

## **DETERMINING A COMBINED SAMPLING PROCEDURE FOR A RELIABLE ESTIMATION OF ARANEIDAE AND THOMISIDAE ASSEMBLAGES (ARACHNIDA, ARANEAE)**

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**ABSTRACT.** As the disappearance of species accelerates, it becomes extremely urgent to develop sampling protocols based on efficient sampling methods. As knowledge of the Iberian spider fauna is extremely incomplete, it is becoming necessary to facilitate reliable and complete species richness inventory collection. In this work the results from six sampling methods (sweeping, beating, pitfall traps, hand collecting at two different heights and leaf litter analysis) in three habitats with different vegetation structure are compared for the inventory of Araneidae and Thomisidae in 1 km<sup>2</sup> sampling plots. A combination of sweeping, beating and pitfall trapping prove to be necessary to achieve a reliable inventory of these two spider families. Hand collecting above knee level contributes to the improvement of the protocol in certain habitats where araneids, concentrated in patches of suitable vegetation, are easy to find.

**RESUMEN.** A medida que se acelera la desaparición de las especies se hace más urgente el desarrollo de protocolos de muestreo basados en métodos eficientes. El conocimiento de la aracnofauna ibérica es bastante escaso, por lo que es necesario desarrollar inventarios fiables y tan completos como sea posible, de una manera rápida y sencilla. En el presente trabajo se comparan seis métodos diferentes de muestreo (manguero, batido, trampas de interceptación, captura directa a dos alturas distintas y análisis de hojarasca) para el inventariado de las familias Araneidae y Thomisidae en parcelas de 1 km<sup>2</sup>, estudiando su comportamiento en tres hábitats con diferente complejidad estructural de la vegetación. Los resultados muestran que para conseguir inventarios fiables de estas dos familias es necesaria la combinación del manguero, batido y de las trampas de caída. En los hábitats en los que la localización de los araneidos es sencilla debido a que se concentran en parches de vegetación concretos, la captura directa a una altura por encima de las rodillas contribuye a mejorar el protocolo.

**Keywords:** Species richness inventory, sampling methods, efficiency, complementarity

Loss of biodiversity, one of the greatest environmental problems (Wilson 1988; May et al. 1995), the outcome of the accelerating destruction of ecosystems, means that many species will be eradicated while still undiscovered or unstudied. Protecting biodiversity implies protecting terrestrial arthropods, a group poorly known but comprising around 80% of the Earth's species and including those denominated as hyperdiverse (Hammond 1992). These groups are the least understood, yet contribute most to the planet's biotic diversity. Conservation of biological diversity requires detailed information on the geographic distribution of organisms. In the case of arthropods, as this information is almost impossible to acquire in the medium-term by means of field sampling (Ehrlich & Wilson 1991; Williams & Gaston 1994), the utilization of

predictive model techniques may be the only possible way to estimate the distribution of biodiversity attributes (Margules et al. 1987; Iverson & Prasad 1998; Guisan & Zimmermann 2000; Lobo & Martín-Piera 2002; etc). However, application of these predictive methods requires reliable biological information; when this is lacking, the design of specific sampling protocols for each taxonomic group that gather the maximum information, most cost-effectively, becomes essential.

About 36,000 species of the order Araneae have been described, while the total number is estimated at between 60,000 and 170,000 (Coddington & Levi 1991; Platnick 1999). This is one of the most diversified orders (Coddington & Levi 1991) and offers the greatest potential to help regulate terrestrial arthropod populations (Marc et al. 1999). Ara-

neids, one of the most successful spider families (approximately 2,600 species; Foelix 1996), are relatively easy to detect due to their size, coloration and their orb webs. Vegetation structure seems to be the most important parameter in determining their presence (Wise 1993). Unlike the araneids, thomisids (crab spiders) do not use webs to capture prey; instead they ambush prey from flowers or leaves (Wise 1993), where their cryptic coloration allows them to go unnoticed. Some genera, like *Xysticus* and *Ozyptila*, are eminently edaphic, capturing prey among leaf litter and herbaceous vegetation.

Arachnological tradition is sorely lacking in the Iberian Peninsula, and spider distribution is extremely poorly understood (1,180 recorded species; Morano 2002). Only in the province of Aragón is there a recent catalogue of arachnological fauna (Melic 2000); the rest of the Iberian catalogues include out-dated records, most of doubtful quality and with erroneous data (Melic 2001). So, it is necessary to augment taxonomic and distributional data on Iberian spiders by using effective and standardized sampling protocols, the design of which involves overcoming some difficulties. As spiders' life history, behavior and morphologic, physiological and ecological adaptation vary widely (Turnbull 1973), sampling method effectiveness depends on the nature of the taxonomic group (Canard 1981; Churchill 1993; Coddington et al. 1996; Costello & Daane 1997; Churchill & Arthur 1999). Furthermore, it must be kept in mind that the effectiveness of the method also depends on the environment (Canard 1981). Thus, in order to inventory reliably and completely, the design of the sampling protocol should combine various sampling methods, selecting the methods promising maximum information and complementarity for each environment and taxonomic group (Coddington et al. 1996; Green 1999; Sørensen et al. 2002). In this work, several sampling methods for Araneidae and Thomisidae species are compared, in habitats with distinct vegetation complexity, in order to determine which combination captures the maximum number of species with the minimum number of sampling techniques.

