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Assessing spider species richness and composition in Mediterranean cork oak forests

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ABSTRACT

Semi-quantitative sampling protocols have been proposed as the most cost-effective and comprehensive way of sampling spiders in many regions of the world. In the present study, a balanced sampling design with the same number of samples per day, time of day, collector and method, was used to assess the species richness and composition of a *Quercus suber* woodland in Central Portugal. A total of 475 samples, each corresponding to one hour of effective fieldwork, were taken. One hundred sixty eight species were captured, of which 150 were recorded inside a delimited one-hectare plot; this number corresponds to around 90% of the estimated species richness. We tested the effect of applying different sampling approaches (sampling day, time of day, collector experience and method) on species richness, abundance, and composition. Most sampling approaches were found to influence the species measures, of which method, time of day and the respective interaction had the strongest influence. The data indicated that fauna depletion of the sampled area possibly occurred and that the inventory was reaching a plateau by the end of the sampling process. We advocate the use of the Chao estimators as best for intensive protocols limited in space and time and the use of the asymptotic properties of the Michaelis–Menten curve as a stopping or reliability rule, as it allows the investigator to know when a close-to-complete inventory has been obtained and when reliable non-parametric estimators have been achieved.

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1. Introduction

Despite their fundamental roles in natural ecosystems, ecosystem services and potential use in identifying conservation priority areas, arthropods have largely been ignored in conservation studies (Franklin, 1993; Kremen et al., 1993; New, 1999a,b). When corrected for knowledge bias, data on arthropods show that risk of extinction is as real for them as it is for vertebrates (Thomas and Morris, 1994; MacKinney, 1999; Dunn, 2005). As a consequence of the current data and knowledge deficit, nowadays most conservation studies and decisions necessarily rely on data predominately from plants, birds and mammals, but their function as good indicators for conservation priorities which ought to be relevant for all other living beings still needs to be proved. When this assumption is tested on comparable datasets, at least birds and mammals appear to be rather ineffective in relation to the use of various arthropod taxa (e.g. Lund and Rahbek, 2000, 2002; Lund, 2002).

Standardization and calibration of sampling methods and protocols are fundamental issues for the comparison of sites and the definition of priority areas for conservation (Stork, 1994; Cannon, 1997; Jones and Eggleton, 2000; Kitching et al., 2001; Pereira and Cooper, 2006). The non-existence of such protocols is one of the reasons why arthropods are usually relegated to a secondary place or even not considered in conservation programs (New, 1999a,b). Nevertheless, standardized protocols have already been proposed and used for some arthropods like ants (Agosti and Alonso, 2000) and carabid beetles (Niemela et al., 2000).

Spiders (order Araneae) are one of the most speciose arthropod orders. It has been estimated that one hectare of tropical forest may support between 300 and 800 species of spiders at any given time (Coddington et al., 1991). They are among the most numerous arthropods in many samples in all kinds of habitats (e.g. Basset, 1991; Borges and Brown, 2004).

Spiders have been mostly sampled by methods originally intended for other arthropods, which allows for comparable samples between sites or dates. However, only a very limited number of methods are simultaneously applied in most studies, leaving many species undetected due to the failure of covering most microhabitats. Spiders are very diverse in their ways of life, and sampling them requires a combination of methods. Also, many sampling programs neglect to consider the time of day as an important variable. Neither do they pay any attention to the collectors' experience nor their influence on the composition of the assemblage collected. For spiders, complete protocols or sampling packages that gather information from a series of semi-quantitative methods have proved to be the most effective (Coddington et al., 1991). They achieve the best results with a minimum of effort. Such methods are adequate for capturing a large number of species as well as large numbers of specimens with replicate samples. Exhaustive sampling protocols directed at spiders, mainly based on semi-quantitative methods, have been experimented with in several parts of the world, i.e. Bolivia (Coddington et al., 1991), Peru (Silva and Coddington, 1996), the USA (Coddington et al., 1996; Dobyns, 1997; Toti et al., 2000), Tanzania (Sørensen et al., 2002) and Denmark (Scharff et al., 2003). However, they were never fully standardized and optimized as the effort concerning the various variables, e.g. time of day, was mostly related to the available resources and personal experience, instead of being a consequence of the thorough analysis of previous results. In this sense, the work now presented is also not fully standardized but it is a first step in a series of similar studies that intend to reach a truly standardized and optimized protocol.

The Mediterranean region is considered one of the most important global biodiversity hotspots, the only one partly located in Europe (Cowling and Samways, 1994; Mittermeier et al., 1998; Médail and Quézel, 1999; Myers et al., 2000; Brooks et al., 2002). Shortcuts for the rapid assessment of spider richness in the Mediterranean have already been proposed by using higher taxa surrogates (Cardoso et al., 2004a) or indicator taxa (Cardoso et al., 2004b), but no field protocol has been proposed for the habitats in the region (but see Jiménez-Valverde and Lobo, 2005, 2006).

The objectives of this study were to determine: (1) the feasibility of sampling the entire community over a limited timeframe; (2) the factors (faunal depletion, time of day, collector experience or method) which most strongly affect collecting productivity and sample composition; and (3) the richness estimators which present the best results for intensive semi-quantitative sampling of spiders. This work is part of a larger project that intends to create a standardized and optimized sampling protocol for Mediterranean spiders; ultimately it will be applicable in most habitats of this region.

2. Materials and methods

2.1. Study site

The study was undertaken in a cork oak (*Quercus suber* L.) woodland of about 10 ha, located in the Vale da Rasca, Arrábida Nature Park (PNA), in central Portugal, at an altitude of 60 m (N 38°30.700, W 008°58.800). The canopy density was relatively sparse, with trees varying from 4 to 8 m in height. The understorey was equally sparse although continuous, and dominated by rockrose (*Cistus ladanifer* L.), heather (*Erica arborea* L.) and other herbaceous plant species typical of Mediterranean habitats. The ground was mostly bare and leaf litter cover was sparse.

2.2. Sampling procedures

A single square sampling plot, 100 m wide and hereafter called "sampling plot" or just "plot", was established in the centre of the habitat, at least 100 m from the edge, in order to reduce spatial edge effects. The majority of the sampling was concentrated in this plot with some additional sampling done outside the area yet still inside the habitat.

Sampling followed a balanced design, which means that the same effort was applied to each factor, i.e., sampling day, time of day, collector and method. These independent variables were tested for their effect on the richness, abundance and composition of samples.

A semi-quantitative sampling design was defined based on the time spent collecting each sample. One person-hour of effective fieldwork was used as a sample unit. All collectors carried a stopwatch and the timer was only counting when each collector was actively searching and processing specimens (transferring them to proper tubes, etc.). In total, 475 samples were collected with 10 different methods (the total dataset). A total of 256 samples were taken inside the plot in eight days, 64 for each of 4 selected methods, i.e., aerial searching, ground searching, beating and sweeping (see below for details). By adding 64 pitfall trap samples that were left open for two weeks at the margins of the plot, we obtained the plot-based dataset on which most of the statistics were based, with a total of 320 samples.

