

# When less means more: Reduction of both effort and survey methods boosts efficiency and diversity of harvestmen in a tropical forest



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## ABSTRACT

Several ecological studies and monitoring programs of biodiversity have shown that using fewer collecting methods in biological surveys is more efficient than several redundant ones. However, in an attempt to increase species detection, researchers are still using as many field methods as possible in the surveys of arthropods and other megadiverse groups of invertebrates. The challenge is to reduce the overall time and effort for surveys while still retaining as much information about species richness and assemblage composition as possible. Researchers usually face a trade-off of loosing some information in order to have more efficient surveys. Here we show that more species were obtained in harvestmen surveys using a reduced version of the traditional method of active nocturnal search. We evaluated both the congruence and efficiency of the beating tray, and three versions of active nocturnal search across a tropical forest area in the Amazon basin. As nocturnal search has long been proved to be the most efficient method to capture arachnids, we tested three variations of this method in an attempt to improve harvestmen survey. A total of 2338 individuals of 23 species, in 20 genera and 10 families, were recorded using all methods together. Just one method, the active cryptic nocturnal search, encountered all taxa sampled with the maximum effort (sum of all methods) and data from this method recovered the ecological patterns found by the more intensive methods. Financial costs and time spent sampling and identifying specimens were reduced by 87% when compared to the maximum effort. We suggest that only one method, active cryptic nocturnal search, is the most efficient method to both sample and monitor harvestmen in Amazon tropical forests.

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## 1. Introduction

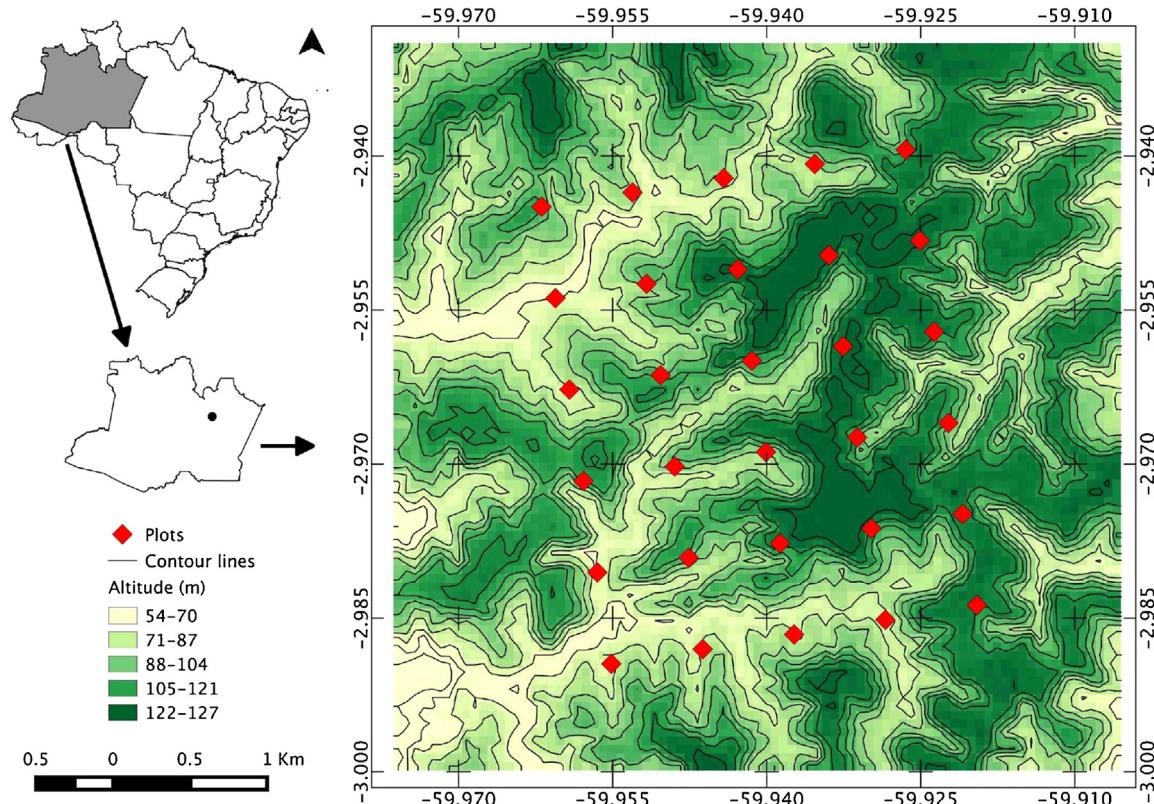
Biological surveys generally use a variety of collecting methods to estimate the species richness and describe assemblage composition of a particular locality (Coddington et al., 1991; Longino and Colwell, 1997; Pinto-da-Rocha and Bonaldo, 2006). Several field methods should provide a larger species data set, however detailed and exhaustive biodiversity surveys are time consuming and very expensive. The costs of biodiversity research in tropical forests are especially high due to complex logistics and difficult in accessing some areas. Therefore a major limitation is inadequate funding (Balmford and Whitten, 2003; Magnusson et al., 2013). When suf-

ficient resources exist for sampling, their more effective use may allow for the more extensive spatial and temporal sampling that is crucial to understand both biogeographic, ecological patterns, processes, and also for biodiversity monitoring (Costa and Magnusson, 2010; Kallimanis et al., 2012).

Efficient field methods are extremely important to obtain data on poorly known faunal groups (such as arthropods, and other invertebrates), so as to detect threats to biodiversity, prioritize areas for conservation and monitoring compositional changes to regional faunas (Longino and Colwell, 1997; Silveira et al., 2010). However, for studies focused on both species richness and composition the use of a combination of several field methods is not always necessary. In fact, several studies have shown that the use of fewer methods may be more efficient and less expensive than several redundant ones (Souza et al., 2012; Azevedo et al., 2013; Tourinho et al., 2014). Nevertheless, usually not as much data is collected if sampling effort is reduced, and then the impacts of reducing the

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**Fig. 1.** Ducke Reserve map showing the position of the 30 curvilinear sampling plots of the PPBio grid (red diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

taxonomic and numerical resolution must be evaluated together with assemblage congruence among different taxonomic groups, especially if there are multiple questions to be answered (Landeiro et al., 2012).

There is a debate about whether we should or should not put a huge effort on very detailed inventories and on species-level identification. Some researchers advocate that the species is the unity carrying most information on organisms and their relationships with environmental variation. Systematists and museum personnel are usually included in this group, as they are most interested in documenting and listing the species diversity (Lenat and Resh, 2001; Marshall et al., 2006; Verdonschot, 2006). Others say that detailed inventories and species-level identification provide little extra information over higher taxonomic levels about community responses to environmental conditions (Warwick, 1993; Bowman and Bailey, 1997; Bailey et al., 2001). Given the global biodiversity and economic crises the ideal is to find a protocol that is cheap, allows rapid surveys, collecting as many species as possible and that is also optimized to meet requirements for both ecological studies and monitoring species occurrence.

