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# Sampling of parasitoid Hymenoptera: influence of the height on the ground

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## Abstract

Parasitoid hymenopterans are a highly diverse group of insects; therefore, the choice of an adequate sampling method becomes important to achieve a representative species richness of a site. The aim of this work is to evaluate the size and diversity of parasitoids in relation to the height of the Malaise trap placement above the ground of a low deciduous forest from Yucatan, Mexico. Parasitoids were collected from September to October 2015, using three Malaise traps at ground level and other three located right above the others, leaving no space between them, at a height of 1.5 m. The collected specimens were identified at family level. A total of 4083 parasitoids belonging to 31 families were collected, representing 93% of the sample's completeness, according to Jack 1 estimator; with differences in richness and abundance between trap heights according to rarefaction and fixed effects multifactorial ANOVA, respectively. Bethylidae, Braconidae and Ichneumonidae were the most abundant families. Besides, when analyzing the differences of each family by separate, there were significant results for Bethylidae, Diapriidae and Ichneumonidae with more individuals in the traps at ground level than in the raised ones. In a further analysis, the effect of body size on the capture height was observed. The specimens of larger size belonging to the families Bethylidae, Sphecidae, Sclerogibbidae and Evaniidae were more collected at ground level, on the other hand, the larger sized Ichneumonidae were collected at raised level.

## Keywords

diversity, families, malaise, neotropics, size

## Introduction

The choice of an adequate sampling method is indispensable to ensure representativeness of the obtained samples and to infer accurate conclusions regarding the diversity of a site. The later becomes particularly important when collecting insects, since the use of different kind of traps are very frequent to quantify them in view of their size and flight capacity (Mazón and Bordera 2008; Aguiar and Santos 2010). Among insects, parasitoids represent one of the most important biological strategies due to their role as population control of other insects, mostly phytophagous. These insects develop and feed during their larval stage on or inside other insects, which die at the end of the process (Godfray 1994). Among these parasitoids, hymenopterans comprise the order with the highest number of species, and it is estimated that given this specialization, this order might be between 2.5 and 3.5 times larger than coleopterans, which is the current order with the highest number of described species within the animal kingdom (Forbes et al. 2018).

In the particular case of parasitoid insects, the use of Malaise traps (Townes 1962) has been one of the most recommended and used methods (Sheikh et al. 2016) in monitoring programs, biodiversity inventories (e.g. Gauld 1991; Longino 1994; Mazon and Bordera 2008; van Achterberg 2009) and to obtain large quantities of parasitoid Hymenoptera (Sääksjärvi et al. 2004, 2006; Fraser et al. 2007; van Achterberg 2009; Lamarre et al. 2012). The Malaise trap is a passive capture system, which works by intercepting insects in flight, becoming especially adequate for capturing hymenopterans, which present positive phototropism; therefore, they fly upwards in search of light when in contact with the trap. For this reason, it is important that the collecting pot be placed towards the maximum illumination; the trap's shape leads the insect towards the collecting jar with alcohol (van Achterberg 2009).

There are several studies which have proven the efficacy of the Malaise trap regarding color (Townes 1972; Campbell and Hanula 2007), mesh size (Darling and Parker 1988), position, design, height (Darling and Parker 1988; Compton et al. 2000; Mederos-López et al. 2012) and sampling effort analysis (Castiglioni et al. 2017). One of the most important aspects for the Malaise trap efficiency is the location; the trap must be placed blocking a corridor, perpendicular to a vegetation barrier: installing them so that the base touches the ground (Sheikh et al. 2016), so its capture span ranges from the ground to about 1.50 m.

