Effectiveness of sampling methods and further sampling for accessing spider diversity: a case study in a Brazilian Atlantic rainforest fragment

GUILHERME H. F. AZEVEDO,[1,2] BÁRBARA T. FALEIRO,[1,2] IVAN L. F. MAGALHÃES,[1,3] ALÍPIO R. BENEDETTI,[4] UBIRAJARA OLIVEIRA,[1,2] JOÃO P. P. PENA-BARBOSA,[4,5] MARCUS T. T. SANTOS,[2] PAULA F. VILELA,[1] MÁRIO DE MARIA[1] and ADALBERTO J. SANTOS[1]   [1]Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Pós-Graduação em Zoologia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3]Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [4]Pós-graduação em Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil and [5]Laboratório Especial de Coleções Zoológicas, Instituto Butantan, São Paulo, Brazil

Abstract. 1. The current biodiversity crisis makes the quantification of the diversity and the description of organism distribution particularly pressing. Biological inventories are among the most effective ways to improve the knowledge about local biota, but they can be very time and money-consuming. The determination of adequate sampling effort and the selection of cost-effective collecting methods are critical issues.

2. In this article, a spider diversity inventory in an Atlantic semi-deciduous forest fragment in Brazil was used to compare the efficiency of three collecting methods in two different seasons in order to propose an optimised sampling protocol. The worthiness of increasing sampling effort in the target area and similar tropical ecosystems was estimated and evaluated in terms of its cost-effectiveness.

3. For a better sampling of the spider community, it is suggested that a proportion of 55, 29 and 16% of total sampling hours should be dedicated to nocturnal hand collecting (NHC), pitfall traps and beating trays, respectively, in the rainy season. If only one method can be applied, the most efficient in terms of species per sampling is the NHC.

4. A completeness of 70% of the estimated spider species richness (as predicted by the Chao1 estimator) was observed in the complete inventory and increasing sampling effort in the studied area may be highly ineffective when the costs involved are considered.

5. Other studies in similar tropical rainforest areas also presented completeness around 70%, which might be a threshold from which the sampling effort necessary to raise the observed species richness substantially starts to be ineffective.

Correspondence: Guilherme H. F. Azevedo, Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais, CEP 31270-901, Brazil. E-mail: ghfazevedo@gmail.com

Introduction

The world has been experiencing drastic reductions in its biodiversity, the so-called sixth mass-extinction event (Chapin *et al.*, 2000; Willis *et al.*, 2007; Barnosky *et al.*, 2011). In this scenario, it is imperative to quantify the diversity and distribution of organisms before they vanish, especially for the megadiverse, but poorly known, arthropods (Kim, 1993; Wheeler, 1995). This is no trivial task, however, since there is a lack of taxonomic working force for most arthropods (Gaston & May, 1992; Marques & Lamas, 2006; Cardoso *et al.*, 2011a) and many areas potentially harbouring undescribed species remain to be sampled for the majority of groups (Kier *et al.*, 2005; Whittaker *et al.*, 2005).

Biological inventories (or surveys) are a way to improve the knowledge on a local fauna at the same time as they provide specimens for museum collections and new species records (Burbidge, 1991; Kim, 1993; Wheeler, 1995; Funk *et al.*, 2005; Cardoso *et al.*, 2011a). Faunal inventories can be, however, very time- and money-consuming (Burbidge, 1991; Oliver & Beattie, 1996; Lawton *et al.*, 1998; Gardner *et al.*, 2008). This problem is particularly accentuated in tropical countries, which harbour much of the world's biodiversity but are frequently also the ones with less financial (Balmford & Whitten, 2003) and human (Gaston & May, 1992; Hawksworth, 1995) resources for taxonomy and biological conservation.

Another problem of biodiversity inventories is that they are a sample of the total richness of an area, as many species will not be collected due to undersampling or other factors (Colwell & Coddington, 1994; Oliver & Beattie, 1996; Magurran & Henderson, 2003; Coddington *et al.*, 2009). Consequently, the observed species richness of taxa will always be a subset of the true richness (Colwell & Coddington, 1994). For most groups, capturing all the species present in a given area would require an enormous investment in sampling effort, well above what has been invested in current diversity surveys (see Coddington *et al.*, 2009). This may be, however, unfeasible, as further sampling substantially increases the cost of an inventory. In Keating *et al.* (1998) words, 'truly complete inventories are not a reasonable goal' (see also Magurran & Queiroz, 2010). Thus, it is important to know when is the best moment to stop sampling an area, so that the majority of the species have been recorded, while the inventory cost-effectiveness is maximised.

This study took place in a fragment of Atlantic forest, a highly threatened biodiversity hotspot (Myers *et al.*, 2000; Ribeiro *et al.*, 2009). Spiders are considered a megadiverse order, with over 43 000 species described to date (Platnick, 2013). Their abundance, high species richness and the relative easiness with which they can be collected and sorted into morphospecies make them good model organisms for ecological studies, such as diversity inventories and definition of conservation priority areas (Coddington *et al.*, 1991; Cardoso *et al.*, 2008).

Many spider species occupy specific micro-habitats (Uetz *et al.*, 1999) or are present as adults only at certain periods of the year (e.g. Scharff *et al.*, 2003), especially in seasonal ecosystems. Therefore, they can only be collected in a specific locality by the use of an appropriate method at the right time. Different methods may sample different subsets (or guilds) of spider assemblages, which may result in different species richness values and/or different species composition, even when sampling effort is similar across methods (Scharff *et al.*, 2003; Cardoso *et al.*, 2008). In some situations, however, there is no time or money to apply a wide array of sampling methods. Thus, to sample the maximum of the local species richness while keeping the time and costs involved as low as possible, it is important to find the combination of the most cost-effective season and collecting methods.

In a scenario of high diversity and low budget, typical of developing tropical countries, the selection of sampling methods and the determination of the cost-effective sampling effort are critical issues. In this article, a spider fauna inventory was conducted at a semi-deciduous forest fragment to compare the efficiency of three collecting methods in two different seasons in terms of both observed and predicted spider species richness. Based on the efficiency of the survey relative to the estimated species richness, an evaluation of the worthiness of increasing sampling effort in the area was made. We hope that our results can help other biologists to design future inventories in a more accurate and cost-effective manner.