Traditional inventories were mainly developed for taxonomic purposes, where the effort is devoted to gain the largest number of species in a single visit to a site (Coddington et al., 1991; Cardoso et al., 2006; Pinto-da-Rocha and Bonaldo, 2006). We have been using harvestmen as models for different biodiversity studies, because this group of arachnids has a moderate local diversity, ranging from 12 to 52 species per locality, that makes species sorting and identification faster than in other megadiverse arthropod groups (Kury, 2011). They also have limited dispersal capability, and a strong relationship with environment conditions, and are thus very sensitive to alterations in temperature, humidity and microhabitat (Bragagnolo et al., 2007; Tourinho et al., 2014).

Six methods normally used to sample arachnids were tested in one of the traditional papers dealing with sampling design and protocols for arthropods surveys in Tropical Ecosystems (Coddington et al., 1991). Those authors chose four protocols that were designed to include basic microhabitat assessment. Two of them, "the looking up" and "the looking down", were variations of the active hand searching method, known as active night searching, which is typically performed during the night when most of the arachnids are active in the forest. These two methods were later fused into one single nocturnal search method that is frequently applied in tropical-forest surveys. In this, the collectors look up and down searching for arachnids in several types of microhabitat in the forest (Bragagnolo et al., 2007; Azevedo et al., 2013; Tourinho et al., 2014).

Even though a combination of three or more methods are constantly used to capture spiders and harvestmen (Bragagnolo and Pinto-da-Rocha, 2003; Bragagnolo and Pinto-da-Rocha, 2006; Bragagnolo et al., 2007; Tourinho et al., 2014), a higher number of spiders is often collected using active nocturnal search (Azevedo et al., 2013). In one recent study evaluating both the method and effort necessary for an effective harvestmen survey in the Amazon region, the authors demonstrated that four methods (beating tray, active nocturnal search, leaf-litter manual sorting and Winkler apparatus) that are regularly used to collect harvestmen documented different assemblages, but the active nocturnal search method have statistically less variance, and was more efficient when compared to other single method, to represent both harvestmen richness and composition (Tourinho et al., 2014).

Here we: tested the redundancy of four field methods (traditional active nocturnal search, modified active nocturnal search, active cryptic nocturnal search and beating tray), two of them newly designed for this study, to investigate how reduction in

number of methods affects the estimates of species richness and composition; test whether the relationships among harvestmen assemblage composition and environmental variables (leaf-litter depth, number of palms trees and soil moisture) will be captured similarly for each method; and investigated the possibility of reducing the cost, time and plot area for surveying harvestmen fauna. We hypothesized that methods more directed to the distribution and habitats used by the harvestmen are going to fulfill the taxonomical, ecological and financial needs of the survey.

## 2. Material and methods

### 2.1. Study area

This study was carried out in the Ducke Reserve, a 10,000 ha area of dense '*terra firme*' rainforest ( $02^{\circ}55' - 03^{\circ}01'S$ ,  $59^{\circ}53' - 59^{\circ}59'W$ ), north of the city of Manaus, Amazonas State, Brazil (Hopkins, 2005) (Fig. 1). The reserve has a north-south central plateau that separates two main drainage basins: the eastern, with water bodies draining into the Amazon River, and the western basin, with water bodies draining into the Rio Negro (Ribeiro et al., 1999). The plateau is at 80–140 m in altitude, and is formed predominantly by yellow latosols, becoming sandy due to increased declivity and lower altitude.

The climate is classified as tropical humid, with a relative humidity of 75–86% and an annual precipitation about 3.000 mm, with heavier rains from November until May. Annual average temperature is  $25.5^{\circ}\text{C}$ , with little variation during the year.

### 2.2. Sample design

Harvestmen were collected between January and February 2008 and also between February and June 2012, in 30 plots set on a grid trail system within the Ducke Reserve. The grid was set by PELD site 1 Project and is now maintained by the Program for Research on Biodiversity (PPBio), and consists of 30 plots distributed evenly at 1 km intervals (Fig. 1). Each plot is 250 m long and to minimize edaphic variation within it follows the altitudinal contour. The spatial design follows the RAPELD system (Magnusson and Lima, 2005; Costa and Magnusson, 2010).

### 2.3. Collecting and identifying harvestmen

Harvestmen were collected in 30 plots between January and June 2012. Beating tray and three variations of active nocturnal search were applied in each plot: (1) active nocturnal search, (2) modified active nocturnal search, and (3) active cryptic nocturnal search.

We use a broader concept of survey effort reduction: (a) reduction in number of data collection used, (b) reduction in number of times the method should be replicate in the same plot, (c) reduction in plot area, and by doing that, (d) reduction in time spent in the field collecting and possibly in the laboratory processing an excessive extra amount of material, and last but not least, (e) reduction in the budget of both field and lab research.

Because the nocturnal search method is responsible for capturing the largest number of species of arachnids (Pinto-da-Rocha and Bonaldo, 2006; Proud et al., 2012; Azevedo et al., 2013; Tourinho et al., 2014), we designed two variations of this method: (a) the first one called active nocturnal search method (Sørensen et al., 2002; Pinto-da-Rocha and Bonaldo, 2006; Resende et al., 2012) is frequently used in most inventories of arachnids in Brazilian Tropical forests and frequently harvestmen, spiders and other arachnids are collected together to maximize the effort and variety of data collected (see Bonaldo et al., 2009; Azevedo et al., 2013; Tourinho et al., 2014). To replicate this methods as it is formally used in past

studies each plot was marked into four sub-plots of 30 m by 10 m, each separated by 30 m and the first located 30 m from the start of the 250 m long plot totaling 1200 m<sup>2</sup> of plot area (Tourinho et al., 2014). Each subplot was surveyed for one hour by one collector. All harvestmen and other arachnids encountered, from the forest floor to a height of 2 m on the trunks and vegetation, were captured. (b) to save time in placing sub-plots and to explore the total length of the plot for the modified active nocturnal search was conducted along the entire length of the plot (250 m), we also reduced the width sampled to 2 m, totaling 500 m<sup>2</sup> of plot survey. Only harvestmen were collected during one hour by one collector, distanced 5 m from the right side of the central line of the plot. All harvestmen encountered, from the forest floor to a height of 2 m on the trunks and vegetation, were captured. (c) the active cryptic nocturnal was also conducted along the entire length of the plot (250 m), with reduced width sampled to 2 m, totaling 500 m<sup>2</sup> of plot survey harvestmen were searched on environments that likely contain cryptic species (such as fallen logs, beneath tree bark, small cavities and holes, suspended leaf litter, on soil leaf litter, cavities and roots). Plots were not paired each collecting method were applied in each plot in different moments.