Sampling was carried out from May 1 to July 11 in 2004; however, most work was done from June 1 to 10. This timeframe was chosen because it is the most species-rich part of the year in Mediterranean areas (Cardoso et al., 2007). Most of the samples were taken between June 1 and June 4 (hereafter days 1–4) and June 7 and June 10 (hereafter days 5–8). Each collector carried out two day and two night samples inside the plot every day (hereafter D1, D2, N1 and N2). The day samples were carried out between 14:00 and 19:00 h, the night samples between 22:00 and 01:00 h. Headlamps were used for night collecting. Eight collectors were chosen for this protocol. Four were considered to be experienced given that they had previously done semi-quantitative sampling (Cardoso, 2004). The others did not have previous experience on semi-quantitative sampling or on the employed methods; however, all collectors had been involved on projects dealing with spiders.

2.3. *Methods*

Ten sampling methods, considered to cover all microhabitats, were used. The already mentioned five ''main'' methods (hereafter named aerial, beat, ground, sweep and pitfall) have been extensively used in similar protocols so we concentrated our efforts on these. The remaining methods were tested for their ability to complement the former methods.

Aerial – This method consisted in collecting all spiders found above knee-level by hand, forceps, pooter or brush and immediately transferring them into alcohol (88 samples, 64 from inside the plot).

Ground – Similar to the aerial method, but it concentrated on all spiders seen below knee level, including those in hidden sites such as below stones or inside hollow trunks (93 samples, 64 from inside the plot).

Beat – A one by one meter square sheet with a glass fiber frame was used as drop-cloth and a one-meter wooden pole was used for beating the branches of trees, as high as they could be reached. The effective time included all the time spent in the activity, like beating and searching for fallen spiders on the sheet (89 samples, 64 from inside the plot).

Sweep – We used a round sweep net with a diameter of 40 cm, 60 cm deep and with a one-meter handle. Only bushes and tall herbs were swept. All time spent sweeping or searching for dislodged spiders was accounted for (92 samples, 64 from inside the plot).

Brush – Brushing the tree bark with a soft large brush was used to dislodge mainly cryptic spiders (8 samples, none from inside the plot).

Canopy – A sweep net, 40 cm wide and 60 cm deep with a four-meter handle, was used to sweep tree canopies (8 samples, none from inside the plot).

Clip – Branch clipping and thorough search for spiders inside a white bag was used to reveal very small or cryptic spiders that could otherwise be overlooked (8 samples, none from inside the plot).

Sieve – Litter was sifted with a 20 cm diameter sieve, with a mesh size of 1 cm, into a similarly sized container to restrain the sifted material (8 samples, none from inside the plot).

Pitfall – 256 pitfall traps were used next to the delimited plot in a square of 16 by 16 traps. Each trap was 5 meters apart from the nearest traps. A sample was a group of 4 pooled traps, so that we had 64 samples in total. The clumping of traps made individual sampling effort reasonably comparable with time-based samples – the effort applied to rig and collect four traps was roughly equivalent to one person-hour of work – and this strategy reduced stochastic heterogeneity among samples. The traps were set outside the plot to avoid interference with the collectors. Most pitfall traps were left in the field for two weeks, from May 31 to June 14. We used 33 cl plastic cups, which were 8 cm wide at the top and 12 cm high. Two-thirds of each cup was filled with a preservative liquid containing 50% of ethylene glycol, covered with a square wooden plate placed about 2 cm above the ground. In all, 80 samples were collected, of those 64 during the intensive period, 8 during the preceding month (4 samples every 2 weeks), and another 8 during the following month.

Bark – Bark traps were made with 50×50 cm cardboards covering the tree trunks, providing a shelter for many species. Four traps were used forming a single sample. They were placed outside the plot from May 31 to June 14.

2.4. *Statistical analysis*

The sorting and the identification of specimens were carried out by the first author. Whenever possible, identifications were made to the species level; otherwise morphospecies were defined. Only adult specimens were considered for statistical purposes given that juveniles cannot usually be identified as belonging to any species. In many of the analyses we have only considered the plot-based samples, the ones that fully complied with a balanced design, either including or not pitfall traps, depending on the specific question.

In order to evaluate the effectiveness of our approach with regard to attaining a thorough representation of the assemblage, we used EstimateS (Colwell, 2005) to calculate randomized accumulation curves of observed species richness, singletons, doubletons, and several different estimators (Chao 1, Chao 2, First and second order Jackknife and Michaelis–Menten). One thousand randomizations were used. To statistically verify if the randomized curves were approaching the asymptote, still increasing or even decreasing by the end of the sampling process, we determined the slope of the final (right end) segment of the curves. The slope value at any point in the accumulation curves is the inverse of the number of individuals that must be captured in order to increase the

species count by one. Values smaller than 0.001 for the end of the curve can be considered as having reached the asymptote, given that more than 1000 individuals are expected to be needed in order to change the richness value. Slopes are easily calculated for non-linear regression curves, including fitted asymptotic curves that are often used to estimate diversity (e.g. Jimenez-Valverde and Lobo, 2006). However, these calculations are inadequate with regard to non-parametric estimator curves. We had to create a new formula that allowed having a slope value at the end of all curves, including those of estimators:

$$\text{Slope} = 1/(n_s - n_{s\pm1})$$

where n_S = final number of individuals for each curve (corresponding to the total richness value S) and $n_{S\pm1}$ = number of individuals corresponding to the point in the curve where the final single species was added or subtracted to S (corresponding to a richness value of $S \pm 1$). If $S \pm 1$ was larger than S, the result was given a negative sign, reflecting a negative slope.

Inventory completeness, defined as observed species richness in relation to estimated richness, was calculated using the Chao 1 estimate, so that completeness values are comparable with previous studies (Sørensen et al., 2002; Scharff et al., 2003). Sampling intensity, defined as the ratio of specimens to species, was calculated as a crude measure of sampling effort (Coddington et al., 1996).

Four-way ANOVAs were made to look for differences in abundance or species richness per sample (dependent variables) with regard to days, times of day, collectors and methods (independent factors). Factors were analysed without interactions, considering all different hypotheses or with these grouped in two sets, according to their characteristics. Sampling days were grouped in two ''weeks'' (days 1–4 and days 5–8), times of day were grouped in day or night periods, collectors in experienced or inexperienced, and methods in ''active search'' (aerial and ground) or ''tool-based'' (beat and sweep) methods. One ANOVA was made considering interactions, with only time of day and collectors grouped. Abundance data were log $(n + 1)$ transformed in all cases to successfully control the heterogeneity of variance (Zar, 1984). The posthoc Tukey HSD test was used to find which possible pairs were significantly different for each factor studied.

We used an analysis of similarity (ANOSIM by Clarke, 1993; implemented at Seaby and Henderson, 2004) and the Spearman rank correlation index to compare sample compositions of all days, times of day, collectors and methods. Abundance data of species per sample were log $(n + 1)$ transformed for the ANOSIM analyses, so that the most common species did not disproportionably influence the results.