Several studies have demonstrated the differences of flying insects assemblies composition comparing the tree canopy and the ground level (Darling and Parker 1988; Compton et al. 2000; Vance et al. 2007; Mederos-López et al. 2012) or ground strata (Lamarre et al. 2012), but all of them using different kind of traps to compare the strata. However, it has not been analyzed whether placing the Malaise trap at ground level, as it is typically done, is the best option or suspending it a few meters over the ground, without reaching the tree canopy, could collect a different variety of parasitoids, considering that they do not only move around the tree canopy, but some also walk on the ground; others, especially the smaller size ones (< 1 mm long) use the air column to scatter at medium heights or over the canopy (Compton et al. 2000). With all these considerations, the aim of this work was to evaluate the diversity and size of

parasitoids in relation to the placement height of the Malaise trap above the ground in a low deciduous forest from the State of Yucatan, Mexico.

## Methods

### Study area and sampling

The present work was conducted in Hacienda Yabucu (20°48'37.55"N, 89°24'48.58"W) located in the municipality of Acanceh in the central part of the State of Yucatan, Mexico. The climate in the area is mainly warm, sub-humid with summer rains; it is one of the hottest zones in the Peninsula, with a mean annual temperature ranging from 28° to 30 °C, reaching their maximum in May (42 °C) and minimum in November (10 °C); with a rainfall from 600 to 700 mm per year. The dominant vegetation type is a low deciduous forest, with a high percentage of trees, which shed their leaves during the dry season; most of the trees are Fabaceae, with a tree layer no taller than 12 m (Rzedowski 2006).

The sampling was performed from September to October 2015, because these are the months with maximum rainfall and the highest abundance peak of parasitoids in the region (González-Moreno and Bordera 2012; González-Moreno et al. 2015; González-Moreno et al. 2018). A total of six Malaise traps were placed at two different heights: three of them were placed in the conventional way, at ground level (**GMT:** Ground Malaise trap) and the others were placed immediately above the first ones, leaving no space between them, at a height of 1.5 m above ground level (**RMT:** Raised Malaise Trap) (Fig. 1). The traps functioned continuously during nine weeks, with weekly cutoffs for recollecting.

Goulet and Huber (1993), and Gibson et al. (1997) keys were used to identify parasitoids families. The collected material was deposited at the Colección Entomológica of Tecnológico Nacional de México/Campus Conkal, Yucatan.

The location map of sampling sites (Fig. 1) was downloaded from https://www.google.es/earth/ and has been used agreeing with terms of use published in https://www.google.com/permissions/geoguidelines/.

### Data analysis

Family richness was described for both trap heights, considering the total of individuals per family and indicating the most abundant families in each trap position. To know how many families are expected for this method and sampling effort, the non-parametric Jackknife 1 richness estimator was calculated, which is used for small samples, with confidence intervals of 95% (Magurran 2004); using the ESTIMATES 9.1.0 software (Colwell 2014). To establish capture differences in terms of richness, a rarefaction analysis was performed, measuring the sampling effort by week and by number of individuals, adjusting it to the smallest sample.
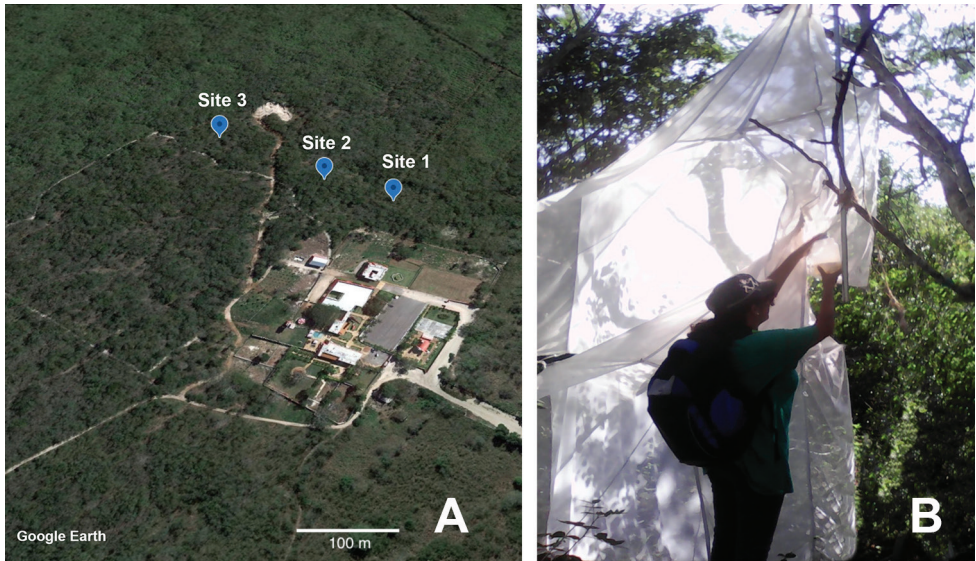
**Figure 1.** Malaise traps position at two different heights **A** location of sampling sites in Hacienda Yabucu **B** trap placement, one placed in the conventional way, at ground level, **GMT**: Ground Malaise trap and the other placed immediately above the first ones, leaving no space between them, **RMT**: Raised Malaise Trap.