Beating tray surveys were conducted during daylight hours, with twenty shrubs up to 3 m tall, where each was struck 20 times with a wooden stick. A wooden frame with 1 m<sup>2</sup> of white fabric, positioned below the shrubs, was used to capture any fallen harvestmen. Species identification was provided by specialists in harvestmen taxonomy (Ana Lúcia Miranda Tourinho, Gonzalo Giribet and Pío Colmenares). All taxonomic material was subsequently deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

### 2.4. Environmental variables

Depth of leaf-litter (cm), the number of palm trees and soil moisture (%) were selected as environmental variables because they have been found previously correlated with diversity, richness and composition of harvestmen assemblage (Curtis and Machado, 2007). The number of palm trees was chosen as a proxy for overall vegetation structure. Depth measurements in each plot were taken with a Marimon-Hay leaf-litter depth meter (Marimon-Junior and Hay, 2008), 5 m away from point zero and 2 m from the left hand side of the center line. In plots where the central line crossed an access trail, point measures located within 2 m of the trail were disregarded and corresponding measures were taken at the end of the plot, using 5 m intervals between points (Rodrigues et al., 2013). For palm trees, the width of each sampling plot was 4 m, giving a total area of 0.1 ha per plot. Each individual palm rooted within a sampling plot was counted, following Guillaumet et al. (2013).

Soil moisture was measured for each plot point by removing the leaf-litter layer while avoiding disturbance to the layers of soil below the litter. Three soil samples were then collected at different depths (0–5, 5–10 and 10–20 cm). The samples were collected with the aid of a 5.5 cm diameter manual auger. Moisture was determined from the difference between the soil sample wet and dry masses (Pimentel and Pezzini, 2013).

### 2.5. Data analysis

To compare the sampling intensity among methods and how effective each was at sampling the total harvestmen assemblage, we constructed species accumulation curves with 999 randomizations (Colwell et al., 2004). We made 999 randomization to minimize the effect of the order of sample units on the shape of the curve.

We considered harvestmen species rare if they were encountered in only one plot. To evaluate if the species composition

**Table 1**

Harvestmen species collected through four collecting methods in Reserva Ducke.

Taxon	Active nocturnal search	Active cryptic nocturnal search	Modified active nocturnal search	Beating tray	Overall
Eupnoi – Sclerosomatidae					
<i>Caluga</i> sp. 1	121	228	59	0	408
<i>Caluga</i> sp. 2	138	126	56	5	325
<i>Geaya</i> sp.	8	2	4	0	14
<i>Prionostemma</i> sp.	15	77	12	0	104
Laniatores – Agoristenidae					
<i>Avima matintapererera</i> (Pinto-da-Rocha, 1996)	15	8	2	0	25
Laniatores – Cosmetidae					
<i>Cynorta</i> sp. 1	0	1	0	0	1
<i>Cynorta</i> sp. 2	60	81	36	0	177
<i>Eucynortella duapunctata</i> Goodnight & Goodnight, 1943	338	187	75	0	600
Laniatores – Cranaidae					
<i>Phareicranaus manauara</i> Pinto-da-Rocha, 1994	29	60	27	0	116
Laniatores – Fissiphalidae					
<i>Fissiphalius martensi</i> Pinto-da-Rocha, 2004	1	21	3	0	25
Laniatores – Gonyleptidae					
<i>Discocyrtus carvalhoi</i> Mello-Leitão, 1941	5	11	2	0	18
<i>Discocyrtus</i> sp.	3	9	9	0	21
Laniatores – Manaosbiidae					
<i>Manaosbia</i> sp.	82	6	0	0	88
<i>Rhopalocranaus</i> sp.	64	40	7	0	111
<i>Saramacia lucasae</i> Jim & Soares, 1991	60	26	11	0	97
Laniatores – Samoidae					
<i>Kalominiua</i> sp.	0	4	0	0	4
Laniatores – Stygnidae					
<i>Protimesius longipalpis</i> Roewer, 1943	32	18	5	0	55
<i>Protimesius</i> sp.	0	5	2	0	7
<i>Ricstygnus</i> sp.	39	6	0	0	45
<i>Stygnus simplex</i> Roewer, 1943	22	33	12	0	67
Laniatores – Zalmoxidae					
<i>Chamaia</i> sp.	10	10	4	0	24
<i>Ethobunus</i> sp.	1	4	0	0	5
<i>Pirassunungoleptes</i> sp.	0	1	0	0	1
Total of individuals	1043	964	326	5	2338
Total de species	19	23	17	1	23

sampled was similar using the three methods, we used the presence or absence matrix of each species obtained in the plots per methods to run a Principal Coordinates Analysis (PCoA). This analysis reduces the dimensionality of the database to axes, as this allowed easier visualization and interpretation of the collected data, the result is the creation of principal coordinates that represents the axes which explains the highest data variation. We ordinated the plots by species composition using the Sørensen Dissimilarity Index (presence/absence). We used the axes obtained by the PCoA as dependent variables in an analysis of variance (ANOVA) to test the congruence of the harvestmen assemblages collected by only one method (low effort) to the community collected by all four methods (maximum effort).

We used redundancy analysis (RDA) (Legendre and Legendre, 1998), which is a direct extension of multiple regression analysis (Borcard et al., 2011), to express the extent to which variance in species composition can be explained by environmental variables, and determine how much of the ecological pattern captured by the four methods combined was obtained by the reduced efforts (i.e. one or two methods). We tested statistical significance in the RDA with 1000 permutations. RDA is a canonical ordering direct gradient analysis, which assumes linear responses of species abundance along environmental gradients. (Legendre and Legendre, 1998).

