepé” settlement (see below) under the Brazilian Forest Code (12.651/12, with alterations in Law 12.727/12).

The site has vegetation consisting of various different associations of plant species. Because plant collections are not permitted at the site, plants that occur frequently in the BPWR were identified based on photographs and the taxonomic literature (Lorenzi 1982; 1994), or by consulting descriptions published in other studies (Accordi and Hartz 2006). The families most commonly observed at the site were: Annonaceae, Apiaceae, Araliaceae, Asteraceae, Araceae, Blechnaceae, Cyperaceae, Euphorbiaceae, Ericaceae, Lauraceae, Malvaceae, Melastomataceae, Fabaceae, Moraceae, Myrsinaceae, Myrtaceae, Onagraceae, Orchidaceae, Phytolaccaceae, Poaceae, Polygonaceae, Pone-tederiaceae, Rubiaceae, Typhaceae, and Verbenaceae.

**Rice field.** The second study site is an approximately 20 ha rice plantation that forms part of the “Filhos de Sepé” Landless Rural Workers Movement Settlement, which surrounds the Banhado dos Pachecos. It is the largest such settlement in the state (9,406 ha) and home to 376 families (SEMA 2011). Because they are located within an Environmental Protection Area, since 2007 these rice plantations have been managed with organic practices.

The rice cultivar IRGA417 was planted with pre-germinated seeds under a layer of water or in mud in October 2011 and harvested in late February 2012.

The rice field has a variety of wild vegetation that grows on the levees and in the paddies outside of planting season. To identify the wild plant species on the levees, reproductively mature material of the most frequently observed species was collected and herbarium specimens were prepared. Plants were identified using the taxonomic literature (Lorenzi 1982; 1994) and with the help of Dr. Ilsí Boldrini of the Postgraduate Program in Botany at the Federal University of Rio Grande do Sul (UFRGS). The most commonly observed families at this site were Asteraceae, Boraginaceae, Commelinaceae, Convolvulaceae, Cyperaceae, Onagraceae, Poaceae, Polygonaceae, and Solanaceae.

**Sampling.** Traps were installed at two sampling points in the BPWR study site. Both points featured “restinga” vegetation, a characteristic ecosystem of the Atlantic Forest biome (Presidência da República Federativa do Brasil 1993). Point 1 (R1) had vegetation resembling that of High Restinga Forest while Point 2 (R2) had vegetation characteristic of Low Restinga Forest (Secretaria do Meio Ambiente do Estado de São Paulo 2013). Two sampling points were also established on the levees of the rice plantation: Point 1 (A1) and Point 2 (A2).

Sampling was carried out every month from May 2011 to April 2012. To collect hymenopteran parasitoids, two Malaise traps (Towes 1972a) and four Moericke traps (Granger 1970) were installed by two transects distant about 400 m from each other and 20 m between them at both the OR site and the BPWR site. The traps were left for 24 hours.

The collected insects were stored in 70% alcohol, labelled with sampling point and trap type, and transported to the Biological Control laboratory of the Plant Health Department.
of the Federal University of Rio Grande do Sul (UFRGS). The insects were separated into morphospecies with a Nikon SMZ445 stereomicroscope. Families of Hymenoptera were identified following the classification system adopted by Goulet and Huber (1993).

Meteorological data of the city of Viamão for mean daily temperature and monthly rainfall were obtained from Brazil’s National Meteorological Institute (INMET 2012).

Data analysis. The average number of hymenopteran parasitoids individuals collected on each sampling occasion was compared between trap types, sampling sites, and seasons using analysis of variance (ANOVA, Kruskal-Wallis test) (Hammer et al. 2001).

Rarefaction curves were constructed to compare richness between the rice field and the native forest. Estimated richness was calculated for each study site via the Chao 1, Jackknife 1, and Bootstrap estimates, using EstimateS software, version 8.2.0 (Colwell 2009). Qualitative differences were demonstrated separating exclusive and shared morphospecies between areas using Venn diagram.

Biological diversity was analysed with the Shannon-Wiener (H’), Simpson (1-D), Margalef (DMg), and Berger-Parker indices, using Past software, version 2.10 (2011) (Hammer et al. 2001).

Results

A total of 430 individual parasitoid wasps were collected at the Banhado dos Pachecos Wildlife Refuge (BPWR), belonging to 203 morphospecies and 20 families. A total of 203 individuals were collected at the organic rice plantation (OR), belonging to 95 morphospecies and 19 families. Platygastroidea, Ichneumonidae, and Braconidae were the most abundant families at the BPWR site, accounting for 30%, 21.40%, and 11.40% of relative frequency of individuals, respectively (Fig. 1). At the OR site, Platygastroidea (26.11%), Braconidae (18.23%), and Encyrtidae (15.27%) (Fig. 1) were the most abundant families.