Materials and methods

Study site and sampling

This study was carried out in Reserva Particular do Patrimônio Natural (RPPN) Mata Samuel de Paula, municipality of Nova Lima, Minas Gerais, southeastern Brazil (20°00′S 43°52′W). The study site is a secondary semi-deciduous tropical forest (Salino *et al.*, 2009) with marked dry (April to September) and rainy (October to March) seasons. The area was sampled once in each season. The rainy season expedition was carried out in October 2006 and the dry season expedition in May 2007. In July 2007, an additional expedition was made to obtain samples that

could not be collected in the dry season due to unexpected torrential rainfall.

Three sampling methods were used: (i) Nocturnal hand collecting (NHC), in which spiders were collected up to five metres away from each side of a 30-m transect during night-time. Each hour of sampling in a transect was considered as a sample. Forty-five NHC samples were obtained in the rainy season and 38 in the dry season. (ii) Beating trays (BT), in which 1 m² trays were placed under the vegetation, which was vigorously hit with a stick until spiders seemed to stop falling down. The spiders obtained in 20 vegetation units were considered a sample. A vegetation unity was composed of a bush, a shrub or a branch haphazardly selected by the collector, which moved freely throughout the area. Each sample lasted 1 hour in average and, therefore, it might be treated as sample-hour in order to be comparable with other methods. Sixty-eight samples were obtained in the rainy season and 58 in the dry season. (iii) Pitfall traps (PT), consisting of plastic cups of 10 cm in diameter containing approximately 250 ml of 70% ethanol. The PT were installed one metre apart from each other in three straight lines, each line separated by approximately 20 m, and left in the field for one week. Each four PT required approximately one hour-person of fieldwork to be installed and removed. Therefore, each four PT were treated as a sample-hour. Thirty-nine samples were obtained in the rainy season and 34 in the dry season. The difference in number of samples between seasons was due to distinct number of collectors available in each expedition and, in the case of PT, due to damage of traps caused by animals.

All specimens were preserved in 70% ethanol. Adult specimens were sorted into morphospecies, identified to the lowest taxonomic level possible and deposited in the arachnid collection of Coleções Taxonômicas da Universidade Federal de Minas Gerais (curator: A.J. Santos).

Species accumulation curves and richness estimates

Estimates 8.0.0 (Colwell, 2006) was used to calculate mean sample-based species accumulation curves from 1000 curves based on random sample addition; and to estimate species richness using the Chao1 and Chao2 estimators. These estimators were chosen because, though relatively more affected by variations in relative abundance distribution, they are more precise than alternative methods (Brose *et al.*, 2003; Walther & Moore, 2005). These procedures were repeated for the total inventory, for each season and for each method separately. The species richness differences between seasons and between methods were preliminarily compared using the confidence interval (CI) of the respective species accumulation curves. Two curves were considered significantly different when the mean of a curve did not overlap the other's CI. To test for differences in species richness independently of differences in sampling effort and abundance, a resampling method developed by Richardson and Richards (2008)

was used. This software tests whether differences in species richness between two or more communities, each with its own total abundance, can be attributed to chance. Species richness between seasons (rainy and dry) and among methods (NHC, PT and BT) was compared, with 10 000 iterations run for each comparison.

Optimised protocol

In order to suggest the best combination of samples per method for an optimised inventory and to verify whether this combination is better than any method alone, we used the software Cobra 1.0 (Cardoso, 2009). This algorithm calculates a species accumulation curve wherein, in each step of the process, it is identified the method that, in average, increases species richness the most. Then, a sample randomly picked from that method is added to build the curve. Therefore, given a number of total samples, it is possible to know the proportion of each sample per method that maximise the species richness (see Cardoso, 2009).

Additional sampling effort

The additional effort that would be necessary to reach higher inventory completeness was calculated using a method proposed by Chao *et al.* (2009). This method estimates the additional sampling effort necessary to reach a determined proportion of the species richness estimated through the Chao1 (in the case of additional individuals necessary) or Chao2 (in the case of additional samples) non-parametric species richness estimators. Calculations were made using a Microsoft Excel® spreadsheet provided as a supplementary file for Chao *et al.* (2009). This was done for (i) the entire inventory; (ii) for each season; and (iii) for each sampling method. Comparisons between season and methods were done in terms of sampling hours. For comparative purposes, the effort needed to increase the completeness of published spider inventories in tropical South America was calculated in terms of individuals, since studies used different methods and some of them were not standardised to hours of sampling. These studies were restricted to those that used more than one sampling method and in which the number of singletons, doubletons, species richness and collected individuals could be accessed: Álvares *et al.* (2004), Podgaiski *et al.* (2007), Ricetti and Bonaldo (2008), Coddington *et al.* (2009) and Bonaldo and Dias (2010).

Species composition and guilds

To compare the spider fauna composition sampled by each method and in each season, a correspondence analysis (CA) was carried out using Past 1.95 (Hammer *et al.*, 2001). All samples with two individuals or less (and one

Table 1. General results and statistics for the entire inventory and for each season and sampling method separately.

Treat.	Mat. Ind.	Samples	Sobs	Exp. Spp.	Sest	Compl.	Add. Ind.	Add. Sam.
Total	2858	282	293	–	416 ± 87*	70*	5709*	–
					425 ± 82[†]	69[†]	–	591[†]
Rainy	2294	152	240	203 ± 6	377 ± 82*	63*	6373*	–
					396 ± 78[†]	60[†]	–	472[†]
Dry	564	130	144	236 ± 6	240 ± 53*	60*	1663*	–
					249 ± 28[†]	58[†]	–	407[†]
BT	835	126	131	172 ± 7	215 ± 44*	61*	2385*	–
					208 ± 42[†]	63[†]	–	319[†]
NHC	1090	83	166	195 ± 7	267 ± 60*	62*	3130*	–
					270 ± 54[†]	62[†]	–	232[†]
PT	933	73	90	182 ± 6	122 ± 27*	73*	1399*	–
					128 ± 24[†]	70[†]	–	128[†]

Treat, treatment; Mat. Ind., mature individuals collected; Samples, total samples obtained; Sobs, observed species richness; Exp. Spp., expected number of species under the assumption of a common species pool [inferred through Richardson and Richards (2008) method], Sest, estimated species richness ± SD, Compl., inventory completeness (%), Add. Ind., additional individuals needed to reach 95% of inventory completeness [estimated through Chao *et al.* (2009) method], Add. Sam., additional samples to reach 95% of inventory completeness [estimated through Chao *et al.* (2009) method]. Treatments: total = entire inventory; rainy = rainy season; dry = dry season; BT = beating trays; NHC = nocturnal hand collecting; PT = pitfall traps.
*Based on the Chao1 estimator.
[†]Based on the Chao2 estimator.

sample with three individuals, each of them a singleton species) were excluded from this analysis since many of these were behaving as strong outliers in a preliminary analysis. Visual inspections of the CI were used to access the significance of differences. Differences in species composition between methods and seasons were analysed through two-way ANOSIM (Clarke, 1993) in Past.