The differences in total abundance of the collected parasitoids at the two different trap heights were analyzed by a fixed effects multifactorial ANOVA, considering the time and trap positions as factors; as variances were not homogeneous, abundance data were transformed to Ln (x), accomplishing homoscedasticity (Levene's test $p$ = 0.77) and residual normality (Shapiro-Wilk's $p$ = 0.20); this analysis was also done individually for each family, to determine if there are any differences between individuals collected at ground level and at raised level. For the comparative analysis of diversity, the non-parametric Shannon index was used, contrasted with the bootstrap method with a confidence interval of 95%.

To determine the size of the specimens collected in each trap, the forewing length (**FW**) of each specimen was measured as an indicator of body size, since both parameters are roughly positively correlated (Grimaldi and Engel 2005). To do this, the software IMAGEJ 1.45 was used, incorporating a millimetric spreadsheet as background and taking photos of each individual (Fig. 2). The size differences were analyzed by a fixed effects multifactorial ANOVA.

## Results

### Family richness

A total of 4083 specimens belonging to 31 families of parasitoid hymenopterans were collected, being Bethylidae, Braconidae and Ichneumonidae the most abundant fami-

**Figure 2.** Measurement of **FW**: fore wing, using millimeter sheet.

lies, representing altogether 40% of the whole sample (Table 1). The Jackknife 1 richness estimator predicted 34 families, using six Malaise traps for nine weeks; so, we collected 93% of the expected families for this method in the low deciduous forest of Yabucu (Table 2).

In terms of family richness, the rarefaction analysis by individuals, with the lower richness estimated at 1881 individuals, was slightly superior at **RMT** with 28 families (SE = 0.12), than below, GMT with 26 families (SE = 0.57). This means that if both samples had equal size, raised level had the highest richness.

**Parasitoid abundance**

Regarding the trap position, **GMT** collected 2202 individuals belonging to 28 families, the most abundant being Bethylidae, Ichneumonidae and Diapriidae, accounting for 54% of the total sample. The families Signiphoridae and Dryinidae were unique to this trap height (Table 1). **RMT** caught 1881 individuals belonging to 29 families, the most abundant being Braconidae, Bethylidae and Eucoilidae, representing 46% of the sample. The families Ceraphronidae, Encyrtidae and Trichogrammatidae were unique to this trap height (Table 1).

There were significant differences in the number of individuals caught at different heights (Table 3). Also, when the differences of each family by separate were analyzed, there were significant differences for Bethylidae, Diapriidae and Ichneumonidae (Tables 4–6); these three families had more individuals caught at ground level than in raised traps (Table 1). This pattern remained constant during all weeks of sampling, in other words, there was no interaction between the factors trap position and time.