Each Malaise trap captured a mean of 13.8 ± 3.46 individuals, significantly more than the Moericke traps (5.5 ± 1.15) (H = 4.84; d.f.= 1; P < 0.05). Ichneumonidae (21.80%), Platygastroidea (18.53%), and Braconidae (16.62%) were the most abundant families captured in the Malaise traps, while Platygastroidea (42.85%), Encyrtidae (18.42%), and Braconidae (9.40%) were the most abundant in Moericke traps. Malaise traps yielded all the families reported in this study, while Moericke traps were more selective, and did not capture individuals of Aphelinidae, Bethylidae, Eucharitidae, Eurytomidae, Evaniidae, Gasteruptiidae, Megaspilidae, Signoniphoridae, Trichogrammatidae, and Torymidae.

Total richness was 203 morphospecies for the BPWR site and 95 for the OR site. Thirty-seven morphospecies were shared between sites (Fig. 2). The highest number (26) was during the crop cycles (October/2011 to February/2012). Non-parametric estimators of species richness are typically based on the richness of rare species, and thus on four variables: singletons and doubletons (i.e., species represented by one or two individuals at a site), and unicates and duplicates (i.e., species collected during just one or two sampling periods) (Colwell 2009). The observed number of species (Sobs) at the most abundant families captured in the Malaise traps, while Platygastroidea (42.85 %), Encyrtidae (18.42 %), and Braconidae (9.40 %) were the most abundant in Moericke traps. Malaise traps yielded all the families reported in this study, while Moericke traps were more selective, and did not capture individuals of Aphelinidae, Bethylidae, Eucharitidae, Eurytomidae, Evaniidae, Gasteruptiidae, Megaspilidae, Signoniphoridae, Trichogrammatidae, and Torymidae.

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![Figure 1](image1.jpg)  
**Figure 1.** Abundance of Hymenopteran parasitoids families in Banhado dos Pachecos Wildlife Refuge (BPWR) and Organic Rice area (OR), Viamão, RS.

![Figure 2](image2.jpg)  
**Figure 2.** Venn diagram showing the composition of parasitoid hymenopteran morphospecies distributed among families exclusive and shared morphospecies between areas, in Banhado dos Pachecos Wildlife Refuge (BPWR) and Organic Rice area (OR), Viamão, RS. Number of morphospecies between parenthesis.

![Figure 3](image3.jpg)  
**Figure 3.** Estimated richness curves (% of estimated richness) of morphospecies to three estimators (Chao 1, Jack 1 and Bootstrap, randomized 500 times) and accumulation curve (Sobs), Banhado dos Pachecos Wildlife Refuge (BPWR), from May 2011 to April 2012, Viamão, RS.
each site indicates that richness was not fully sampled, as
illustrated by the ascending curves in Figs 4 and 5. At the
BPWR site there were 137 singletons, 36 doubletons, 153
unicates, and 32 duplicates, while at the OR site there were
64 singletons, 14 doubletons, 72 unicates, and 14 duplicates.
The richness estimators suggested that 40 % to 78 % of total
richness was collected at the two sites (Figs. 3 and 4).

The rarefaction curve for the abundance data shows
species richness differing between the two sites (Fig. 5), and
the Margalef, Shannon, Simpson, and Berger-Parker indices
(Table 1) also indicated higher diversity at the BPWR site.

As expected, abundances at both study sites varied over
time throughout the study (Fig. 6). June, July, and August
had the fewest captures and were very rainy months, but no
correlation between rainfall and number of captured insects
was observed. Abundances peaked in November for both
study sites.

The Pearson correlation coefficient indicated a positive
correlation between abundance and temperature for the OR
site ($P < 0.05$, $R^2 = 0.393$) and the BPWR site ($P < 0.05$; $R^2 = 0.383$).

**Discussion**

The most abundant family and morphospecies shared in this
study, Platygastridae, has been recorded in several other
studies of well preserved environments in Brazil’s Atlantic
Forest, even when different sampling methods were used
e.g., sweep nets), or when all three methods (Malaise,
Moericke, and sweep nets) were used (Azevedo and Santos
2000; Azevedo et al. 2002; Azevedo et al. 2003). Likewise,
some families found to be rare in this study (e.g. Aphelinidae,
Chrysididae, Eumniidae, Eurytomidae, Torymidae, and
Trichogrammatidae), have also been reported to have
abundances below 1 % in other studies (Azevedo and Santos
2000; Azevedo et al. 2002).