Since taxonomic composition might change across assemblages while functional diversity stays stable (Cardoso *et al.*, 2011b), the methods were also compared in terms of guilds sampled. Species were classified in guilds according to the criterion proposed by Cardoso *et al.* (2011b). The number of species from each guild sampled by each method was counted and put into a contingency table. The null hypothesis of no association between sampling method and guilds was tested with a Chi-square test of independence in Past.

Results

Overall results

Two hundred and eighty-two samples were collected, of which 268 samples contained mature individuals, yielding 2858 adult spiders (1237 females and 1621 males), belonging to 43 families and 293 morphospecies (Table 1). Rare morphospecies represented more than half the total richness: 110 (37.9%) morphospecies were singletons and 49 (16.9%) were doubletons. A complete list of the spider species collected in the area and their abundance per method may be found in Table S1. Neither the species accumulation curves nor the species richness estimators reached an asymptote, and the curves

of singletons and doubletons for the entire inventory did not intersect (Fig. 1). The Chao1 and Chao2 estimators predicted a total richness of approximately 416–425 species, leaving the inventory completeness around 70% (Table 1). The sampling effort needed to reach progressively higher inventory completeness grows exponentially both in terms of individuals and samples (Fig. 2a). Data from other studies also show similar pattern of sampling effort needed to reach progressively higher inventory completeness. For an increment of 10% in completeness it would be necessary around 1.46 times the sampling effort
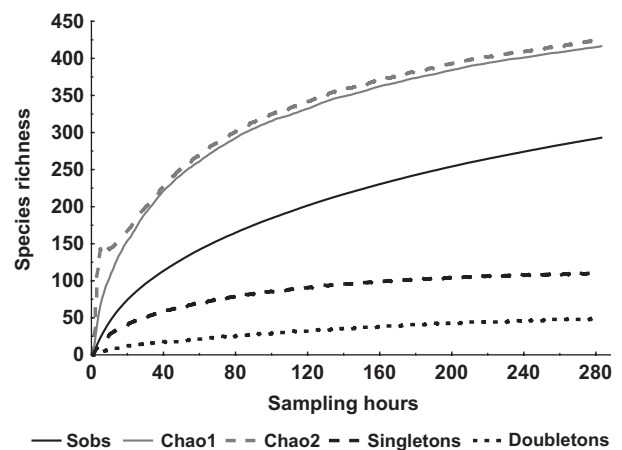

Fig. 1. Sample-based species accumulation curve, estimated species richness (based on Chao1 and Chao2 estimators) and cumulative number of singletons and doubletons for the entire inventory. All curves based on 1000 sequences of random sample addition.
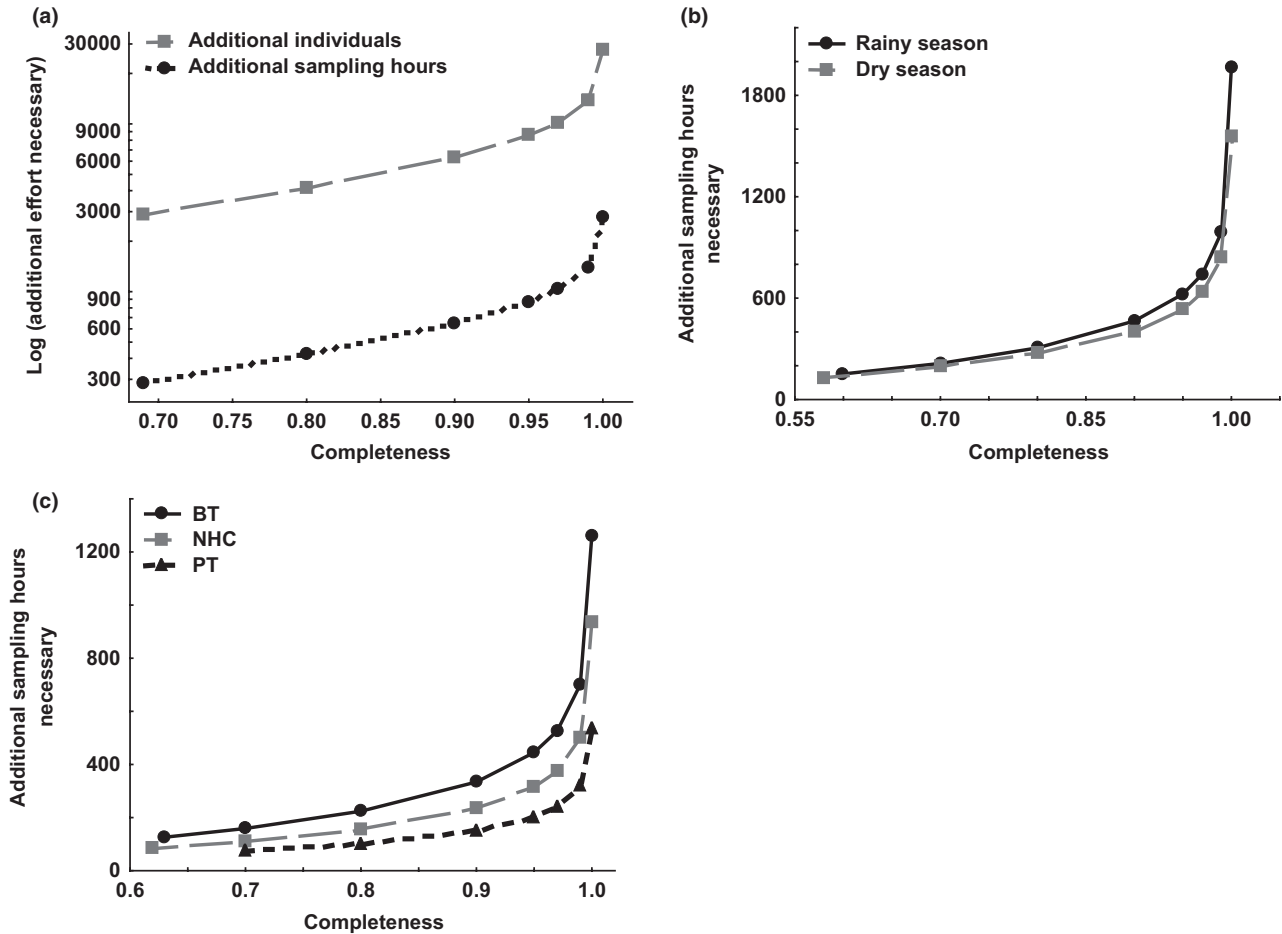
Fig. 2. Additional sampling effort needed to reach different levels of inventory completeness, based on species richness estimators Chao1 (individuals) and Chao2 (samples). In all graphs, the first point represents the actual effort made in this study and its corresponding completeness. (a) Entire inventory. Additional effort measured in terms of additional individuals (dashed line) and samples (solid line) necessary. *Y*-axis in logarithmic scale. (b) Comparison between sampled seasons. Additional effort measured in terms of additional samples necessary. (c) Comparison between sampling methods. Additional effort measured in terms of additional samples necessary. BT, beating trays; NHC, nocturnal hand collecting; PT, pitfall traps.