For most of the calculations (except for the ANOSIM) we used the Statistica 6 package (StatSoft Inc., 2001).

3. Results

Overall, the 475 samples included a total 23,704 spiders, of which 7423 (31%) were adults. These specimens represent 168 species of 120 genera and 32 families (Appendix 1). One of these species is new to the Iberian Peninsula: *Lathys simplex* (Simon, 1884). An additional 5 species are new to Portugal: *Drassodes lutescens* (C.L. Koch, 1839), *Leptorchestes peresi* (Simon, 1868), *Philodromus longipalpis* Simon, 1870, *Phrurolithus szilyi* Herman, 1879 and *Theonina cornix* (Simon, 1881). The latter was also the first record of the genus *Theonina* Simon, 1929 for the country. All material is deposited at the Natural History Museum of Denmark, Zoological Museum, University of Copenhagen (ZMUC).

The sampling intensity consisted in 44 individuals per species, with 18% of singletons (Table 1). The species accumulation curve (Fig. 1a) presented a final slope of 0.005 indicating that probably more than 200 additional specimens had to be captured in order to increase the number of observed species. The estimated spider species richness for the site at the time of collecting was around 200 species. This value was not supported by the Michaelis–Menten curve which estimated a value even lower than the observed richness (Fig. 1a). By the end of the randomized accumulation curves, none of the estimators reached an asymptote, but Chao 1 and 2 were very close to it with slopes of 0.002 and 0.001 respectively (Fig. 1a). The singletons and doubletons curves are approaching each other but are not crossing (Fig. 1a).

Inside the plot (including the pitfall traps), 18,017 spiders were collected, with 5548 adults representing 150 species (Table 1). Plot-based sampling intensity was lower than the overall, but the percentage of singletons was also lower. The final slope of the species accumulation curve presented a value of 0.005, which is similar to the curve with the complete data. The estimated richness was around 170 species, but the Michaelis–Menten curve, once again, crosses the observed species curve (Fig. 1b). These two curves crossed at the value of 4854 individuals, equivalent to 280 or 87.5% of the samples. With more individuals the estimator was in fact inferior to the observed richness. It was also around this number of individuals that both Chao 1 and Chao 2 reached the asymptote, maintaining their values of 162 and 166 respectively until

Table 1 – Summary data for the overall captures of this study

	Inside sampling plot		Total
	Pitfall excluded	Pitfall included	
Samples	256	320	475
Individuals (inc. juv.)	3733 (14,685)	5548 (18,017)	7423 (23,704)
Individuals/sample	14.6	17.3	15.6
Species	128	150	168
Species/sample	7.2	7.8	7.3
Sampling intensity	29	37	44
Singletons	30	26	30
Doubletons	17	25	21
Estimates			
Chao 1 ± SD	152 ± 10	162 ± 6	188 ± 9
Chao 2 ± SD	154 ± 11	166 ± 7	192 ± 10
Jackknife 1 ± SD	159 ± 6	179 ± 6	202 ± 6
Jackknife 2	173	184	214
Michaelis–Menten	129	149	164
Completeness	84%	92%	89%

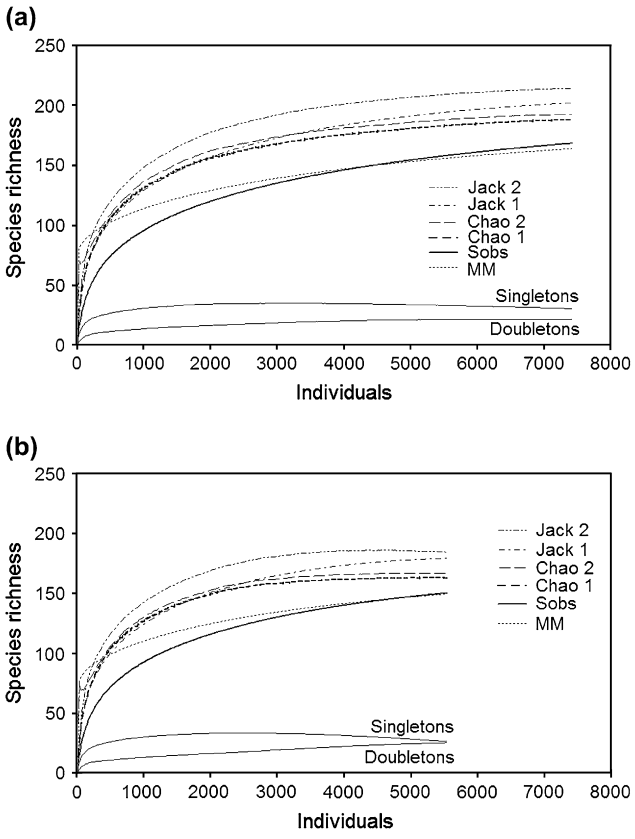

Fig. 1 – Randomized accumulation curves of observed species richness, singletons, doubletons and several estimators for: (a) total captures; and (b) captures inside the sampling plot.

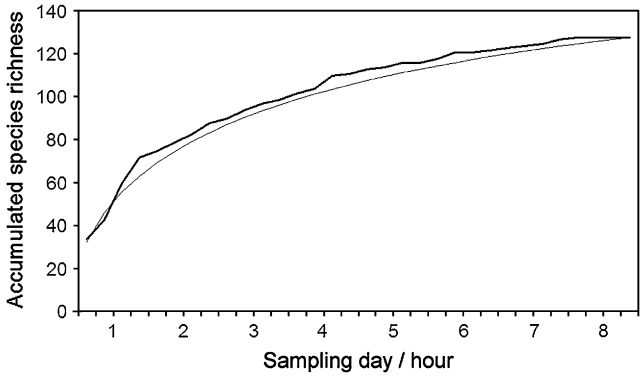

Fig. 2 – Chronological accumulation curve (thick line) of species richness inside the sampling plot, with the randomized curve for comparison (thin line). Subdivisions on the *x*-axis represent one collecting hour (with eight samples each, one by each collector).

the end of the curves (slope values are close to 0 in both cases; Fig. 1b). Jackknife 1 was still rising (slope = 0.002) while Jackknife 2 was decreasing by the end of the curve (slope = − 0.003). The singletons and doubletons curves almost crossed (Fig. 1b).

3.1. *Sampling days*

A non-randomized collecting curve gives a better idea of the gain of species along time than a randomized curve (Fig. 2). In our case a steep increase in richness was observed during the first day, a moderate addition of species happened during the next three days, followed by a much lower addition of species from the fifth to the seventh days and a single species during the last day, in the first hour of sampling.

The abundance and richness per sample significantly decreased along time (Table 2) with the Tukey HSD test revealing differences between the first and the last days in both values. The same pattern was found when comparing the two ''weeks'' (Table 2). However, species composition was similar for all the days, as indicated both by ANOSIM ($p > 0.05$ in all paired comparisons; full data $R = -0.013$, $p = 0.995$) and Spearman correlation results ($p < 0.001$ in all paired comparisons).