The low abundance of Ichneumonidae in the rice plantation
may be explained by the fact that species of the family are
more numerous in temperate and wet tropical regions (Townes
1972b). Humidity is higher in forested environments, and
is associated with the abundance of these insects (Townes
1972b). Although the rice plantation is irrigated, it is an open
environment more vulnerable to variation in environmental
conditions, which may have contributed to its lower species
richness in this family. By contrast, the BPWR site has more
shade due to its denser vegetation, which offers a more humid

![Figure 4](https://via.placeholder.com/150)

**Figure 4.** Estimated richness curves (% of estimated richness) of
morphospecies to three estimators (Chao 1, Jack 1 and Bootstrap,
randomized 500 times) and accumulation curve (Sobs), at the organic
rice site (OR), from May 2011 to April 2012. Viamão, R.S.

![Figure 5](https://via.placeholder.com/150)

**Figure 5.** Rarefaction curves of Hymenopteran parasitoids collected at
the organic rice area (OR) and at the Banhado dos Pachecos Wildlife
Refuge (BPWR), from May 2011 to April 2012. Viamão, R.S.

![Figure 6](https://via.placeholder.com/150)

**Figure 6.** Fluctuation of individuals and data of mean temperature
monthly and precipitation at the organic rice site (OR) and at the
Banhado dos Pachecos Wildlife Refuge (BPWR), from May 2011 to
April 2012. Viamão, R.S.

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<td>Berger-Parker</td>
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<td>Margalef</td>
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*p significant < 0.05 (Bootstrap).
microclimate with less extreme variations in temperature and humidity inside the forest (Tanque et al. 2010). It would thus appear that oscillations in environmental conditions affect the ichneumonid community to a lesser degree at the BPWR site. This result is similar to that of another survey carried out in an organic rice plantation in Rio Grande do Sul state, in the towns of Capivari do Sul, Eldorado do Sul, and Cachoeira do Sul. That study found Eulophidae (131 individuals), Braconidae (69 individuals), and Platygastridae (39 individuals) to be the most common families (Fritz et al. 2011). Species in these families were also found in our study and have been noted in other studies to attack pests in rice plantations.

A parasitism rate of 32 % was found in *Tribra ca limbativentris* Stål, 1860, parasitized by Telenomus podisi (Ashmead) and Trissolcus urichi (Crawford) (Platygastridae) and Oencyrtus submetallicus (Howard) (Encyrtidae) (Maciel et al. 2007). This natural parasitism is important since Pentatomidae have been reported throughout Brazil and can reduce crop productivity by up to 90 % (Ferreira et al. 1997). In rice crops in southern Brazil, *T. limbativentris* was found to be parasitized by *T. uruchi* (7.7 %) and *T. podisi* (75 %) (Riffel et al. 2010; Idalgo et al. 2013), and eggs of *O. poecilus* were found to be parasitized by *T. podisi* (Krinski et al. 2011).

The composition of families and species of parasitoid communities can also vary between sites and between crops due to their pest hosts, which may vary dramatically from one crop to another. The most abundant parasitoid found by Bambaradeniya and Edirisinghe (2008) in a study of rice in Sri Lanka, for example, was the family Mymaridae (39 %), which attack the insects *Nilaparvata lugens* (Stål, 1854) and *Sogatella furcifera* (Horváth, 1899) (Hemiptera: Delphacidae), important pests in that region, different from our work in which this family came fourth in abundance.

In rice fields, Braconidae is known by parasite Spodoptera frugiperda (Smith, 1979), Elasmostalpus lignosellus (Zeller) (Lepidiota: Pyralidae) and Cotesia flavipes Cameron [= Apanteles flavipes (Cameron)] (Braconidae) has been reported to D. saccharalis (Embrapa Arroz e Feijão 2004).

The highest number of morphospecies shared, 39 % of OR site, indicates the importance of Legal Reserve Area like natural enemies repository. The diversity vegetation increase in agricultural landscape can be a solution for beneficial insects increase (Thomas et al. 1991).

Trap efficiency can also depend on the surrounding vegetation, species’ habitats, and specific attributes such as flight capacity and host type (Southwood 1978). In our study, Malaise traps were installed in areas with sparse vegetation cover that may serve as corridors for dispersing insects (i.e., levees and resting forest), and this could have resulted in the greater number of individuals captured in the Malaise traps. In heterogeneous environments such as tropical forest, using multiple sampling methods is recommended for assessing the diversity of hymenopteran parasitoids (Noyes 2001). Malaise traps are efficient at collecting winged parasitoids because they intercept any insect in flight, while Moericke traps depend on the degree to which each group is attracted to colour (Mazón and Borda 2008).

Marchiori et al. (2003) obtained similar results to ours. They found that the Ichneumonidae family accounted for the largest number of individuals in Malaise traps (35.1 %), followed by Braconidae (19.1 %), while Diapriidae (26.7 %) and Encyrtidae (11.5 %) were the most abundant families in Moericke traps in south eastern Brazil. Thus, inventories designed to compare insect diversity and abundance should use sampling methods based on the sampling site and the group under study, both of which influence which types of insects will be captured.