applied, which would imply a mean increase of only 14% in observed species richness (Table 2).

Season comparisons

Despite the similar sampling efforts in the two seasons, the number of individuals and species collected is much greater in the rainy season (Fig. 3; Table 1). The rainy season rendered much more species per sampling hour than the dry season. The resampling method also indicates differences in species richness between seasons that are not attributable to differences in the abundance (Table 1). Finally, the estimated species richness is also much higher in the rainy season (Table 1). Despite differences in species richness between seasons, both of them showed similar inventory completeness (~60%; Table 1).
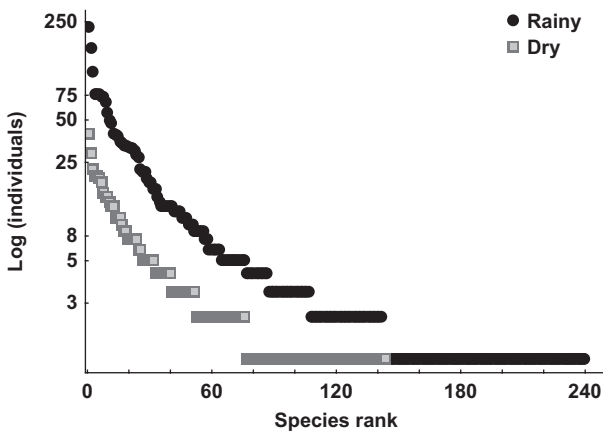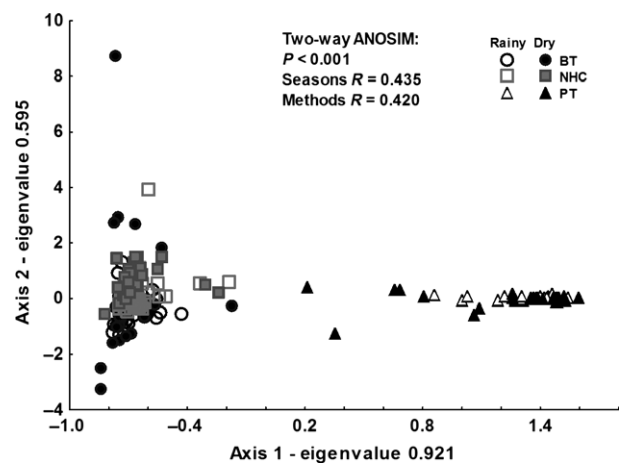
A similar proportion of additional effort would be needed to reach higher inventory completeness in both seasons (Fig. 2b; Table 1).

The two-way ANOSIM showed that the difference in species composition between seasons are probably not due to chance ($R = 0.435$; $P < 0.001$). The rainy season has a higher proportion of exclusive species (149/240 against 53/144 in the dry season) and a lesser proportion of them are rare species (three or less individuals; 114/149, against 49/53 in the dry season). Also, most species which occur in both seasons are more abundant in the rainy season (paired *t*-test, $t = 3.907$, $P < 0.001$). The community equitability is greater in the dry season, which has fewer dominant species (Fig. 4). Despite the differences between seasons, the CA shows that variation in species composition is greater between methods than between seasons (Fig. 5).

Table 2. Sampling effort needed to increase the inventory completeness of South American tropical rain forest spider surveys [estimated through Chao et al. (2009) method].

Study	Mat. Ind.	Sobs	Sest	Compl. Obs.	% Add. Eff.	% Und. Spp.
This study	2858	293	416	0.7	0.44	0.14
Podgaiski et al. (2007)	2946	447	626	0.71	0.39	0.13
Álvares et al. (2004)	2310	223	361	0.61	0.22	0.15
Bonaldo and Dias (2010)	1612	393	561	0.71	0.38	0.16
Ricetti and Bonaldo (2008)	2750	427	627	0.68	0.55	0.15
Coddington et al. (2009)	5965	352	452	0.78	0.61	0.13
Mean ± SD					0.43 ± 0.13	0.14 ± 0.01

Mat. Ind., mature individuals collected; Sobs, observed species richness; Sest, estimated species richness (based on Chao1 estimator); Compl. Obs., observed completeness; % Add. Eff., percentage of additional sampling effort (in terms of Mat. Ind.) needed for an increase of 0.1 in completeness; % Und. Spp., percentage of undetected species expected for an increase in 0.1 in completeness.
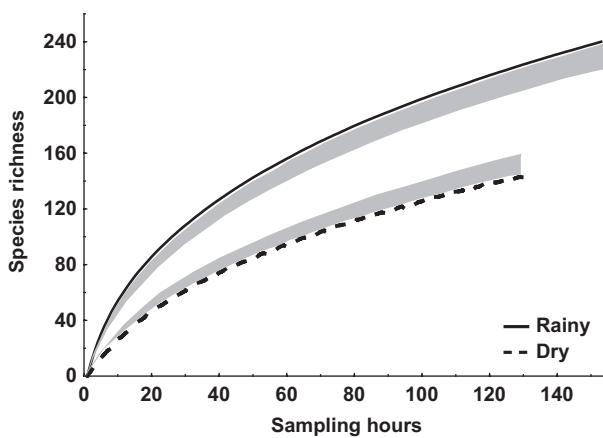
Fig. 3. Sample-based species accumulation curves for the rainy and dry seasons, based on 1000 sequences of random sample addition. Shaded areas represent the lower and upper CI limits of the rainy and dry seasons respectively.