**Table 1.** Individuals' number collected in two Malaise trap height: **GMT, RMT** and ANOVA F and *p* value. Values of probabilities were significant at 0.05\*. Families without statistics values had not enough data for the analysis.

| | Individuals in GMT | Individuals in RMT | Total individuals | F | *p* |
|---|---|---|---|---|---|
| Bethylidae | 422 | 216 | 638 | 6.64 | 0.01* |
| Braconidae | 204 | 365 | 569 | 1.63 | 0.24 |
| Ichneumonidae | 280 | 121 | 401 | 6.37 | 0.01* |
| Diapriidae | 249 | 58 | 307 | 16.60 | 0.00* |
| Eucoilidae | 81 | 200 | 281 | 2.35 | 0.16 |
| Sclerogibbidae | 128 | 148 | 276 | 0.18 | 0.68 |
| Chalcididae | 145 | 108 | 253 | 2.24 | 0.14 |
| Scelionidae | 125 | 119 | 244 | 0.07 | 0.80 |
| Platygastridae | 83 | 113 | 196 | 0.48 | 0.50 |
| Eupelmidae | 101 | 72 | 173 | 1.54 | 0.23 |
| Eucharitidae | 77 | 44 | 121 | 2.66 | 0.14 |
| Evaniidae | 44 | 53 | 97 | 0.56 | 0.47 |
| Mymaridae | 19 | 66 | 85 | 4.29 | 0.07 |
| Aphelinidae | 38 | 28 | 66 | 2.58 | 0.127 |
| Eurytomidae | 25 | 36 | 61 | 1.22 | 0.29 |
| Perilampidae | 37 | 21 | 58 | 3.45 | 0.10 |
| Chrysididae | 35 | 17 | 52 | 2.25 | 0.17 |
| Sphecidae | 24 | 22 | 46 | 0.04 | 0.86 |
| Elasmidae | 28 | 5 | 33 | 1.57 | 0.24 |
| Figitidae | 17 | 13 | 30 | 0.44 | 0.52 |
| Torymidae | 14 | 11 | 25 | 0.07 | 0.79 |
| Eulophidae | 8 | 14 | 22 | 3.24 | 1.09 |
| Pteromalidae | 11 | 9 | 20 | 2.98 | 0.09 |
| Ceraphronidae | 0 | 6 | 6 | – | – |
| Gasteruptiidae | 1 | 5 | 6 | – | – |
| Rhopalosomatidae | 1 | 5 | 6 | – | – |
| Encyrtidae | 0 | 4 | 4 | – | – |
| Stephanidae | 2 | 1 | 3 | – | – |
| Dryinidae | 2 | 0 | 2 | – | – |
| Trichogrammatidae | 0 | 1 | 1 | – | – |
| Signiphoridae | 1 | 0 | 1 | – | – |
| **TOTAL** | **2202** | **1881** | **4083** | | |

**Table 2.** Species richness expected according Jackknife 1 estimator and percentage of specimens collected.

| Site / Malaise trap height | Total of families observed | Total of families expected | % families collected |
|---|---|---|---|
| **1 GMT** | 24.33 | 28.52 | 85.30 |
| **1 RMT** | 27.53 | 31.05 | 88.66 |
| **2 GMT** | 28.95 | 31.83 | 90.95 |
| **2 RMT** | 29.87 | 32 | 91.37 |
| **3 GMT** | 30.5 | 33.15 | 92.00 |
| **3 RMT** | 31 | 33.5 | 92.53 |

## Diversity and size of parasitoids

In terms of diversity, there were no differences between families collected at ground level (**GMT** *H'*: 2.68) and raised traps (**RMT** *H'*: 2.71) (*p* = 0.08).