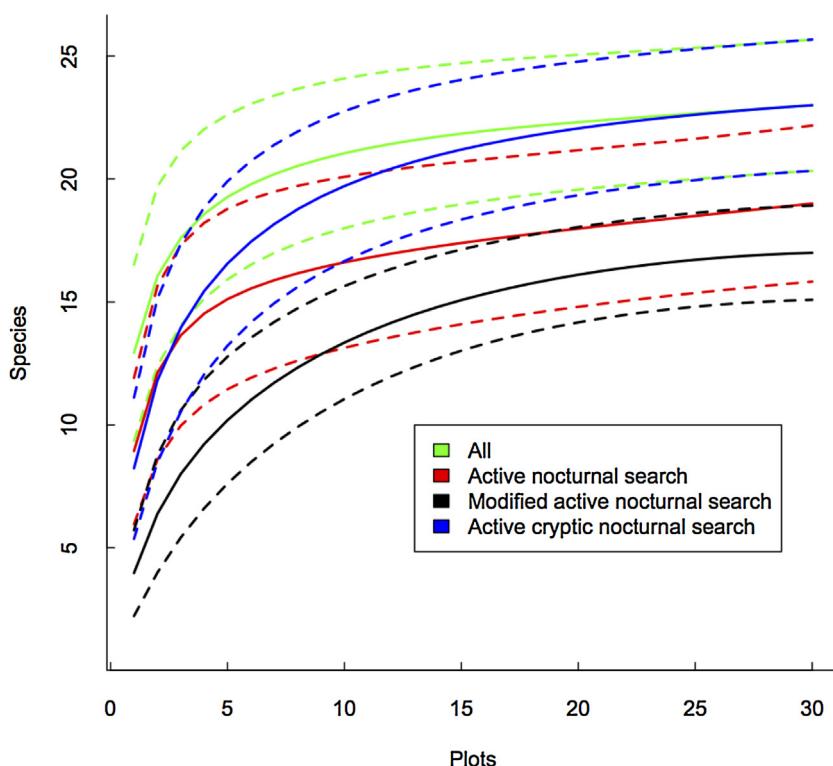
## 2.6. Project costs

The time spent collecting in the field, and the screening and identification in the laboratory, plus financial costs for the combined use of the four methods were considered as maximal effort (100%), and fractions of this cost were calculated for the other methods following Souza et al. (2012).

## 3. Results

### 3.1. Harvestmen diversity

We collected 2338 harvestmen, 10 families, 19 genera, 23 species (Table 1). The undescribed species are here referred as the name of the genus followed by sp. The number of individuals captured per plot ranged from 13 to 131 and the number of species per plot ranged from 1 to 18. The most abundant families were Sclerosomatidae and Cosmetidae (recorded in all plots), with an abundance of 851 and 778 individuals, respectively. Using the active nocturnal search method alone the abundance of Cosmetidae (398 individuals) was higher than Sclerosomatidae (282 individuals). The most frequent and abundant species, occurring in all plots, were *Eucynortella duapunctata* Goodnight & Goodnight, 1943 (Cosmetidae), with 600 individuals, followed by *Caluga* sp. 1 (Sclerosomatidae), with 408 individuals.



**Fig. 2.** Harvestmen species accumulation curve. Dashed lines indicate the 95% confidence interval.

**Table 2**

Variance proportion of the harvestmen community explained by the environmental variables through RDA.

Methods	R <sup>2</sup>	p
All	0.020	0.023
Active nocturnal search	0.183	0.019
Active cryptic nocturnal search	0.184	0.049
Modified active nocturnal search	0.135	0.220

We recorded 17 genera and 19 species, using the active nocturnal search; 14 genera and 17 species using the modified active nocturnal search, and 19 genera and 23 species with the active cryptic nocturnal search. We only collected one species, five individuals, using the beating tray. We recorded only two singletons: *Cynorta* sp. 1 (Cosmetidae) and *Pirassunungoleptes* sp. 1 (Zalmoxidae), we did not include them in the analysis.

The rarefaction curve for species based on 23 taxa sampled in 30 plots, approached the number of species (26) obtained by the Jackknife estimate (Fig. 2). An overlap between the curves indicates methodological overlap in the species being captured by the different field methods, and that the same 23 taxa were found by the active cryptic nocturnal search as with all the other methods combined. Active nocturnal search and modified active nocturnal search collected 19 and 17 taxa, respectively.

### 3.2. Congruence between field methods

In this work the two PCoA axes represent the species composition and together they explained 41% of data variation (PCoA axis 1: 24%, PCoA axis 2: 17%), we used the PCoA axis 1 to perform the ANOVA analyses because it explained alone 24% of data variation. The PCoA (Fig. 3) demonstrated significant variation between the species composition obtained with the different field methods (ANOVA using PCoA axis 1: F = 26.87, p < 0.001).

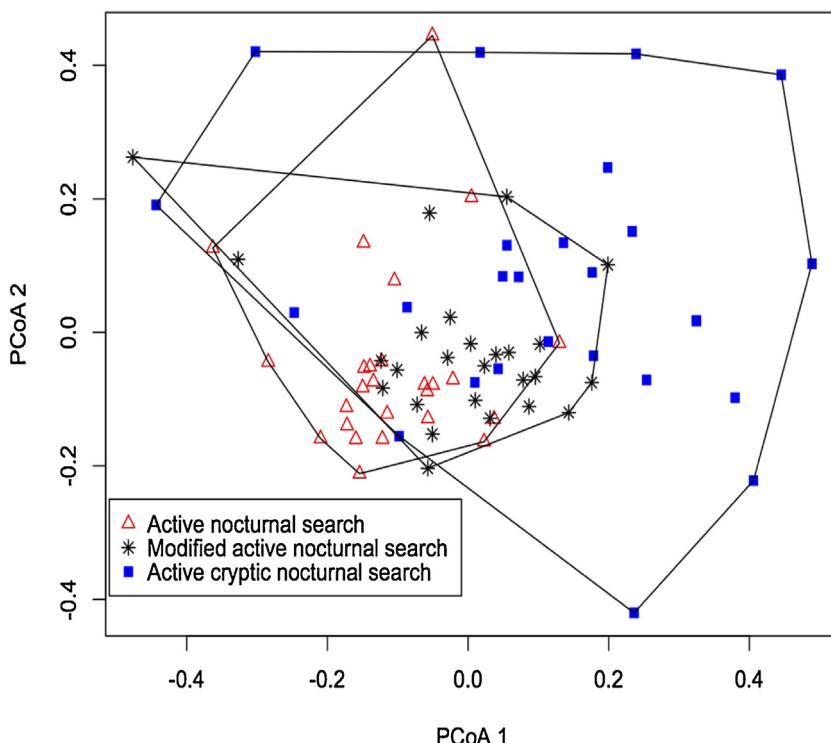
A significant variation were observed between the modified active nocturnal search vs. active cryptic nocturnal search (ANOVA for PCoA axis 1 p = 0.01), and between the active nocturnal search vs. modified active nocturnal search (ANOVA for PCoA axis 1 p < 0.001), since the modified active nocturnal search was less efficient at capturing the richness and abundance of the harvestmen community. There was no difference between the active nocturnal search and active cryptic nocturnal search (ANOVA for PCoA axis 1 p = 0.1), the methods that yielded the highest richness of species and abundance of specimens.

Despite the differences in species composition between the field methods, active nocturnal search and active cryptic nocturnal search methods are congruent because there was an overlap in the species they collected (Fig. 3). In terms of methodological complementarity, active cryptic nocturnal search is the most effective method increasing the number of species sampled by 23% compared to the other methods.

**Table 3**

Partial values of the RDA results, indicating that the environmental variables have an explanatory power on the ecological patterns observed in each field method tested.