Fig. 5. First two axes of a correspondence analysis of the spider species composition, excluding samples with two individuals or less. CIs omitted for clarity. Samples were grouped by sampling methods (BT + NHC vs. PT) rather by season, although there are equivalent dissimilarities between seasons as revealed by a two-way analysis of similarity (see text for details). The first and second axis account for 3.06 and 1.97% of data variation respectively. BT, beating trays; NHC, nocturnal hand collecting; PT, pitfall traps.

Method comparisons

The species accumulation curves by method showed that NHC was the most efficient, with the highest observed and estimated species richness per sample. The performance of PT and BT was similar (Fig. 6). The re-sampling analysis showed that the differences in observed richness between methods cannot be attributed to the variation in abundance (Table 1).

The application of the method of Cardoso (2009) to our data indicated that the optimum proportion of samples of each method seems to stabilise after approximately 90 total samples (Fig. 7). Therefore, we used the mean proportion between 90 and 150 samples as guide protocol suggested to optimise sampling effort. In an optimum
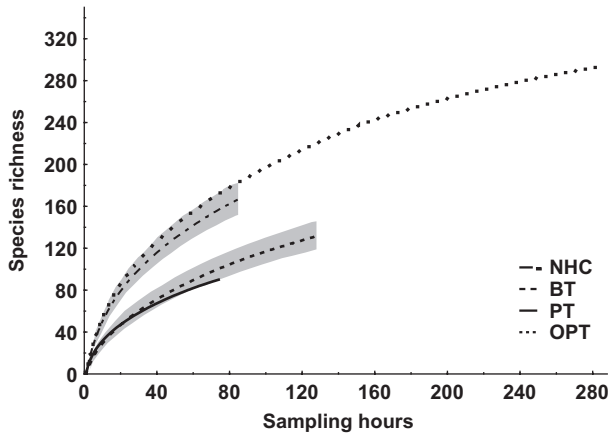

Fig. 4. Species abundance rank of the rainy (black dots) and dry (grey squares) seasons.

Fig. 6. Sample-based species accumulation curves for the three sampling methods and optimised protocol, based on 1000 sequences of random sample addition. Shaded areas represent the lower and upper CI limits of nocturnal hand collecting and beating trays. BT, beating trays; NHC, nocturnal hand collecting; OPT, optimised protocol; PT, pitfall traps.
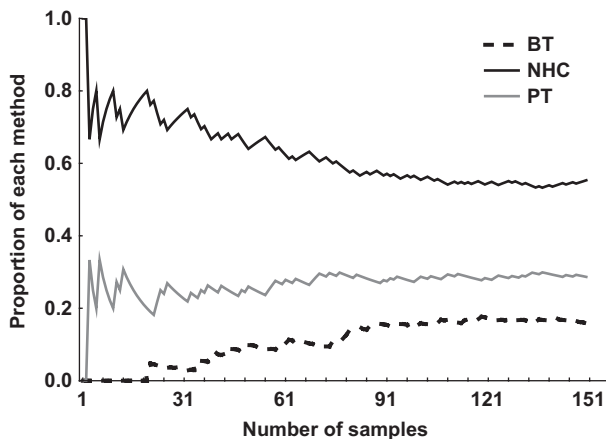

Fig. 7. Optimal combination of methods for maximising species richness. For each given number of samples, a different optimal proportion of samples of different methods was estimated. From ~90 samples on, a stable 'golden proportion' is achieved, in which 55, 29 and 16% of the samples should be of NHC, PT and BT respectively. BT, beating trays; NHC, nocturnal hand collecting; PT, pitfall traps.

protocol, around 55% of total samples should be dedicated to NHC, 29% to PT and 16% to BT. Although this combination of methods optimise effort, the optimised protocol is not better than NHC alone regarding the number of species collected by sample (Fig. 6).

The further sampling effort curve for the methods was similar to seasons and total inventory curves, growing exponentially (Fig. 2c). BT is the method which requires most additional samples to reach its estimated richness, while PT requires the least (Fig. 2c).
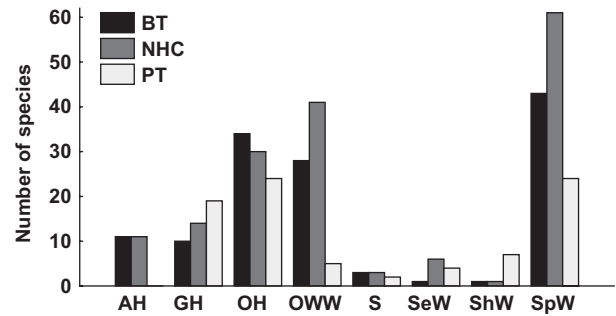
Fig. 8. Number of spider species of each guild sampled by each method. There is a strong association between guilds and sampling methods (independence test: $\chi^2 = 53.464$; d.f. = 14; $P < 0.001$). AH, ambush hunters; GH, ground hunters; OH, other hunters; OWW, orb web weavers; S, specialists; SeW, sensing web weavers; ShW, sheet web weavers; SpW, space web weavers; BT, beating trays; NHC, nocturnal hand collecting; PT, pitfall traps.

Regarding the species composition, samples were grouped in two sets separated along the first axis of the CA, one composed of PT samples and the other composed of NHC and BT samples (Fig. 5). All methods yielded unique species (PT, 65 spp.; BT, 57; NHC, 88), most of them singletons or doubletons, and the differences between the methods were significant according to the two-way ANOSIM ($R = 0.43588$; $P < 0.001$).

The differences in taxonomic composition among methods are reflected in the fact that each of them is better at sampling different spider guilds ($\chi^2_{14}=53.464$; $P < 0.001$; Fig. 8). For instance, orb-web weavers were abundantly sampled by BT and NHC, but not by PT (Fig. 8: OWW), while ground hunters were more abundantly sampled by PT than by the other two methods (Fig. 8: GH).

Discussion

Our results show that the three sampling methods and seasons differ in species richness, and differences in composition between seasons are subtle and mainly due to rare species. Species richness estimators showed that all of the treatments fail to sample all of the predicted richness, but that continuing to collect to reach this goal may be unfeasible due to the steep increase in the additional sampling effort necessary. It is important to notice that estimators usually underestimate true richness, particularly in uneven communities (Brose *et al.*, 2003). Thus, the 70% completeness is conservative and reaching true richness may be even more costly.

Seasonality

If it is desired to sample the greatest number of spider species with less effort in a site, it is preferable to sample

during the rainy season. This season has higher observed and estimated spider richness and abundance when compared with the dry season. With the same sampling effort, an inventory during the rainy season renders much more species than during the dry season.