**Table 3.** Analysis of variance for the number of individuals of the Hymenoptera parasitoids for Malaise trap height: **GMT, RMT** and sampling week. Values of probabilities were significant at 0.05*.

| Main effects | Sum of squares | DF | F | p |
|---|---|---|---|---|
| Sampling Week | 6.23 | 8 | 0.59 | 0.78 |
| Malaise Height | 15.61 | 1 | 11.73 | 0.0015* |
| Interaction | 3.67 | 8 | 0.35 | 0.94 |
| Residuals | 50.7 | 38 | | |
| Total | 96.2 | | | |

**Table 4.** Analysis of variance for the number of individuals of Bethylidae for Malaise trap height: **GMT, RMT** and sampling week. Values of probabilities were significant at .05*.

| Main effects | Sum of squares | DF | F | p |
|---|---|---|---|---|
| Sampling week | 314.50 | 8 | 0.36 | 0.93 |
| Malaise Height | 718.6 | 1 | 6.64 | 0.01* |
| Interaction | 231.82 | 8 | 0.27 | 0.97 |
| Residuals | 3895.33 | 36 | | |
| Total | 5160.31 | 53 | | |

**Table 5.** Analysis of variance for the number of individuals of Diapriidae for Malaise trap height: **GMT, RMT** and sampling week. Values of probabilities were significant at 0.05*.

| Main effects | Sum of squares | DF | F | p |
|---|---|---|---|---|
| Sampling week | 128.48 | 8 | 0.39 | 0.92 |
| Malaise Height | 675.57 | 1 | 16.60 | 0.00* |
| Interaction | 98.93 | 8 | 0.30 | 0.96 |
| Residuals | 1464.67 | 36 | | |
| Total | 2367.65 | 53 | | |

**Table 6.** Analysis of variance for the number of individuals of Ichneumonidae for Malaise trap height: **GMT, RMT** and sampling week. Values of probabilities were significant at 0.05*.

| Main effects | Sum of squares | DF | F | p |
|---|---|---|---|---|
| Sampling week | 487.37 | 8 | 0.83 | 0.58 |
| Malaise Height | 468.167 | 1 | 6.37 | 0.016* |
| Interaction | 148.333 | 8 | 0.25 | 0.98 |
| Residuals | 2647.33 | 36 | | |
| Total | 3751.2 | 53 | | |

**Table 7.** Differences in individual wing length of five parasitoid families collected in traps set at different heights: **GMT, RMT**.

| Parasitoid family | GMT | RMT | T (p) |
|---|---|---|---|
| | Mean mm (SE) | Mean mm (SE) | |
| Bethylidae | 2.31(.028) | 1.96(0.038) | 7.22(<0.05) |
| Ichneumonidae | 4.34(.10) | 5.47(0.15) | -6.35(<0.005) |
| Sphecidae | 3.85(.18) | 2.85(0.19) | 3.83(<0.0005) |
| Sclerogibbidae | 1.7(.03) | 1.5(0.03) | 4.9(<0.0005) |
| Evaniidae | 2.9(.09) | 2.26(0.08) | 5.23(<0.0001) |

Only in five of the total 31 collected families, there were differences in relation to size. The largest Bethylidae, Sphecidae, Sclerogibbidae and Evaniidae were collected at **GMT**; on the other hand, the larger Ichneumonidae were collected in **RMT** (Table 7).

## Discussion

The total of collected families comprise 97% of 32 families recorded for Yucatan Peninsula (Delfín-González and Chay-Hernández 2010) and 70% of those recorded in the Neotropic (Fernández and Sharkey 2006), resulting in an optimum sampling effort since the family richness observed (31 families) is 91% of the estimated richness (34 families). Therefore, the representativeness of the results is sufficient to make comparisons at higher taxa level, in agreement with the results by Mazon (2016) on diversity of parasitoid subfamilies. In the current study, taking into account the minimum sampling effort, the results allow to state that keeping a Malaise trap for two months provides 85% of the parasitoids' representativeness in the site at family level. However, it is important to consider the time of the year when the sampling is done, as it must coincide with the seasonal maximum populations. In temperate weathers, they follow a bimodal pattern with maxima in the Spring and Fall, or unimodal, with one population peak in Spring-Summer, related to the yearly balmy temperatures (Gaasch et al. 1998; Rodríguez-Berrío et al. 2010; Mazon et al. 2011). At the tropics, the highest abundances follow a unimodal pattern around the rainy season (Gauld 1991). Particularly for the area of study, previous works have proven that the months from August to October have the highest parasitoid abundance and diversity (González-Moreno and Bordera 2012; González-Moreno et al. 2015; González-Moreno et al. 2018).