3.2. *Time of day*

Night samples revealed more species and specimens than day samples, either when analysing each time of day separately or when establishing day and night periods (Table 3). The Tukey HSD tests revealed highly significant differences between day and night periods ($p < 0.001$ in most paired comparisons, all cases with $p < 0.009$) and no differences when comparing day or night samples among each other ($p > 0.692$ in all cases). However, the number of unique species per time of

Table 2 – Species richness and abundance over time. The different groups revealed by the Tukey HSD test results are indicated for individuals and species per sample

	Day (ANOVA individuals/sample $p = 0.009$, species/sample $p < 0.001$)								Week (ANOVA individuals/sample $p = 0.005$, species/sample $p < 0.001$)	
	1	2	3	4	5	6	7	8	1st	2nd
Samples	32	32	32	32	32	32	32	32	128	128
Individuals	545	452	512	466	492	447	461	358	1975	1758
Individuals/sample	17.0[a]	14.1[a,b]	16.0[a]	14.6[a,b]	15.4[a,b]	14.0[a,b]	14.4[a,b]	11.2[b]	15.4	13.7
Species	72	66	60	65	61	62	63	49	111	100
Unique species	7	4	5	5	3	5	5	1	28	17
Species/sample	8.8[a]	7.6[a,b]	7.4[a,b]	7.9[a,b]	7.2[a,b]	6.7[b]	6.2[b]	6.0[b]	7.9	6.5
Sampling intensity	8	7	9	7	8	7	7	7	18	18

Table 3 – Species richness and abundance found at any time of the day (D1 and D2 are the two consecutive day samples, N1 and N2 the two consecutive night samples). The different groups revealed by the Tukey HSD test results are indicated for individuals and species per sample

	Time of day (ANOVA individuals/sample $p < 0.001$, species/sample $p < 0.001$)				Period (ANOVA individuals/sample $p < 0.001$, species/sample $p < 0.001$)	
	D1	D2	N1	N2	Day	Night
Samples	64	64	64	64	128	128
Individuals	922	834	1021	956	1756	1977
Individuals/sample	14.4[a]	13.0[a]	16.0[b]	14.9[b]	13.7	15.4
Species	80	75	83	89	101	106
Unique species	12	8	9	7	22	27
Species/sample	6.5[a]	6.4[a]	8.2[b]	7.9[b]	6.4	8.0
Sampling intensity	12	11	12	11	17	19

day did not follow the same pattern, only if analysed per period (Table 3). The Spearman correlation index did not detect differences in species composition ($r_s > 0.534$, $p < 0.001$ in all paired comparisons) but the ANOSIM revealed significant differences between day and night samples, in all cases with p-values around 0.01 (full data $R = 0.033$, $p < 0.001$).

3.3. *Collectors*

Only the most and least productive collectors were significantly, albeit marginally, different (Table 4). All other comparisons were insignificant. If collectors were grouped according to their experience, only marginally significant differences occur in both dependent variables, with experienced collectors being the most productive (Table 4). The species composition was similar for all collectors, as indicated both by ANOSIM ($p > 0.05$ in all paired comparisons; full data $R = -0.023$, $p = 1$) and Spearman correlation results ($r_S > 0.515$, $p < 0.001$ in all paired comparisons).

3.4. *Methods*

The ANOVA results were corroborated by all paired comparisons of richness and abundance (all $p < 0.001$ except aerial and ground with $p > 0.257$), revealing that the choice of methods proved to be the most important factor (Table 5). Despite such differences per sample, the total number of species captured by each method was similar, with all methods capturing around 40% of the observed species (Table 5).

The accumulation curves for aerial (Fig. 3a) revealed that the Chao estimators were approaching the asymptote, but given the low number of specimens the slope was relatively steep, 0.018 for both estimators. With about the same number of species and specimens, ground produced curves that were still clearly rising (Fig. 3b), even for the estimators, all with slope values between 0.025 and 0.042. Beat, on the contrary, presented Chao estimators that were close to asymptote (Fig. 3c, slope = 0.003 in both cases) revealing that the estimates were fairly reliable. In this method the Michaelis–Menten curve crossed the observed curve, just like in the overall datasets (Fig. 1). Sweep was the method that captured most species. However, it was the one that presented the lowest ratio of completeness, with a considerably higher estimation of richness than all other methods. It also showed a relatively low sampling intensity, similar to those of aerial and ground (Table 5). The final value of the observed richness slope of sweep (0.024) was also closer to the ones of aerial (0.042) and ground (0.036) than to the values of beat and pitfall (0.008 in both). Nevertheless, Fig. 3d revealed that the Chao estimators were already asymptoting (Chao 1 slope = 0.008, Chao 2 slope = 0.004). Despite the high sampling intensity of pitfall (Table 5), the estimators were all still rising by the end of the curves (Fig. 3e). Even so, the Chao were the ones closer to asymptote (Chao 1 slope = 0.006, Chao 2 slope = 0.009).

Pitfall was the method that captured the highest number of unique species, which is in accordance with the larger differences found in terms of species composition with ANOSIM ($R > 0.727$, $p < 0.001$ in all paired comparisons of pitfall and

Table 4 – Species richness and abundance captured by each collector and with collectors grouped according to experience. The different groups revealed by the Tukey HSD test results are indicated for individuals and species per sample

	Collector (ANOVA individuals/sample $p = 0.003$, species/sample $p = 0.073$)								Experience (ANOVA individuals/sample $p = 0.027$, species/sample $p = 0.038$)	
	1	2	3	4	5	6	7	8	Experienced	Inexperienced
Samples	32	32	32	32	32	32	32	32	128	128
Individuals	717	432	397	486	457	354	458	432	2032	1701
Individuals/sample	22.4[a]	13.5[a,b,c]	12.4[b,c]	15.2[a,b]	14.3[a,b,c]	11.1[c]	14.3[a,b,c]	13.5[a,b,c]	15.9	13.3
Species	69	59	65	64	62	56	55	71	109	104
Unique species	6	3	8	3	5	2	1	6	24	19
Species/sample	8.1[a]	7.5[a,b]	7.2[a,b]	7.5[a,b]	7.1[a,b]	6.2[b]	6.7[a,b]	7.6[a,b]	7.6	6.9
Sampling intensity	10	7	6	8	7	6	8	6	19	16