The existence of many exclusive abundant species in the rainy season suggests that they might: (i) have a reproductive peak during the rainy season; (ii) be extremely inactive during the dry season; and/or (iii) explore different microhabitats in each season. Unfortunately, many of these species have not even been described yet and nothing is known of their natural history (see Cardoso *et al.*, 2011a). Spider inventories carried out in an area which lacks clearly defined rainy and dry seasons failed to find significant differences in species richness and abundance throughout the year (e.g. Dias *et al.*, 2006), while inventories in areas with marked pluvial regimes (as our study area) did found a seasonal difference in spiders abundance and species richness (e.g. Battirola *et al.*, 2004; Cardoso *et al.*, 2007; Podgaiski *et al.*, 2007; Ferreira-Ojeda *et al.*, 2009). It has been demonstrated that insect communities in areas with low and unpredictable rainfall tend to fluctuate more in abundance and the dry season may have a strong effect on the seasonal distribution of species, even in tropical sites (Wolda, 1978a, 1980, 1992). Wolda (1978b) suggested that the rainfall patterns might affect leaf productivity, which in turn might control the abundance of insects that feed on leaves. Once insects are an important resource for spiders, the abundance of the former may strongly influence the abundance of the latter (see Nakamura *et al.*, 2005; Langlands *et al.*, 2006). Habitat structure may also be important in controlling spider abundance (Halaj *et al.*, 1998, 2000). Since the studied area is a semi-deciduous forest, seasonal changes in habitat structure, for instance in the leaf litter depth, can affect spider abundance fluctuation through time. Nevertheless, nothing is known about the magnitude of this seasonal habitat structural changes and how it affects spider community, and this particular aspect deserves future investigation.

In neither of the seasons, it would be worthy to improve sampling effort in order to collect a higher number of species. Our results show that it would be necessary to double the current sampling effort to reach a completeness of 80%, which means an increase of 32% (rainy season) and 38% (dry season) in the observed species richness. If one wishes to continue sampling the area, the best decision would be, however, to do so in the rainy season. Although more samples are needed to reach 95% completeness in the rainy season (472) than in the dry season (407), the number of undetected species in the former is much higher.

Optimal protocol

Nocturnal hand collecting was the most efficient method regarding the number of species and individuals collected per sample. Additionally, in terms of species collected per sample, NHC alone is as efficient as an optimised protocol with the best combination of the three methods. Thus anyone willing to choose only one method for sampling spiders in a particular area should pick NHC. It is important to notice that all methods yielded unique species and sampled different spider guilds. An optimised protocol yields a better sampling of the community composition and it would be as expensive as a protocol that uses only NHC, given the same amount of effective fieldwork. Therefore, it is suggested to dedicate around 55, 29 and 16% of the total sampling hours to NHC, PT and BT respectively. This proposed protocol will permit a better assessment of the spider fauna of an area and a better knowledge of the ecological characteristics of the community.

Further sampling and stopping rule

Increasing sampling effort in order to increase observed species richness seems to be unfeasible. For instance, for BT and NHC, it would be necessary around 1.8 times the applied effort to reach a completeness of 80%, which means an increase of around 30% of the observed richness (35 species for BT and 50 for NHC). Regarding PT, to reach the same completeness it would take 1.3 times the number of samples used. Although this may seem reasonable, it would increase the observed species richness in only 14% (12 species). Gardner *et al.* (2008) estimated at approximately US$ 7 000.00 the cost of an epigeic arachnid (excluding Acari) inventory in a tropical forest, with a completeness (or 'sample representation') of 75% and 3176 individuals collected. The estimated time spent, including field and laboratory work, was around 1200 hours × person. Considering this cost as an approximation of the cost of inventory presented in this study (which received an initial funding, at current rates, corresponding roughly to US$ 9 000.00, salaries not considered), an increase of 50% in the sampling effort (to reach 80% of completeness) would cost additional US$ 4 500.00 and would take additional 600 hours × person. Therefore, if one wish to continue sampling the studied site, it would be more interesting to use other sampling methods capable of reaching different habitats and guilds of spiders, as canopy fogging, for example. This is especially interesting because many of the 'rare' species in one habitat may be, in fact, common in another, rarely sampled habitat (Novotný & Basset, 2000; but see Coddington *et al.*, 2009).

Most surveys in tropical rainforests show inventory completeness similar to the one observed here (60–80%; e.g. Álvares *et al.*, 2004; Ricetti & Bonaldo, 2008; Coddington *et al.*, 2009; Bonaldo & Dias, 2010). This is explained by the high proportion of rare species found in tropical areas (Magurran & Henderson, 2003; Coddington *et al.*, 2009). Since the estimated richness is exponentially related to the proportion of rare species, given an

observed species richness, the completeness will be lower where the number of rare species is higher. The completeness around 70% found here and in these other similar tropical rainforest studies might be a threshold from where the sampling effort necessary to raise effectively the observed richness starts to be worthless. It is also important to notice that these yet undetected species are usually rare or not easily collected by the methods applied (Magurran & Henderson, 2003). Keeping sampling this same area might contribute to provide more specimens of common species, which may be useful for studying population parameters. On the other hand, besides providing common species, sampling another area may contribute to the knowledge of the biodiversity in two more aspects. First, it could improve our knowledge about species distributions by providing new records of widely distributed species from poorly explored localities. Second, new areas are more likely to yield undescribed species, once the more distant (or the more ecologically different) are the areas, the higher is the expected difference in composition. Therefore, in cases where this inventory completeness is reached, it may be worthier to stop sampling the area and invest money and time in other areas rather than going back to field to try to reach the asymptote of the species accumulation curve.

Such a 'stopping-rule' for biological surveys based on the estimated richness has been suggested before (Peterson & Slade, 1998), as it is widely recognised that sampling all species in an area may be unfeasible (Keating *et al.*, 1998). We recognise, however, that it is very difficult to anticipate how much sampling effort should be applied in an area, since it is not possible to know, during fieldwork, whether the threshold inventory completeness has been reached. On the basis of the data presented here, we suggest an initial protocol with 90–150 hours of field work in the rainy season with around 55% of the time dedicated to NHC, 29% dedicated to PT and 16% dedicated to BT.