Methods	Number of palms trees		Leaf-litter depth (cm)		Soil moisture (%)	
	F	p	F	p	F	p
All	3.392	0.030	2.638	0.010	0.525	0.810
Active nocturnal search	0.688	0.040	3.129	0.230	2.008	0.300
Active cryptic nocturnal search	3.038	0.050	1.455	0.480	1.380	0.750
Modified active nocturnal search	2.727	0.640	0.788	0.009	0.542	0.074



**Fig. 3.** Relationship among the 3 field methods summarized in two axes of the PCoA. Active nocturnal search (triangle), active cryptic nocturnal search (square), modified active nocturnal search (asterisk).

**Table 4**  
Cost and time spent to sample harvestmen in each method.

Methods	Costs (%)	Time (%)
All	100	100
Active nocturnal search	38	37.5
Active cryptic nocturnal search	12	12.5
Modified active nocturnal search	12	12.5
Beating tray	38	37.5

**Table 5**  
Procrustes rotation indicating correlations into the methods with 999 permutation.

Methods	Correlation	p
All × Active nocturnal search	0.421	0.007
All × Active cryptic nocturnal search	0.579	0.001
All × Modified active nocturnal search	0.429	0.006
Active cryptic nocturnal search × Modified active nocturnal search	0.434	0.050
Active nocturnal search × Active cryptic nocturnal search	0.367	0.404
Active nocturnal search × Modified active nocturnal search	0.267	0.960

pared to the active nocturnal search and by 30% compared to the modified active nocturnal search method.

The species composition in each plot was obtained with Procrustes rotation, where we noted that use of all methods together has the composition of all the variations of collection methods, and the active cryptic nocturnal search has similar composition modified active nocturnal search (Table 5).

### 3.3. Relationships with environmental variables

Correlation with environmental variables varied depending on the field method used. The influence of the environmental variables on the composition of the harvestmen community, based on RDA

scores, is given in Table 3. Overall, the composition of harvestmen captured with the three methods was correlated with the environmental variables tested (leaf-litter depth, number of palm trees and soil moisture) ( $RDA = 0.020$ ,  $p < 0.023$ ) (Table 2), and similar patterns were found when the harvestmen community was sampled with active nocturnal ( $RDA = 0.183$ ,  $p < 0.01$ ) and active cryptic nocturnal searches ( $RDA = 0.184$ ,  $p < 0.049$ ) (Table 2). However, the associations differed when modified active nocturnal search was used, as no correlation was observed with the tested environmental variables ( $RDA = 0.135$ ,  $p = 0.220$ ).

When the three methods were combined (maximum effort), the number of palm trees was the variable that best explained the distribution of the harvestmen community (Table 3). This was also the case with the active nocturnal search ( $p = 0.040$ ), and active cryptic nocturnal search ( $p = 0.050$ ), but not with the modified active nocturnal search ( $p = 0.640$ ). When all methods were combined, harvestmen community distribution was also explained by leaf-litter depth ( $p = 0.010$ ). This was also the case with the modified active nocturnal search ( $p = 0.009$ ), but not for the other two methods. Soil moisture content alone did not have any significant correlation with the methods used, thus, it could not explain the harvestmen distribution.

We covered an area of  $1200 \text{ m}^2$  per plot using the active nocturnal search,  $36,000 \text{ m}^2$ . We covered  $500 \text{ m}^2$  per plot using the modified active nocturnal search and cryptic active nocturnal search,  $15,000 \text{ m}^2$ . We reduced in  $21,000 \text{ m}^2$  (58%) of the initial area while using the modified active nocturnal search or the cryptic active nocturnal search.

### 3.4. Financial costs

Active nocturnal search and beating tray consumed most financial resources. They also took the most time to complete. Each consumed 38% of the financial resources for the study dedicated to sampling and 37.5% of the collection time for all methods. The mod-

ified active nocturnal search and active cryptic nocturnal search consumed only 12% of the financial resources and 12.5% of the time used for sampling (Table 4).

#### 4. Discussion

We generally use a combination of at least three field methods in arthropods surveys, in an attempt to access a variety of microhabitats, and sample the fauna associated with them. Our purpose in this paper was to evaluate how far could we go on reducing the sampling effort and also to assess whether it is possible to capture the ecological patterns that would be achieved with comprehensive surveys (Santos et al., 2008; Souza et al., 2012).

On the whole, we found that, unlike the results of previous studies, one method alone, the active cryptic nocturnal search, not only collected more species than all other methods, but also collected the same number and the same species as using the maximum effort (=the four methods combined). This method was also designed to reduce both search area (in 58%) and time spent searching this area. Comparing the plot area and the number of species sampled in each site, a 0.527 species/km<sup>2</sup> ratio was found in the active nocturnal search method; 1.53 species/km<sup>2</sup> with the cryptic active nocturnal search and 1.133 species/km<sup>2</sup> with the modified active nocturnal search. We collected a higher number of species in a much smaller area using active cryptic nocturnal search. The reduction of the sampled area, for the same time interval, allows the collector to better explore the site and find cryptic species. Plot shape affects the number of species included for a given area, long thin plots tend to contain more species than square or circular plots for two reasons: organisms tend to occur in higher densities in some habitats than others, and long plots have more chance of intercepting different habitats than short plots (Magnusson et al., 2013).

We recorded 23 taxa in Reserva Ducke using the maximum effort, active cryptic nocturnal search alone recovered all the 23 species, while 19 species were captured using the traditional active nocturnal search method, 17 using modified active nocturnal search, and beating tray was the least efficient and resulted in only one species. As most harvestmen are nocturnal and cryptic and therefore their struggle for life goes essentially undetected by humans (Cokendolpher and Mitov, 2007), active nocturnal cryptic search has an advantage over all the other methods because it focuses directly and only on the specific microhabitats in which harvestmen conceal themselves, enhancing detection chance per time unit.

In this study we showed that in a harvestmen survey it was possible to reduce the number of methods used to one and the area sampled, saving time and money, and still capture the ecological patterns that would be achieved with all methods combined. To the best of our knowledge this is the first study to show reduction of methods to minimum effort with no loss of information about species richness. Several studies suggest that the loss of information about species richness is compensated by the saving costs and time (Santos et al., 2008; Souza et al., 2012), or that we should avoid using several redundant methods in ecological studies; they should only be used when a more detailed survey is needed (Tourinho et al., 2014).