There are several factors that affect the insect diversity among the different vertical strata of a forest; for example, time, microclimate, light intensity, movement capacity (scattering), interspecific competition, natural enemies, quality of food resources and foliage (Basset 1992). In the present study, differences in family richness and abundance were observed, probably because hymenopteran families have different searching patterns; some studies have reported that very small size parasitoids such as many Chalcidoidea, fly frequently by the vegetation canopy, taking advantage of the air column for their dispersion, whereas other families such as Mymaridae are restricted to the lower levels in the forest (Compton et al. 2000). Also, insect herbivores are more abundant and speciose in the upper canopy than in the understory (Basset et al. 2001), so it is more likely to find different families of parasitoids looking for its herbivorous insect hosts.

Two of the most abundant families were Braconidae and Ichneumonidae, which are considered hyper-diverse groups (Fernández and Sharkey 2006); furthermore, they have been recorded as the two largest families of Hymenoptera in the Yucatan Peninsula (Delfín-González and Chay-Hernández 2010) and the rest of the world (Quicke 2015), with more than 46,500 valid described species (Yu et al. 2015). In the case of Bethylidae, its abundance can be explained by the fact that they are gregarious

parasitoids (Fernández and Sharkey 2006). This means it is probable that if traps are placed where the hosts are located, a high number of individuals from this family can be caught.

Regarding exclusive families at each height level, these results should be taken with caution due to the extremely low abundances, which may not represent a preference for a given height. However, some studies have recorded preferences of Trichogrammatidae at 8 m high and Encyrtidae at 12 m (e.g., Mederos-López et al. 2012); others have recorded Encyrtidae as one of the most abundant parasitoid family at "high levels", finding higher abundances at 18, 27 and 36 m above the ground (Compton et al. 2000). In this study, Encyrtidae, Trichogrammatidae and Ceraphronidae, fell exclusively in the traps placed at 1.5 m above the ground, which could be related to these these preferences. On another hand, the families Dryinidae and Signiphoridae fell only in traps at ground level.

The results from the analysis of families separately, demonstrated that for Bethylidae, Diapriidae and Ichneumonidae, trap height is important to collect a better representation in terms of abundance. Bethylidae attacks mainly Coleoptera larvae, which dwell on the ground (Vargas-Roja and Terayama 2006), so it is more likely that bethylid wasps fly near the ground. Diapriidae attacks mainly immature stages of Diptera; adults are found in humid habitats, in the shade and on the ground or near water (Masner 2006), so it is also highly probable to catch them near the ground. Lastly, for the Ichneumonidae, the difference in abundances could be also due to the host-searching strategy closer to the ground (Giraldo-Vanegas and García 1994; Kasparyan and Ruiz-Cancino 2008).

In relation to size, results suggest than this factor can also have an influence in the flight height of several families. The largest Bethylidae, Sphecidae, Sclerogibbidae and Evaniidae were collected at **GMT**; while the larger size of Ichneumonidae was collected at **RMT**. However, the explanation of this behavior is not easy at the family level, since these groups have a large range of sizes, especially Sphecidae and Evaniidae, and particularly Ichneumonidae. A possible explanation could perhaps be found from a more detailed study of these families at the genus or species level. In this way, the biology of these species itself could explain better the differences found.

## Conclusion

In conclusion, there were no differences in the diversity of parasitoid families collected at different heights from the ground; therefore, placing a Malaise trap at ground level is an adequate method for collecting parasitoids. However, a trend to more family richness could be observed in raised traps than in those at ground level. The trap position on the ground has influence on the abundance of collected Bethylidae, Diapriidae and Ichneumonidae, and also on the body size of Bethylidae, Sphecidae, Sclerogibbidae, Evaniidae and Ichneumonidae. It would be convenient to conduct these studies in other types of habitats to verify if this trend persists.

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