Conclusions

The urgency of basic biodiversity research and the lack of funding to apply in this discipline lead to the need of designing inventories with the highest cost-effectiveness. Therefore, in order to obtain more spider species with less sampling effort, the NHC method in the rainy season seems to be the best option in a tropical, seasonal rainforest ecosystem, especially for low-budget surveys. If there is the possibility of combining methods, a proportion of 55, 29 and 16% of NHC, PT, BT, respectively, should be applied because they assess different components of the spider fauna.

Finally, combining great sampling effort of different methods in different seasons provides a more complete knowledge on the species diversity of a site, and this should be done whenever there is time and money available. The time to stop sampling an area is, however, also crucial and may prevent ineffective money spent. Here, 70% of the

estimated spider species richness were sampled and increasing sampling effort in the studied area, although promising for sampling undetected species, may be highly ineffective when the costs involved are considered.

Acknowledgements

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12061:

Table S1. List of spider species collected at RPPN Mata Samuel de Paula, Nova Lima, Minas Gerais, Brazil, and respective abundances by sampling season and method.

References

Álvares, E.S.S., Machado, E.O., De Maria, M. & Azevedo, C.S. (2004) Composition of the spider assemblage in an urban forest reserve in southeastern Brazil and evaluation of two sampling method protocols of species richness estimates. *Revista Ibérica de Aracnologia*, **10**, 185–194.

Balmford, A. & Whitten, T. (2003) Who should pay for tropical conservation, and how could the costs be met? *Oryx*, **37**, 238–250.

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B. & Ferrer, E.A. (2011) Has the Earth's sixth mass extinction already arrived? *Nature*, **471**, 51–57.

Battirola, L., Marques, M. & Adis, J. (2004) Aspectos ecológicos da comunidade de Araneae (Arthropoda, Arachnida) em copas da palmeira *Attalea phalerata* Mart. (Arecaceae) no Pantanal de Poconé, Mato Grosso, Brasil. *Revista Brasileira de Entomologia*, **48**, 421–430.

Bonaldo, A.B. & Dias, S.C. (2010) A structured inventory of spiders (Arachnida, Araneae) in natural and artificial forest gaps at Porto Urucu, Western Brazilian Amazonia. *Acta Amazonica*, **40**, 357–372.

Brose, U., Martinez, N.D. & Williams, R.J. (2003) Estimating species richness: sensitivity to sample-coverage and insensitivity to spatial patterns. *Ecology*, **84**, 2364–2377.

Burbidge, A.A. (1991) Cost constraints on surveys for nature conservation. *Nature Conservation: Cost-Effective Biological Surveys and Data Analysis* (ed. by C.R. Margules and M.P. Austin), pp. 3–6. CSIRO, Collingwood, Victoria, Australia.

Cardoso, P. (2009) Standardization and optimization of arthropod inventories – the case of Iberian spiders. *Biodiversity and Conservation*, **18**, 3949–3962.

Cardoso, P., Erwin, T.L., Borges, P.A.V. & New, T.R. (2011a) The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, **144**, 2647–2655.

Cardoso, P., Pekár, S., Jocqué, R. & Coddington, J. (2011b) Global patterns of guild composition and functional diversity of spiders. *PLoS One*, **6**, 1–10.

Cardoso, P., Scharff, N., Gaspar, C., Henriques, S.S., Carvalho, R., Castro, P.H., Schmidt, J.B., Silva, I., Szüts, T., De Castro, A. & Crespo, L.C. (2008) Rapid biodiversity assessment of spiders (Araneae) using semi-quantitative sampling: a case study in a Mediterranean forest. *Insect Conservation and Diversity*, **1**, 71–84.

Cardoso, P., Silva, I., Oliveira, N.G. & Serrano, A.R.M. (2007) Seasonality of spiders (Araneae) in Mediterranean ecosystems and its implications in the optimum sampling period. *Ecological Entomology*, **32**, 516–526.

Chao, A., Colwell, R.K., Lin, C.-W. & Gotelli, N.J. (2009) Sufficient sampling for asymptotic minimum species richness estimators. *Ecology*, **90**, 1125–1133.

Chapin, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. & Díaz, S. (2000) Consequences of changing biodiversity. *Nature*, **405**, 234–242.

Clarke, K.R. (1993) Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Coddington, J.A., Agnarsson, I., Miller, J.A., Kuntner, M. & Hormiga, G. (2009) Undersampling bias: the null hypothesis for singleton species in tropical arthropod surveys. *Journal of Animal Ecology*, **78**, 573–584.

Coddington, J.A., Griswold, C.E., Dávila, D.S., Peñaranda, E. & Larcher, S.F. (1991) Designing and testing sampling protocols to estimate biodiversity in tropical ecosystems. *The Unity of Evolutionary Biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology* (ed. by E.C. Dudley), pp. 44–60. Dioscorides Press, Portland, Oregon.

Colwell, R.K. (2006) EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.0.0. < purl.oclc.org/estimates > 28th February 2011.

Colwell, R.K. & Coddington, J.A. (1994) Estimating terrestrial biodiversity through extrapolation. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*, **345**, 101–118.

Dias, S., Brescovit, A. & Couto, E. (2006) Species richness and seasonality of spiders (Arachnida, Araneae) in an urban Atlantic Forest fragment in Northeastern Brazil. *Urban Ecosystems*, **9**, 323–335.

Ferreira-Ojeda, L., Flórez-D, E. & Sabogal-Gonzáles, A. (2009) Orbweaving spiders of subtropical humid forest of the Sierra Nevada of Santa Marta (Magdalena, Colombia). *Caldasia*, **31**, 381–391.

Funk, V.A., Richardson, K.S. & Ferrier, S. (2005) Survey-gap analysis in expeditionary research: where do we go from here? *Biological Journal of the Linnean Society*, **85**, 549–567.

Gardner, T.A., Barlow, J., Araujo, I.S., Ávila-Pires, T.C., Bonaldo, A.B., Costa, J.E., Esposito, M.C., Ferreira, L.V., Hawes, J., Hernandez, M.I.M., Hoogmoed, M.S., Leite, R.N., Lo-Man-Hung, N.F., Malcolm, J.R., Martins, M.B., Mestre, L.A.M., Miranda-Santos, R., Overal, W.L., Parry, L., Peters, S.L., Ribeiro-Junior, M.A., da Silva, M.N.F., da Silva Motta, C. & Peres, C.A. (2008) The cost-effectiveness of biodiversity surveys in tropical forests. *Ecology Letters*, **11**, 139–150.

Gaston, K.J. & May, R.M. (1992) Taxonomy of taxonomists. *Nature*, **356**, 281–282.