Our results were consistent with other studies showing that efficiency would be considerably higher in most of the monitoring programs by using just one method. In the specific case of harvestmen our results were similar to other studies using arachnids pointing nocturnal search as the most efficient method (e.g. Tourinho et al., 2014). However, our findings also indicate that this method may be improved and designed to reach a higher potential if we focus on specific active cryptic nocturnal searches. Active nocturnal search is the most used method in tropical forest sur-

veys (Coddington et al., 1991; Pinto-da-Rocha and Bonaldo, 2006; Tourinho et al., 2014), this method was modified from Coddington et al. and consists of merging the looking up and looking down methods proposed in that paper, it is usually performed to capture both spiders and harvestmen at the same time to save time and money. Our results have shown that less species of harvestmen were obtained using active nocturnal search, spending more time when compared to active cryptic nocturnal search. Using modified active nocturnal search we collected two species less than using the active nocturnal search but still in less time and in smaller area.

In general, we observed that, proportionately, the financial and temporal investment for collecting and sorting the material sampled with this method was twice that of both variations of the night search methods proposed and tested in this study. One explanation for that is that the habitat complexity accessed using this method is possibly higher, because collectors must inspect all kinds of habitats available looking up the vegetation and the ground. The area to be covered using this method is also larger, that meaning additional work and additional habitats to explore. The active cryptic nocturnal search method provided direct access to microhabitats characterized basically by leaf-litter, fallen logs, roots, termite nests, and suspended litter at the base of the palm leaves. Such habitats are those favored by harvestmen, providing ideal conditions of humidity and temperature for their development (Mestre and Pinto-da-Rocha, 2004; Curtis and Machado, 2007; Curtis, 2007; Proud et al., 2012). Therefore, focusing on search methods that are tailored to the ecological preferences of the faunal group, it becomes unnecessary to use a broad spectrum of collection methods, either for taxonomic or ecological purpose and we were also able to acquire better performance for existing methods.

Our results are in line with other studies suggesting that harvestmen respond to variation in habitat structure (Bragagnolo et al., 2007; Proud et al., 2012; Tourinho et al., 2014). There was correlation between environmental variables (number of palm trees, leaf-litter depth and soil moisture) and the harvestmen assemblages when the methods were combined, with the exception of the beating tray method. Data obtained by only active nocturnal search or active cryptic nocturnal search was also correlated with these variables, indicating that neither of these methods can yield the same ecological information as all three field methods combined. The positive relation between harvestmen captured and palm tree density were also recovered in previous study in the Amazon, it seems that palms, which increase habitat complexity and being correlated with well-structured soils (Vasconcelos, 1990; Emilio et al., 2013) are an important component for harvestmen community reflecting the variation in microhabitat structures for harvestmen species (Tourinho et al., 2014). Also, most abundant harvestmen species, such as *Eucynortella duapunctata* and *Cynorta* sp. 1, were found mostly on palm trees. They are especially common in the litter that accumulates at the base of the fronds. These suspended litter microhabitats accumulate both fallen dry leaves and rainwater, forming highly moist and protected microhabitats that are beyond the reach of many of the predators present in the soil and the forest-floor leaf-litter layers (e.g. Vasconcelos, 1990; Franken et al., 2013).

Both maximum effort and active nocturnal search correlated with depth of the leaf-litter to harvestmen abundance. This microhabitat is favored by several species of harvestmen in the Amazon, such as *Saramacia lucasae* and *Eucynortella duapunctata*, 85% of the species collected in this study were represented in the leaf-litter. This microhabitat provides harvestmen with shelter and substrate moisture, and is also rich in food resources (e.g. Adams, 1984; Lo-Man-Hung and Gardner, 2008; Curtis and Machado, 2007). Since depth of leaf-litter is related to its humidity, and many species of harvestmen prefer high humidity (Curtis and Machado, 2007),

leaf-litter may have had an indirect effect on the abundance and composition of the local harvestmen community.

Financial costs often limit the scope of biodiversity studies, especially in logistically-challenging areas such as the Amazon basin (Costa and Magnusson, 2010). Overall, the active cryptic nocturnal search method retrieved a higher number of taxa than all other methods combined using 12.5% of the total time used by all four methods together, although there was no loss of taxonomic or ecological information when using this method. This method had the advantage of not using subplots, so it reduced sampling time by 87.5% when compared to the other field methods. Consequently, there will be a concomitant reduction in the time spent on identification, preparation and processing of animals captured, and in field costs. However, results from previous studies have shown that, even traditional active night search was the most effective method, still there were a small percentage of specific species that are only accessed if sifting and/or leaf litter search are used (Tourinho et al., 2014).

Coddington et al. (1991) recommended the use of a suite of field survey methods to investigate the environments used by arachnids. Recent studies suggested that nocturnal search alone is an efficient alternative for adequately sampling harvestmen assemblage, even though there was a loss of 2–5 species in both sites tested, there was no redundancy, sampling was faster and less expensive, more efficient to test environmental relationships of species assemblages. However, we have shown that a more efficient version of this method, the reduction of the sampled area and time will probably reduce this loss of information or even work at no loss. For surveying harvestmen fauna in Tropical forests we consider that the use of active cryptic nocturnal search will be, in terms of time and financial resources, the most effective way to obtain reliable results.