Table 5 – Species richness and abundance per method

	Method (ANOVA individuals/sample $p < 0.001$, species/sample $p < 0.001$)					Method type (pitfall excluded) (ANOVA individuals/sample $p < 0.001$, species/sample $p < 0.001$)	
	Aerial	Ground	Beat	Sweep	Pitfall	Search	Tool
Samples	64	64	64	64	64	128	128
Individuals	475	508	1878	872	1815	983	2750
Individuals/sample	7.4	7.9	29.3	13.6	28.4	7.7	21.5
Species	57	54	58	68	57	88	87
Unique species	10	5	10	15	22	41	40
Species/sample	5.2	4.4	11.2	8.3	10.1	4.8	9.7
Sampling intensity	8	9	32	13	32	11	32
Singletons	18	17	13	21	12	22	23
Doubletons	8	9	8	9	9	14	10
Estimates							
Chao 1 ± SD	74 ± 9	68 ± 8	67 ± 6	89 ± 11	64 ± 5		
Chao 2 ± SD	74 ± 9	69 ± 8	69 ± 7	85 ± 9	72 ± 9		
Jackknife 1 ± SD	76 ± 5	72 ± 4	72 ± 4	89 ± 5	72 ± 4		
Jackknife 2	86	81	79	99	81		
Michaelis–Menten	64	66	58	72	57		
Completeness	77%	80%	87%	76%	90%		

other methods). Pitfall presented a strong negative correlation with most of the other methods ($r_s < -0.358$, $p < 0.001$ in all paired comparisons), except ground with which it presented a highly positive correlation ($r_s = 0.354$, $p < 0.001$). Nonetheless, the ANOSIM results indicate that all methods captured a different part of the community ($p < 0.001$ in all paired comparisons; full data $R = 0.774$, $p < 0.001$), even pitfall and ground ($R = 0.727$).

3.5. *Methods and time of day interaction*

The ANOVA results revealed a strong interaction between method and time of day, meaning that different methods behaved differently when comparing their day and night catches (for individuals $F_{3,128} = 16.736$, $p < 0.001$, for species $F_{3,128} = 12.464$, $p < 0.001$). Analyses did not show other significant interactions.

Obvious dissimilarities regarding the different levels of productivity of the methods used according to sampling period were present (Table 6). Aerial was remarkably more productive during the night, both in terms of captured specimens and species per sample as well as in absolute numbers (Table 6, $p < 0.001$). Ground at night was only more productive than during the day with regard to the number of specimens ($p = 0.018$) but not concerning the number of species per sample ($p = 0.454$). In absolute terms, though, the number of species captured during the night was considerably higher (Table 6). Beating presented no differences in abundance or richness per sample or even in absolute values (Table 6, $p > 0.842$). Sweeping presented some advantages during night sampling, but this was not significant (Table 6, $p > 0.192$).

For all methods, although day and night abundances of species were always correlated ($r_s > 0.441$, $p < 0.001$), we found significant differences in composition ($p < 0.05$ in all paired comparisons; full data $R = 0.630$, $p < 0.001$). Such differences were higher for aerial and ground, the two active search methods ($R > 0.301$, $p < 0.001$). On the other hand, the difference in composition had intermediate values for sweep ($R = 0.153$, $p < 0.001$) and was almost marginal for beat ($R = 0.093$, $p < 0.05$).

4. Discussion

The spider sampling accomplished for this study was one of the most exhaustive ever carried out that adopted a semi-quantitative methodology (Table 7). It was also the most balanced regarding the number of samples per day, time of day, collector or method. The exhaustiveness of the sampling can probably explain the very low percentage of singletons obtained and the very high completeness. The behaviour of the Chao estimators is noteworthy, as it indicates that the estimated richness is accurate.

A sampling intensity of around 30 specimens per species would probably be enough to provide very high completeness values and estimator accuracy, if executed inside the delimited area of one hectare. Nevertheless, this ratio may not be enough if richness or species/abundance relationships are different, in which case even higher intensities may not provide the same quality of results (Gotelli and Colwell, 2001; Scharff et al., 2003). Dobyns (1997) argues that effort should be restricted in space for optimal results, as a spreading of effort can result in only finding the common species and in missing many cryptic or locally rare species. Besides, for comparative purposes, a similar area surface should always be sampled. The delimitation of the sampled area in semi-quantitative sampling programs is therefore advisable for feasibility, productivity and comparability.

The conjecture that the abundance and even the richness could decrease in the course of the protocol was confirmed.

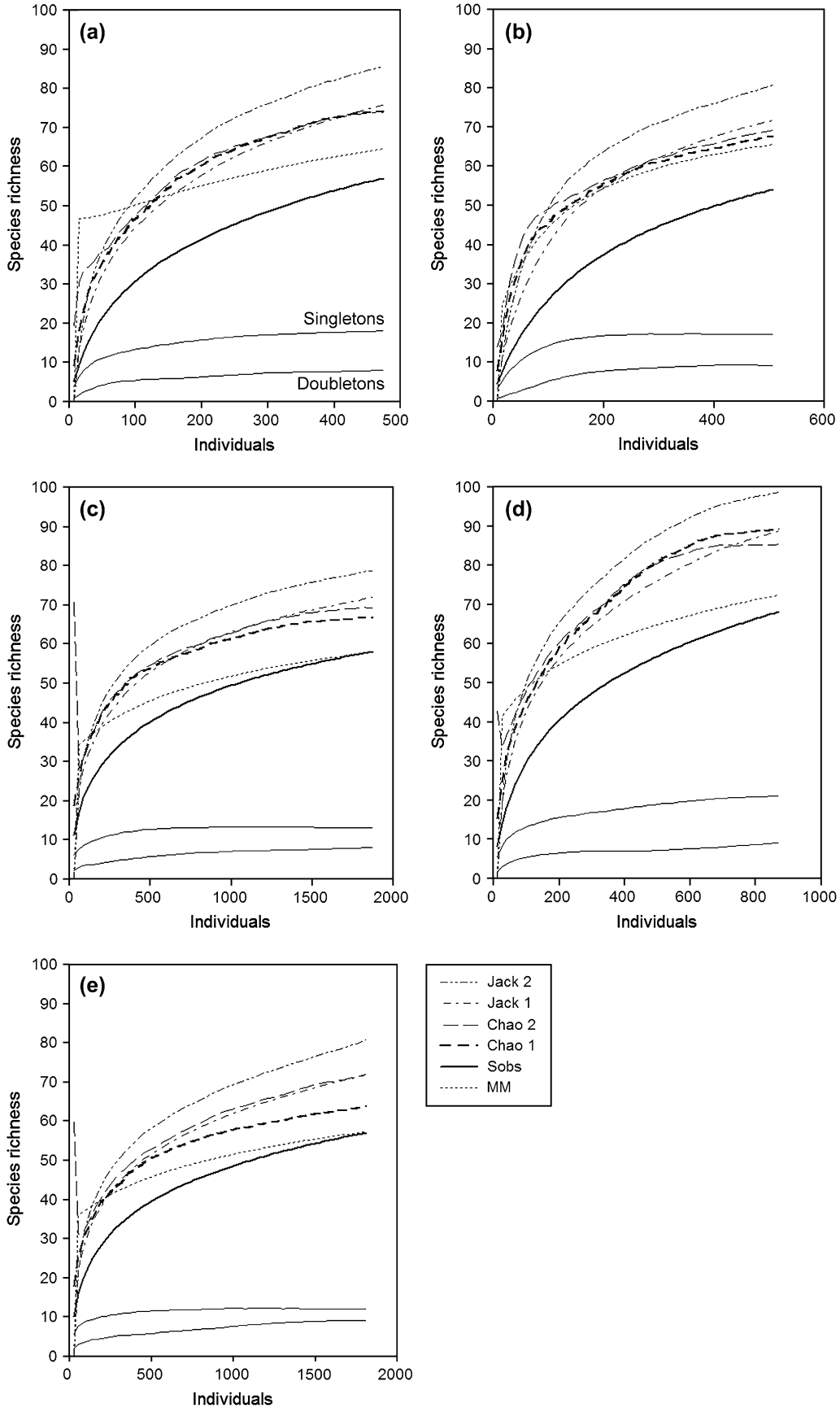

Fig. 3 – Randomized accumulation curves of observed species richness, singletons, doubletons and several estimators for: (a) aerial; (b) ground; (c) beat; (d) sweep; and (e) pitfall methods. Only captures inside the sampling plot are included.