The estimated richness of the study sites appears similar to those published in other studies of Neotropical environments (Querino et al. 2011). The variation in diversity values obtained with the estimators reflects the different parameters used in each. The first-order Jackknife estimator uses the unicates (Heltshe and Forrestor 1983). By contrast, the Chao 1 estimator is a function of the ratio between singletons and doubletons. At both study sites there were large numbers of singletons, which played an important role in defining the value of the Chao 1 estimator (Figs. 4 and 5).

Differences between the diversity indices of BPWR and OR were expected. An increase in the index value may reflect higher richness, greater evenness, or both (Magurran 2011). In a survey of hymenopteran parasitoid diversity in soybean crops, for example, Lara et al. (2009) found H’ values of 0.48, much lower than that found in our study, even though those authors recorded 22 families. The authors argued that these low values reflect the low evenness of their sample. Since Margalef’s index reflects richness based on the number of individuals (Moreno 2001) and both number of species and abundance were more than twice as high at the BPWR site than at the OR site, this index showed a strong difference between the sites. The only index that did not find a difference between the sites was Simpson’s, which is strongly affected by the most dominant species in the sample and less sensitive to species richness (Magurran 2011). As both sites had few very abundant species and many rare species, this index found no difference between the two.

In our study, the observed diversity in the smaller community did not reach the 95 % confidence interval of the larger community, by rarefaction technique. The comparison is made at the point at which the abundance level of the larger community is identical to that of the smaller community (Gotelli and Entsminger 2001). The difference in the diversity of hymenopteran parasitoids between the OR and BPWR sites may reflect their different vegetation structure. Although the OR site is managed organically, the agricultural environment is still less complex (Gliessman 2001) and the differences in plant families and genera between the two sites are apparent and well documented (Accordi and Hartz 2006). Diversity in vegetation structure and species is one of the factors that create niche diversity (Wilson 1994), and consequently resources for adult parasitoids to find shelter and food. Several studies have shown that the abundance and richness of entomophagous insects within an agricultural environment are closely related to the nature of the surrounding vegetation (Altieri et al. 2003). Adult hymenopteran parasitoids are also free-living and feed on honey and pollen (Jervis et al. 1993), which are provided by several wild plants, especially during flowering season.

Species richness, abundance, and composition can vary both with niche-related spatial heterogeneity and with temporal variation in weather and seasonal changes within a given area. Studies with hymenopteran parasitoids have shown highest abundance in austral spring and summer,

The same authors found Ichneumonidae and Braconidae to be the most common parasitoid families, and also reported a 70% correlation between temperature and abundance. No correlation was found with rainfall, corroborating our data from the BPWR site. While these abiotic factors are important, other parameters unrelated to weather, such as food availability, can also affect seasonal diversity patterns (Wolda 1988).

The peak abundance in November at both sites was strongly influenced by the abundance of Platygasteridae and Braconidae. The greater abundance of Braconidae can be explained by the family’s preference for open vegetation (like the rice plantation and “restinga”) and for mean temperatures between 20 and 24 °C (Cirelli and Penteado-Dias 2003).

Platygastridae reached its highest abundance in the austral summer and spring, at both the BPWR and OR sites. Peak abundance in this family could potentially be related to host availability. The months with the greatest abundance of platygastrids were those in which the rice crop was growing (November-February). The BPWR site showed high abundance during the same months and also in October, when the rice was starting to be planted. This may be additional evidence that the BPWR can supply the crop with parasitoids during the season in which it is most vulnerable to pest attacks.

Parasitoid abundance in the rice plantation is affected not only by temperature but also by the presence or absence of the crop. The months between November and February had the highest abundance, since as rice matures there is more food available for the pests that serve as parasitoid hosts. Once seedlings emerge after November, these form a type of vegetation that is different from that of the winter landscape. During this same time wild plants growing on the levees, mostly Asteraceae and Cyperaceae, flower and provide resources for adult parasitoids (Coombes and Sotherton 1986; Corbett and Planta 1993; Jervis et al. 1993).

Given that there are 61 families of hymenopteran parasitoids in the word, and that several are restricted to specific zoogeographical regions such as the Australian and the Holarctic (Azevedo and Santos 2000), and considering that 36 hymenopteran families with parasitizing species and Chrysidoidea have been recorded in Brazil (De Santis 1980), the sites we studied appear to have a high parasitoid diversity and to play a key role in maintaining the diversity of these natural enemies.

**Conclusions**

The Malaise traps yielded more hymenopteran parasitoids and a higher diversity of those parasitoids than the Moericke traps. Parasitoid diversity was higher at the Banhado dos Pachecos Wildlife Refuge than at the organic rice crop. Parasitoid abundance was highest in the rice crop during the months in which the crop was growing at the site.

**Literature cited**


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