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## References

- Adams, J., 1984. The habitat and feeding ecology of woodland harvestmen (Opiliones) in England. *Oikos* 42, 361–370.
- Azevedo, G.H.F., Faleiro, B.T., Magalhães, I.L.F., Benedetti, A.R., Oliveira, U., Pena-Barbosa, J.P.P., Santos, M.T.T., Vilela, P.F., de Maria, M., Santos, A.J., 2013. Effectiveness of sampling methods and further sampling for accessing spider diversity: a case study in a Brazilian Atlantic rainforest fragment. In: Dytham, C., DeVries, P. (Eds.), *Insect Conservation and Diversity*, pp. 1–11, Retrieved June 20, 2014, from <http://doi.wiley.com/10.1111/icad.12061>.
- Bailey, R.C., Norris, R.H., Reynoldson, T.B., 2001. Taxonomic resolution of benthic macroinvertebrate communities in bioassessments. *J. North Am. Benthol. Soc.* 20, 280–286 (Retrieved September 23, 2014, from <http://www.jstor.org/stable/1468322?origin=crossref>).
- Balmford, A., Whitten, T., 2003. Who should pay for tropical conservation, and how could the costs be met? *Oryx* 37, 238–250, Retrieved February 28, 2013, from [http://www.journals.cambridge.org/abstract\\_S0030605303000413](http://www.journals.cambridge.org/abstract_S0030605303000413).
- Bonaldo, A.B., et al., 2009. Inventário e história natural dos aracnídeos da Floresta Nacional de Caxiuaná. In: Lisboa, P.L.B. (Ed.), *Caxiuaná: Desafios Para a Conservação De Uma Floresta Nacional Na Amazônia*. Museu Paraense Emílio Goeldi, Belém, pp. 545–588.
- Borcard, D., Gillet, F., Legendre, P., 2011. *Numerical Ecology with R*. Springer New York, New York, NY, Retrieved July 14, 2014, from <http://link.springer.com/10.1007/978-1-4419-7976-6>.
- Bowman, M.F., Bailey, R.C., 1997. Does taxonomic resolution affect the multivariate description of the structure of freshwater benthic macroinvertebrate communities? *Can. J. Fish. Aquat. Sci.* 54, 1802–1807, Retrieved July 10, 2014, from <http://www.nrcresearchpress.com/doi/abs/10.1139/f97-085>.
- Bragagnolo, C., Pinto-da-Rocha, R., 2003. Diversidade de opilhões do parque nacional da serra dos Órgãos, rio de Janeiro, Brasil (Arachnida: Opiliones). *Biota Neotrop.* 3, 1–20, Retrieved from <http://www.biotaneotropica.org.br/v3n1/pt/fullpaper?bn=0203012003+pt>.
- Bragagnolo, C., Nogueira, A.A., Pinto-da-Rocha, R., Pardini, R., 2007. Harvestmen in an Atlantic forest fragmented landscape: evaluating assemblage response to habitat quality and quantity. *Biol. Conserv.* 139, 389–400, Retrieved July 22, 2011, from <http://linkinghub.ehlvier.com/retrieve/pii/S0006320707002807>.
- Cardoso, P., Borges, P.A.V., Gaspar, C., 2006. Biotic integrity of the arthropod communities in the natural forests of Azores. *Biodivers. Conserv.* 16, 2883–2901, Retrieved July 11, 2014, from <http://link.springer.com/10.1007/s10531-006-9078-x>.
- Coddington, J.A.J.A., Griswold, C.E., Dávila, D.S., Peñaranda, E., Larcher, S.F., 1991. *Designing and testing sampling protocols to estimate biodiversit in tropical ecosystems*. In: Dudley, E.C. (Ed.), *The Unity of Evolutionary Biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology*. Dioscorides Press, Portland, pp. 44–60.
- Cokendolpher, J.C., Mitov, P.G., 2007. Natural enemies. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, pp. 339–373.
- Colwell, R., Mao, C., Chang, J., 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85, 2717–2727, Retrieved April 11, 2013, from <http://www.esajournals.org/doi/abs/10.1890/03-0557>.
- Costa, F., Magnusson, W., 2010. The need for large-scale, integrated studies of biodiversity—the experience of the Program for Biodiversity Research in Brazilian Amazonia. *Nat. Conserv.* 8, 3–12, Retrieved April 4, 2013, from <http://doi.editoracubo.com.br/10.4322/natcon.00801001>.
- Curtis, D.J., Machado, G., 2007. *Ecology. Harvestmen: The Biology of Opiliones*. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.). Harvard University Press, Cambridge, pp. 280–308.
- Curtis, D.J., 2007. Methods and techniques of study. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, pp. 489–494.
- Emilio, T., Quesada, C.A., Costa, F.R.C., Magnusson, W.E., Schiatti, J., Feldpausch, T.R., Brienen, R.J.W., Baker, T.R., Chave, J., Álvarez, E., Araújo, A., Bánki, O., Castilho, C.V., Honorio, C., E.N., Killeen, T.J., Malhi, Y., Oblitas Mendoza, E.M., Monteagudo, A., Neill, D., Alexander Parada, G., Peña-Cruz, A., Ramírez-Angulo, H., Schwarz, M., Silveira, M., Ter Steege, H., Terborgh, J.W., Thomas, R., Torres-Lezama, A., Vilanova, E., Phillips, O.L., 2013. Soil physical conditions limit palm and tree basal area in Amazonian forests. *Plant Ecol. Divers.* 7, 215–229, Retrieved September 23, 2014, from <http://www.tandfonline.com/doi/abs/10.1080/17550874.2013.772257>.
- Franken, E.P., Baccaro, F., Gasnier, T.R., 2013. Is there a refuge for ants in litter accumulated at the base of *Attalea attaleoides* (Barb. Rodr.) Wess. Boer (Arecaceae)? *Entomotropica* 28, 27–37.
- Guillaumet, J.L., Lima, A., Costa, F., 2013. Composição Da Comunidade De Palmeiras Da Reserva Ducke, Retrieved from <http://ppbio.inpa.gov.br/knb/metacat/melo.92.12/ppbio>.
- Hopkins, M.J.G., 2005. *Flora da Reserva Ducke, Amazonas, Brasil*. Rodriguésia 56, 9–25.
- Kallimanis, A.S., Mazaris, A.D., Tsakanikas, D., Dimopoulos, P., Pantis, J.D., Sgardelis, S.P., 2012. Efficient biodiversity monitoring: which taxonomic level to study? *Ecol. Indic.* 15, 100–104, Retrieved June 8, 2014, from <http://linkinghub.ehlvier.com/retrieve/pii/S1470160x11003116>.
- Kury, A.B., 2011. Classificação De Opiliones, Museu Nacional/UFRJ Website. <http://www.museunacional.ufrj.br/mndi/Aracnologia/>.
- Landeiro, V.L., Bini, L.M., Costa, F.R.C., Franklin, E., Nogueira, A., de Souza, J.L.P., Moraes, J., Magnusson, W.E., 2012. How far can we go in simplifying biomonitoring assessments? An integrated analysis of taxonomic surrogacy, taxonomic sufficiency and numerical resolution in a megadiverse region. *Ecol. Indic.* 23, 366–373, Retrieved May 23, 2014, from <http://linkinghub.ehlvier.com/retrieve/pii/S1470160x12001859>.
- Legendre, P., Legendre, L., 1998. *Numerical Ecology*. In: *Developments in Environmental Modelling*, 20, Second English edition. Elsevier.
- Lenat, D.R., Resh, V.H., 2001. Taxonomy and stream ecology: the benefits of genus- and species-level identifications. *J. North Am. Benthol. Soc.* 20, 287–298, Retrieved September 23, 2014, from <http://www.jstor.org/stable/1468323?origin=crossref>.