Table 6 – Species richness and abundance captured by each combination of method and time of the day. All percentages are relative to the plot-based sampling, excluding pitfall traps

	Aerial		Ground		Beat		Sweep	
	Day	Night	Day	Night	Day	Night	Day	Night
Samples	32	32	32	32	32	32	32	32
Individuals	120	355	204	304	1030	848	402	470
Individuals/sample	3.8	11.1	6.4	9.5	32.2	26.5	12.6	14.7
Species	37	45	31	43	48	48	48	52
Unique species	5	5	3	11	5	1	9	5
Species/sample	3.0	7.3	3.7	5.0	11.6	10.7	7.5	9.0
Sampling intensity	3	8	7	7	21	18	8	9
Singletons	17	14	12	16	12	12	16	15
Doubletons	8	8	5	8	10	9	5	6
Estimates								
Chao 1 ± SD	52 ± 9	55 ± 7	42 ± 8	56 ± 8	54 ± 4	55 ± 5	68 ± 11	67 ± 9
Chao 2 ± SD	58 ± 11	60 ± 9	44 ± 8	54 ± 6	58 ± 6	54 ± 5	65 ± 10	64 ± 7
Jackknife 1 ± SD	55 ± 4	60 ± 5	44 ± 3	58 ± 4	62 ± 3	60 ± 4	64 ± 4	68 ± 4
Jackknife 2	67	69	51	64	67	63	73	74
Michaelis–Menten	57	51	40	57	50	51	56	59
Completeness	71%	82%	74%	77%	89%	87%	71%	78%

This has not occurred during previous studies employing considerable effort (e.g. Sørensen et al., 2002; Scharff et al., 2003). In such studies, the spider abundance and richness remained constant during the entire sampling process. Only with regard to other invertebrate taxa, like epigean carabids, depletion was ever verified (Digweed et al., 1995). In our case, the decrease of the captured specimens and species along the sampling days may have three possible explanations. Firstly, there can be a real depletion, at least of the number of adults, the most easily captured group due to their larger size and higher mobility when compared to juveniles. Secondly, the most accessible places for sampling may have been progressively exhausted by the employed methods. Thirdly, collectors may have become tired. We believe that the first hypothesis is supported and the third refuted by the fact that the number of juveniles inside the plot did not decrease with time (results not presented). Additionally, the richness outside the plot did not decrease, although the adult abundance did tend to diminish (results not presented). The second hypothesis is plausible but it would have been more obvious if methods that caused more disturbance, like sweeping, were more affected than methods that caused very little disturbance, such as aerial. In fact, all methods showed a significant decrease in similar proportion.

Species richness is often found to be greater during the night (Green, 1999; Sørensen et al., 2002), but this seems to happen mainly in (sub-) tropical forests, and it is less likely to occur in temperate forests (Coddington et al., 1996; Dobyns, 1997; Green, 1999; Scharff et al., 2003). In this Mediterranean habitat, however, spiders seem to behave in the same way as in the tropics, presenting higher abundance and richness during the night.

Collector experience has influenced both the abundance and richness in the studies of Coddington et al. (1996) and Dobyns (1997). Yet several authors have claimed that inexperienced collectors can become statistically indistinguishable from experienced collectors in a very short time (Coddington et al., 1991; Scharff et al., 2003). In Arrábida, all collectors had similar background in the sampling of arthropods, although sometimes not limited to spiders. Among all tested factors, collector or experience was the one that least influenced the results. The differences found should be due to

Table 7 – Comparison of selected semi-quantitative sampling protocols following a similar strategy

Reference	Coddington et al., 1996	Dobyns, 1997	Sørensen et al., 2002	Scharff et al., 2003	This study	This study (inside plot)
Site	U.S.A.	U.S.A.	Tanzania	Denmark	Portugal	Portugal
Samples	133	157	370	149	475	320
Abundance	1629	2842	9096	8710	7423	5548
Species	89	92	170	66	168	150
Sampling intensity	18	31	53	132	44	37
Singletons	29%	20%	19%	29%	18%	17%
Estimated richness (Chao 1)	123	112	197	81	188	162
Completeness	72%	82%	86%	81%	89%	92%

purely stochastic factors and we believe that they are not influencing the results.

All published studies, including this work, found that different methods present significant differences in both richness and abundance of spiders per sample (Coddington et al., 1991, 1996; Dobyns, 1997; Sørensen et al., 2002; Scharff et al., 2003). The higher number of specimens and species per sample found by beat and pitfall compared with the other methods can result in a decrease in complementarity between samples, given that complementarity between two samples (or sites) usually diminishes with the increase in effort (Colwell and Coddington, 1994; Cardoso and Borges, submitted). Consequently, these two methods also present lower final slopes of the species accumulation curves and the estimators. The higher sampling intensity of beat and pitfall is leading to a higher robustness of estimates and higher completeness than aerial, ground or sweep methods.

As expected and concurrent to previous works (Coddington et al., 1996; Toti et al., 2000; Sørensen et al., 2002), methods for collecting in similar microhabitats are found to be closer in species composition (correlation and similarity analyses). Aerial, beat and sweep are directed towards vegetation spiders, while ground and pitfall concentrate on epigean spiders. Nevertheless, no two methods are similar, all of them present unique species and none of the five main methods could have been left out of the survey.

Regarding the five methods that were used only outside the delimited plot with a low effort, only canopy sweeping provided unique species, *Araneus sturmi* (Hahn, 1831) and *Oedothorax fuscus* (Blackwall, 1834). Both are singletons in this study and had already been captured by the first author on other occasions while using different methods in varying habitat types. In the present study, they were most probably captured in marginal areas of the habitat and can be considered as false rare due to spatial edge effects. Although it did not result in providing unique species in this study, a sieve can be used in some types of habitat while doing ground searching. This can be potentially useful in northern Mediterranean forests where a deep leaf litter may hinder the sighting of small spiders. This is not the case in most of the Mediterranean habitats, however, where leaf litter is scarce and the method is not productive (e.g. Jiménez-Valverde and Lobo, 2005). Moreover, the spiders captured by sieving can probably be captured by pitfall traps, a method that proved to be very productive, with a very high completeness. Brush, bark and clip, besides providing very few specimens, did not provide different species which makes them dispensable.