Halaj, J., Ross, D.W. & Moldenke, A.R. (1998) Habitat structure and prey availability as predictors of the abundance and community organization of spiders in western Oregon forest canopies. *Journal of Arachnology*, **26**, 203–220.

Halaj, J., Ross, D.W. & Moldenke, A.R. (2000) Importance of habitat structure to the arthropod food-web in Douglas-fir canopies. *Oikos*, **90**, 139–152.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001) PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, **4**, 1–9.

Hawksworth, D.L. (1995) The resource base for biodiversity assessments. *Global Biodiversity Assessment* (ed. by V.H. Heywood), pp. 545–606. Cambridge University Press, Cambridge, UK.

Keating, K.A., Quinn, J.F., Ivie, M.A. & Ivie, L.L. (1998) Estimating the effectiveness of further sampling in species inventories. *Ecological Applications*, **8**, 1239–1249.

Kier, G., Mutke, J., Dinerstein, E., Ricketts, T.H., Kuper, W., Kreft, H. & Barthlott, W. (2005) Global patterns of plant diversity and floristic knowledge. *Journal of Biogeography*, **32**, 1107–1116.

Kim, K.C. (1993) Biodiversity, conservation and inventory: why insects matter. *Biodiversity and Conservation*, **2**, 191–214.

Langlands, P.R., Brennan, K.E.C. & Pearson, D.J. (2006) Spiders, spinifex, rainfall and fire: long-term changes in an arid spider assemblage. *Journal of Arid Environments*, **67**, 36–59.

Lawton, J., Bignell, D., Bolton, B., Bloemers, G., Eggleton, P., Hammond, P., Hodda, M., Holt, R.D., Larsen, T.B., Mawdsley, N.A., Stork, N.E., Srivastava, D.S. & Watt, A.D. (1998) Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature*, **391**, 72–76.

Magurran, A.E. & Henderson, P.A. (2003) Explaining the excess of rare species in natural species abundance distributions. *Nature*, **422**, 714–716.

Magurran, A.E. & Queiroz, H. (2010) Evaluating tropical biodiversity: do we need a more refined approach? *Biotropica*, **42**, 537–539.

Marques, A.C. & Lamas, C.J.E. (2006) Taxonomia zoológica no Brasil: estado da arte, expectativas e sugestões de ações futuras. *Papéis Avulsos de Zoologia*, **46**, 139–174.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Nakamura, M., Utsumi, S., Miki, T. & Ohgushi, T. (2005) Flood initiates bottom-up cascades in a tri-trophic system: host plant

regrowth increases densities of a leaf beetle and its predators. *Journal of Animal Ecology*, **74**, 683–691.

Novotný, V. & Basset, Y. (2000) Rare species in communities of tropical insect herbivores: pondering the mystery of singletons. *Oikos*, **89**, 564–572.

Oliver, I. & Beattie, A.J. (1996) Designing a cost-effective invertebrate survey: a test of methods for rapid assessment of biodiversity. *Ecological Applications*, **6**, 594–607.

Peterson, A.T. & Slade, N.A. (1998) Extrapolating inventory results into biodiversity estimates and the importance of stopping rules. *Diversity and Distributions*, **4**, 95–105.

Platnick, N.I. (2013) *The World Spider Catalog, Version 13.5*. American Museum of Natural History, New York City, New York. < http://research.amnh.org/iz/spiders/catalog > 14th January 2013.

Podgaiski, L., Ott, R. & Rodrigues, E. (2007) Araneofauna (Arachnida; Araneae) do Parque Estadual do Turvo, Rio Grande do Sul, Brazil. *Biota Neotropica*, **7**, 197–212.

Ribeiro, M.C., Metzger, J.P., Martensen, A.C., Ponzoni, F.J. & Hirota, M.M. (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation *Biological Conservation*, **142**, 1141–1153.

Ricetti, J. & Bonaldo, A.B. (2008) Diversidade e estimativas de riqueza de aranhas em quatro fitofisionomias na Serra do Cachimbo, Pará, Brasil. *Iheringia. Série Zoologia*, **98**, 88–99.

Richardson, J.M.L. & Richards, M.H. (2008) A randomisation program to compare species-richness values. *Insect Conservation and Diversity*, **1**, 135–141.

Salino, A., Heringer, G., Andrade, L.E., Melo, P.H.A. & Almeida, T.E. (2009) Flora/Florístico. *Biodiversidade da Mata Samuel de Paula* (ed. by AngloGold Ashanti Brasil Mineração), pp. 32–133. Belo Horizonte, Brazil, Formato Artes Gráficas. < http://www.anglogoldashanti.com.br/PDFs/Livros/Biodiversidade%20da%20Mata%20Samuel%20de%20Paula.pdf > 18th November 2013.

Scharff, N., Coddington, J.A., Griswold, C.E., Hormiga, G. & Bjørn, P.D.P. (2003) When to quit? Estimating spider species richness in a northern European deciduous forest. *Journal of Arachnology*, **31**, 246–273.

Uetz, G.W., Halaj, J. & Cady, A.B. (1999) Guild structure of spiders in major crops. *Journal of Arachnology*, **27**, 270–280.

Walther, B.A. & Moore, J.L. (2005) The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. *Ecography*, **28**, 815–829.

Wheeler, Q.D. (1995) Systematics, the scientific basis for inventories of biodiversity. *Biodiversity and Conservation*, **4**, 476–489.

Whittaker, R.J., Araújo, M.B., Paul, J., Ladle, R.J., Watson, J.E.M. & Willis, K.J. (2005) Conservation biogeography: assessment and prospect. *Diversity and Distributions*, **11**, 3–23.

Willis, K.J., Araújo, M.B., Bennett, K.D., Figueroa-Rangel, B., Froyd, C.A. & Myers, N. (2007) How can a knowledge of the past help to conserve the future? Biodiversity conservation and the relevance of long-term ecological studies. *Philosophical transactions of the Royal Society of London Series B, Biological Sciences*, **362**, 175–187.

Wolda, H. (1978a) Fluctuations in abundance of tropical insects. *American Naturalist*, **112**, 1017–1045.

Wolda, H. (1978b) Seasonal fluctuations in rainfall, food and abundance of tropical insects. *The Journal of Animal Ecology*, **47**, 369–381.

Wolda, H. (1980) Seasonality of tropical insects. *The Journal of Animal Ecology*, **49**, 277–290.

Wolda, H. (1992) Trends in abundance of tropical forest insects. *Oecologia*, **89**, 47–52.