- Lo-Man-Hung, N., Gardner, T., 2008. The value of primary, secondary, and plantation forests for Neotropical epigaeic arachnids. *J Arachnol* 36, 394–401, Retrieved January 21, 2013, from <http://www.bioone.org/doi/abs/10.1636/CT07-136.1>.
- Longino, J., Colwell, R., 1997. Biodiversity assessment using structured inventory: capturing the ant fauna of a tropical rain forest. *Ecol. Appl.* 7, 1263–1277, Retrieved April 10, 2013, from [http://www.esajournals.org/doi/pdf/10.1890/1051-0761\(1997\)007\[1263:BAUSIC\]2.0.CO;2](http://www.esajournals.org/doi/pdf/10.1890/1051-0761(1997)007[1263:BAUSIC]2.0.CO;2).
- Magnusson, W.E., Lima, A.P., 2005. RAPELD: a modification of the gentry method for biodiversity surveys in long-term ecological research. *Biota Neotrop.* 5, 1–6.
- Magnusson, W., Braga-Neto, R., Pezzini, F., Baccaro, F., Bergalho, H., Penha, J., Rodrigues, D., Verdade, L.M., Lima, A., Albernaz, A.L., Hero, J.-M., Lawson, B., Castilho, C., Drucker, D., Franklin, E., Mendonça, F., Costa, F., Galdino, G., Castley, G., Zuanon, J., Vale, J., Santos, J.L.C., Luizão, R., Cintra, R., Barbosa, R.I., Lisboa, A., Koblitz, R.V., Cunha, C.N., Pontes, A.R.M., 2013. *Biodiversidade E Monitoramento Ambiental Integrado Biodiversity and Integrated Environmental Monitoring*. In: Magnusson, W., Pezzini, F. (Eds.), 1st edn. Áttema Editorial, Santo André.
- Marimon-Junior, B.H., Hay, J.D., 2008. A new instrument for measurement and collection of quantitative samples of the litter layer in forests. *For. Ecol. Manage.* 255, 2244–2250, Retrieved March 7, 2013, from <http://linkinghub.elsevier.com/retrieve/pii/S0378112708000121>.
- Marshall, J.C., Steward, A.L., Harch, B.D., 2006. Taxonomic resolution and quantification of freshwater macroinvertebrate samples from an australian dryland river: the benefits and costs of using species abundance data. *Hydrobiologia* 572, 171–194, Retrieved July 10, 2014, from <http://link.springer.com/10.1007/s10750-005-9007-0>.
- Mestre, L.A.M., Pinto-da-Rocha, R., 2004. Population dynamics of an isolated population of the harvestman *ilhiaia cuspidata* (Opiliones, gonyleptidae), in araucaria forest (Curitiba, paraná, Brazil). *J. Arachnol.* 32, 208–220, Retrieved from <http://www.bioone.org/doi/abs/10.1636/M02-61>.
- Pimentel, T.P., Pezzini, F.F., 2013. Metadados Das Coletas E Análises físico-químicas Do Solo De 72 Parcelas Permanentes Instaladas Na Reserva Florestal Adolpho Ducke – AM. <http://ppbio.inpa.gov.br/knb/metacat/fecosta.4.5/p>.
- Pinto-da-Rocha, R., Bonaldo, A.B., 2006. A structured inventory of harvestmen (Arachnida, opiliones) at Juruti River Plateau, State of Pará, Brazil. *Revista Ibérica de Aracnología* 13, 155–162, Retrieved January 21, 2013, from <http://repositorio.museu-goeldi.br/jspui/handle/123456789/305>.
- Proud, D.N., Felgenhauer, B.E., Townsend, V.R., Osula, D.O., Gilmore, W.O., Napier, Z.L., Van Zandt, P.A., 2012. Diversity and habitat use of neotropical harvestmen (Arachnida: opiliones) in a costa rican rainforest. *ISRN Zoology* 2012, 1–16, Retrieved September 13, 2012, from <http://www.isrn.com/journals/zoology/2012/549765/>.
- Resende, L.P.A., Pinto-da-Rocha, R., Bragagnolo, C., 2012. *The harvestmen fauna (Arachnida: opiliones) of the parque estadual carlos botelho, and the floresta nacional de ipanema, São paulo, Brazil*. *Biota Neotrop.* 12, 1–10.
- Ribeiro, J.E.L.S., Hopkins, M.J.G., Vicentini, A., Sothers, C.A., Costa, M.A.S., Brito, J.M., Souza, M.A.D., Martins, L.H.P., Lohmann, L.G., Assunção, P.A.C.L., Pereira, E.C., Silva, C.F., Mesquita, M.R., Procópio, L.C., 1999. *Flora Da Reserva Ducke: Guia De Identificação Das Plantas Vasculares De Uma Floresta De Terra-firme Na Amazônia Central*.
- Rodrigues, F.R., de, O.R., Costa, F., 2013. Medidas De Massa Seca(g) E Profundidade(cm) De Serrapilheira Nas 30 Parcelas Da Grada PPBio Na Reserva Ducke. *Fecosta.50.3*, Retrieved February 1, 2013, from <http://ppbio.inpa.gov.br/knb/metacat/fecosta.50.3/>.
- Sørensen, L.L., Coddington, J., Scharff, N., 2002. Inventorying and estimating subcanopy spider diversity using semiquantitative sampling methods in an afromontane forest. *Environ. Entomol.* 31, 319–330, Retrieved September 13, 2012, from <http://www.bioone.org/doi/abs/10.1603/0046-225X-31.2.319>.
- Santos, E.M.R., Franklin, E., Magnusson, W.E., 2008. Cost-efficiency of subsampling protocols to evaluate oribatid-Mite communities in an amazonian savanna. *Biotropica* 40, 728–735.
- Silveira, L., Beisiegel, B., Curcio, F., 2010. Para que servem os inventários de fauna? Estudos Avançados 24, 173–207, Retrieved May 20, 2013, from [http://www.scielo.br/scielo.php?pid=S0103-40142010000100015&script=sci\\_arttext&tlang=en](http://www.scielo.br/scielo.php?pid=S0103-40142010000100015&script=sci_arttext&tlang=en).
- Souza, J.L.P., De Baccaro, F.B., Landeiro, V.L., Franklin, E., Magnusson, W.E., 2012. Trade-offs between complementarity and redundancy in the use of different sampling techniques for ground-dwelling ant assemblages. *Appl. Soil Ecol.* 56, 63–73, Retrieved November 9, 2012, from <http://linkinghub.elsevier.com/retrieve/pii/S0929139312000145>.
- Tourinho, A.L., Laçá, L., de, S., Baccaro, F.B., Dias, S.C., 2014. Complementarity among sampling methods for harvestman assemblages. *Pedobiologia*, 1–9.
- Vasconcelos, H., 1990. Effect of litter collection by understory palms on the associated macroinvertebrate fauna in Central Amazonia. *Pedobiologia* 34, 157–160.
- Verdonschot, P.F.M., 2006. Evaluation of the use of Water Framework Directive typology descriptors, reference sites and spatial scale in macroinvertebrate stream typology. *Hydrobiologia* 566, 39–58, Retrieved September 24, 2014, from <http://link.springer.com/10.1007/s10750-006-0071-x>.
- Warwick, R.M., 1993. Environmental impact studies on marine communities: pragmatical considerations. *Austral Ecol.* 18, 63–80, Retrieved July 10, 2014, from <http://doi.wiley.com/10.1111/j.1442-9993.1993.tb00435.x>.