4.1. *Estimator performance*

The behavior of the Michaelis–Menten equation that we have found for our data, crossing the observed species curve (Fig. 1), was already mentioned by different authors (Soberón and Llorente, 1993; Colwell and Coddington, 1994; Magurran, 2004). According to Colwell and Coddington (1994), it is caused by the Eadie-Hofstee transformation of the original formula, implemented in EstimateS software. Some species accumulation curves that have a rapid initial increase force this transformation to produce estimates that fall below the observed values for a large number of samples.

Henderson and Magurran (in Magurran, 2004) have mentioned, but not demonstrated, a possible use for this particular property of the estimator: to use it as a stopping rule. They suggest that when the Michaelis–Menten curve intercepts the observed species accumulation curve, the non-parametric estimators should have reached the asymptote and can be used as reliable estimates of species richness. If the Michaelis–Menten curve has not intercepted the observed curve, the non-parametric estimators are probably underestimating the true richness of the area. This was verified inside the sampling plot (Fig. 1b). With all data included (Fig. 1a), the Michaelis–Menten crossed the observed curve even before the other estimators reached an asymptote. These data, however, are much more heterogeneous, with some samples taken on the margins of the habitat or out of the plot-based collection time-frame. The same situation seems to occur with the two methods that captured higher abundances, beat and pitfall. In both, the Chao estimators almost reached an asymptote and the Michaelis–Menten is just crossing the observed species accumulation curve. However, the reason why the Michaelis–Menten presents this valuable property is obscure and more tests must be performed in order to know if this behavior is repeated in other sites or habitats.

We continue to advocate the use of Chao 1 as the best estimator for short term semi-quantitative sampling programs in delimited, relatively uniform areas. It provided the lowest and most realistic estimate, as possible faunal depletion occurred and only one new species was added on the last day. It also reached the asymptote or low slope values more often than the jackknife estimators did. Chao 2 can be a good alternative for sample-based estimations (incidence data) with behavior and values very similar to Chao 1.

4.2. *Conclusions*

It is important to stress that the results and conclusions presented in this work where derived from a single sampling site. However, results obtained for different habitats in Portugal (Cardoso et al., in press) do support the conclusions.

The chosen time of the year for sampling matches the annual species richness peak in Portugal and probably in the entire Mediterranean region, independently of the habitat (Cardoso et al., 2007). Short term sampling during this period, if done in a thorough way, is likely to capture around 50% of the annual spider diversity of a site in the Iberian Peninsula (Jiménez-Valverde and Lobo, 2006; Cardoso et al., 2007) and probably the rest of Europe (e.g. Scharff et al., 2003). Although this is a coarse estimate, the single hectare sampled here may be found to harbour more than 300 species over a full year. This corresponds to almost half of the approximately 750 species known to occur in Portugal (Cardoso, 2007). However, this last number still falls short of the ''guesstimated'' 1100–1300 species of the country (Cardoso, unpublished data).

Our results seem to largely corroborate previous work done with semi-quantitative sampling of spiders (Coddington et al., 1991, 1996; Sørensen et al., 2002; Scharff et al., 2003). This includes the major importance of method and time of day, the low effect of experience in collecting, the low return on investment in more specialized collecting techniques, the importance of intensity and completeness statistics and the

adequacy of the Chao estimators. It seems therefore that the design and implementation of sampling protocols for spiders is approaching scientific maturity. Only with a much more solid knowledge of the distribution and status of species that the implementation of these protocols will provide, will it be possible, even if only in the distant future, to acknowledge arthropods in general and spiders in particular, the bulk of biodiversity, in conservation programs.

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Appendix 1

List of the captured species grouped by family and respective adult abundances

Agelenidae (6 spp.)	253
Malthonica lusitanica Simon, 1898	207
Tegenaria aff. *ramblae*	3
Tegenaria atrica C. L. Koch, 1843	1
Tegenaria feminea Simon, 1870	2
Tegenaria montigena Simon, 1937	37
Tegenaria picta Simon, 1870	3
Anyphaenidae (1 spp.)	6
Anyphaena sabina L. Koch, 1866	6
Araneidae (14 spp.)	202
Agalenatea redii (Scopoli, 1763)	2
Araneus sturmi (Hahn, 1831)	1
Araniella cucurbitina (Clerck, 1757)	12
Cyclosa algerica Simon, 1885	36
Cyclosa insulana (Costa, 1834)	1
Cyrtophora citricola (Forskål, 1775)	1
Gibbaranea bituberculata (Walckenaer, 1802)	3
Gibbaranea gibbosa (Walckenaer, 1802)	4
Hypsosinga sanguinea (C.L. Koch, 1844)	3
Leviellus kochi (Thorell, 1870)	44
Mangora acalypha (Walckenaer, 1802)	30
Neoscona subfusca (C.L. Koch, 1837)	1
Nuctenea umbratica (Clerck, 1757)	3
Zilla dioidia (Walckenaer, 1802)	61
Clubionidae (4 spp.)	14
Clubiona comta C.L. Koch, 1839	9
Clubiona diniensis Simon, 1878	2
Clubiona genevensis L. Koch, 1866	2
Clubiona leucaspis Simon, 1932	1
Corinnidae (3 spp.)	299
Liophrurillus flavitarsis (Lucas, 1846)	259
Phrurolinillus lisboensis Wunderlich, 1995	38
Phrurolithus szilyi Herman, 1879	2

Appendix (*continued*)

Dictynidae (6 spp.)	234
Dictyna civica (Lucas, 1850)	94
Lathys humilis (Blackwall, 1855)	2
Lathys simplex (Simon, 1884)	4
Lathys sp.	1
Marilynia bicolor (Simon, 1870)	1
Nigma puella (Simon, 1870)	132
Dysderidae (5 spp.)	308
Dysdera fuscipes Simon, 1882	3
Dysdera lusitanica Kulczynski, 1915	61
Dysdera machadoi Ferrández, 1996	5
Harpactea subiasi Ferrández, 1990	99
Rhode scutiventris Simon, 1882	140
Filistatidae (1 spp.)	14
Pritha cf. *nana* (Simon, 1868)	14
Gnaphosidae (20 spp.)	458
Callilepis concolor Simon, 1914	58
Drassodes lapidosus (Walckenaer, 1802)	2
Drassodes lutescens (C.L. Koch, 1839)	2
Haplodrassus sp.	1
Leptodrassus femineus (Simon, 1873)	2
Leptodrassus simoni Dalmas, 1919	5
Micaria brignolii (Bosmans and Blick, 2000)	3
Micaria dives (Lucas, 1846)	136
Micaria formicaria (Sundevall, 1831)	1
Nomisia excerpta (O.P.-Cambridge, 1872)	76
Phaeocedus sp.	2
Poecilochroa senilis (O.P.-Cambridge, 1872)	3
Scotophaeus blackwalli (Thorell, 1871)	3
Trachyzelotes fuscipes (L. Koch, 1866)	58
Trachyzelotes holosericeus (Simon, 1878)	2
Zelotes denisi Marinaro, 1967	23
Zelotes medianus Denis, 1935	57
Zelotes sp. 1	12
Zelotes sp. 2	1
Zelotes thorelli Simon, 1914	11
Hahniidae (1 spp.)	2
Hahnia candida Simon, 1875	2
Linyphiidae (23 spp.)	1588
Araeoncus humilis (Blackwall, 1841)	8
Diplocephalus sp.	16
Erigone dentipalpis (Wider, 1834)	2
Frontinellina frutetorum (C.L. Koch, 1834)	21
Gongylidiellum vivum (O.P.-Cambridge, 1875)	2
Hybocoptus corrugis (O.P.-Cambridge, 1875)	202
Lepthyphantes minutes (Blackwall, 1833)	2
Linyphiidae sp. 1	1
Linyphiidae sp. 2	10
Meioneta pseudorurestris (Wunderlich, 1980)	156
Microctenonyx subitaneus (O.P.-Cambridge, 1875)	1
Microlinyphia pusilla (Sundevall, 1830)	1
Microlinyphia sp.	1
Neriene furtiva (O.P.-Cambridge, 1871)	1
Oedothorax fuscus (Blackwall, 1834)	1
Ostearius melanopygius (O.P.-Cambridge, 1879)	2
Palliduphantes stygius (Simon, 1884)	10
Prinerigone vagans (Audouin, 1826)	5
Sintula sp.	3
Styloctetor romanus (O.P.-Cambridge, 1872)	1
Tenuiphantes tenuis (Blackwall, 1852)	1139
Theonina cornix (Simon, 1881)	1
Tiso vagans (Blackwall, 1834)	2

(*continued on next page*)

Appendix (*continued*)	
Liocranidae (3 spp.)	20
Agroeca inopina O.P.-Cambridge, 1886	4
Apostenus sp.	4
Scotina celans (Blackwall, 1841)	12
Lycosidae (3 spp.)	113
Alopecosa albofasciata (Brullé, 1832)	1
Arctosa sp.	11
Pardosa hortensis (Thorell, 1872)	101
Mimetidae (1 spp.)	73
Ero aphana (Walckenaer, 1802)	73
Miturgidae (1 spp.)	5
Cheiracanthium sp.	5
Nemesiidae (3 spp.)	12
Iberesia machadoi Decae and Cardoso, 2005	5
Nemesia athiasi Franganillo, 1920	4
Nemesia sp.	3
Oecobiidae (1 spp.)	7
Uroctea durandi (Latreille, 1809)	7
Oonopidae (1 spp.)	10
Oonops sp.	10
Oxyopidae (1 spp.)	77
Oxyopes lineatus Latreille, 1806	77
Philodromidae (4 spp.)	454
Philodromus longipalpis Simon, 1870	419
Philodromus pulchellus Lucas, 1846	4
Philodromus rufus Walckenaer, 1826	27
Tibellus oblongus (Walckenaer, 1802)	4
Pholcidae (1 spp.)	1
Pholcus opilionoides (Schrank, 1781)	1
Salticidae (18 spp.)	540
Aelurillus luctuosus (Lucas, 1846)	5
Ballus chalybeius (Walckenaer, 1802)	64
Chalcoscirtus infimus (Simon, 1868)	15
Cyrba algerina (Lucas, 1846)	52
Euophrys rufibarbis (Simon, 1868)	47
Euophrys sp.	6
Euophrys sulphurea (L. Koch, 1867)	1
Evarcha jucunda (Lucas, 1846)	14
Heliophanus cupreus (Walckenaer, 1802)	4
Heliophanus rufithorax Simon, 1868	9
Icius hamatus (C.L. Koch, 1846)	190
Leptorchestes peresi (Simon, 1868)	1
Macaroeris nidicolens (Walckenaer, 1802)	13
Phlegra bresnieri (Lucas, 1846)	1
Pseudeuophrys erratica (Walckenaer, 1826)	14
Salticidae sp.	3
Salticus scenicus (Clerck, 1757)	80
Salticus zebraneus (C.L. Koch, 1837)	21
Scytodidae (1 spp.)	19
Scytodes velutina Heineken and Lowe, 1832	19
Segestriidae (1 spp.)	4
Segestria florentina (Rossi, 1790)	4
Sparassidae (1 spp.)	17
Olios argelasius (Walckenaer, 1805)	17
Tetragnathidae (2 spp.)	21
Metellina merianae (Scopoli, 1763)	2
Tetragnatha obtusa C.L. Koch, 1837	19
Theridiidae (26 spp.)	1560
Achearanea lunata (Clerck, 1757)	1

Appendix (*continued*)	
Anelosimus pulchellus (Walckenaer, 1802)	202
Anelosimus vittatus (C.L. Koch, 1836)	6
Argyrodes argyrodes (Walckenaer, 1842)	20
Dipoena melanogaster (C.L. Koch, 1837)	58
Dipoena sp.	1
Episinus maculipes Cavanna, 1876	58
Episinus truncatus Latreille, 1809	1
Euryopis sp.	24
Keijia tincta (Walckenaer, 1802)	121
Kochiura aulica (C.L. Koch, 1838)	2
Lasaeola testaceomarginata Simon, 1881	1
Paidiscura pallens (Blackwall, 1834)	332
Phoroncidia paradoxa (Lucas, 1846)	4
Rhomphaea nasica (Simon, 1873)	4
Simitidion simile (C.L. Koch, 1836)	151
Steatoda nobilis (Thorell, 1875)	7
Theridiidae sp.	2
Theridion blackwalli O.P.-Cambridge, 1871	3
Theridion hemerobium Simon, 1914	3
Theridion melanurum Hahn, 1831	1
Theridion mystaceum L. Koch, 1870	276
Theridion nigropunctatum Lucas, 1846	3
Theridion pinastri L. Koch, 1872	7
Theridion sp.	260
Theridion varians Hahn, 1833	12
Thomisidae (8 spp.)	248
Misumena vatia (Clerck, 1757)	34
Runcinia grammica (C.L. Koch, 1837)	36
Synema globosum (Fabricius, 1775)	46
Thomisus onustus Walckenaer, 1805	15
Tmarus piger (Walckenaer, 1802)	8
Tmarus sp.	1
Tmarus staintoni (O.P.-Cambridge, 1873)	104
Xysticus tortuosus Simon, 1932	4
Uloboridae (4 spp.)	27
Hyptiotes paradoxus (C.L. Koch, 1834)	5
Polenecia producta (Simon, 1873)	14
Uloborus plumipes Lucas, 1846	3
Uloborus walckenaerius Latreille, 1806	5
Zodariidae (2 spp.)	822
Zodarion atlanticum Pekár and Cardoso, 2005	806
Zodarion styliferum (Simon, 1870)	16
Zoridae (1 spp.)	2
Zora spinimana (Sundevall, 1833)	2
Zoropsidae (1 spp.)	13
Zoropsis spinimana (Dufour, 1820)	13

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