#### METHODS

**Study site.**—The study was carried out from 2 May–14 June 2002 in three localities

in the Comunidad de Madrid (central Spain), with vegetation differing in structural complexity as follows: 1) A grassland zone subjected to intense pasturing pressure, with small shrub patches, at 980 m elevation in the municipality of Colmenar Viejo (latitude 40.69, longitude  $-3.77$ ). Its potential vegetation is the holm-oak forest (supra-mesomediterranean-siliceous series of *Quercus ilex rotundifolia*; Rivas-Martinez, 1987). 2) An extensive and dense zone of shrub located in El Berrueco (latitude 39.97, longitude  $-3.53$ ), at 940 m elevation. The area belongs to the same vegetation series as the former (Rivas-Martinez, 1987); nevertheless, human activity has caused the original vegetation to be replaced by the *Cistus ladanifer* series, with patches of *Lavandula pedunculata* and *Thymus* spp. 3) A Holm-oak forest zone in Cantoblanco (latitude 40.51, longitude  $-3.65$ ) at an elevation of 700 m, composed of some tall (6–8 m) specimens of *Quercus ballota*, though the majority of the trees are between 3–4 m tall. An old plantation of *Pinus pinea*, which dates from the 1930s, occupies one part of the forest.

**Sampling methods.**—In each habitat a 1 km<sup>2</sup> sampling plot divided into 2,500 subplots of 400 m<sup>2</sup> was established; 20 of these subplots were chosen at random, and a sampling effort unit carried out in each. For the capture of species in these two families, six cheap, easy and widely used sampling methods were employed: sweeping, beating, pitfall traps, above-knee-level visual search, below-knee-level visual search, and leaf litter analysis. A sampling effort unit was defined as one of the following: 1) A one-person sweep of the herbaceous vegetation and shrub during 15 minutes. The opening of the sweep net was 37 cm in diameter, and it was emptied at regular intervals to avoid loss and destruction of the specimens. 2) A one-person beating of bushes and small trees and branches during 15 minutes with a heavy stick; the specimens fell on a 1.25 × 1.25 m white sheet. In cases where the structure of the vegetation made the use of the sheet difficult a 41 × 29 cm plastic pail was employed. 3) A one-person visual search from knee level to as high as one can reach (above visual search, AVS) during 15 minutes. 4) A one-person visual search from ground to knee level (below visual search, BVS) during 15 min. Stones were lifted up because tho-

misids, especially females after laying eggs (Levy 1975; Hidalgo 1986), from the genera *Xysticus* and *Ozyptila* usually dwell under them. 5) Analysis during 15 min. of leaf litter poured in a white pail, justifiable because this is the habitat of the genus *Ozyptila* (Thomisidae) (Urones 1998). 6) The running of 4 open pitfall traps during 48 hours. These traps were 11.5 cm wide and 1 liter in volume, each 10 m apart from the others in order to avoid interference effects and to maximize the efficacy of each trap (Samu & Lövei 1995). Traps were filled with water, and a few drops of detergent added to break the surface tension so as to prevent the spiders from escaping.

Spiders were sucked up with an aspirator to reduce damage and were transferred to 70% alcohol. Sampling was always done by the same person in order to avoid possible differences due to the effect of the collector (Norris 1999); rainy and windy days were avoided in order to prevent a reduction in the efficiency of the sampling methods (see Gyenge et al. 1997; Churchill & Arthur 1999). All specimens are deposited in the Museo Nacional de Ciencias Naturales collection (Madrid, Spain). All together, sampling involved running 240 pitfall traps (3 sampling plots  $\times$  20 subplots  $\times$  4 pitfall traps) and one-person fieldwork during 75 hours (0.25 hours  $\times$  5 methods  $\times$  3 sampling plots  $\times$  20 subplots).

**Data analysis.**—The cumulative number of species found by different sampling efforts (species accumulation curves) was studied to evaluate the accuracy of the species inventories obtained in each of the three sampling plots (see Gotelli & Colwell 2001). The number of sampling effort units (i.e. the number of subplots) was used as the measure of sampling effort, and the order in which sampling unit inventories were added was randomized 500 times to build smoothed curves using the EstimateS 5.0.1 software (Colwell 1997). The asymptotic value of the accumulation curves obtained was estimated using the Clench equation (Soberon & Llorente 1993; Colwell & Coddington 1994). This score, together with the species richness estimations produced by three nonparametric methods, was used to test if the total number of species caught in each sampling plot underestimated the true species richness. The nonparametric species richness estimators used are the first-order jackknife, the abundance-based coverage

(ACE), and the incidence-based coverage estimator (ICE). Detailed descriptions of the estimators can be found in Colwell (1997) and Colwell & Coddington (1994).

In order to study the effects of sampling method and the interaction of method and habitat on the number of species and individuals collected per sampling effort unit, a factorial ANOVA was performed. As data were not normally distributed, they were transformed by  $\log(n+1)$ , and a Tukey test (HDS) was used to determine pairwise significant differences ( $P < 0.05$ ). STATISTICA package (1999) was used for all statistical computations.

**Other methodological considerations.**—As Norris (1999) pointed out, the inclusion of immature specimens is the factor which has the most significant effect on community trends. It cannot be assumed that the abundance distribution of juveniles is the same as that for adults, and the relative abundance of species in a community can be highly altered if juveniles are considered. However, since our objective was to find all the species inhabiting the sampling plots, juveniles that could be identified to the species level were included in the analysis. Sometimes genera represented only by immature states did appear, in which case, they were also included. Rejecting juveniles would have involved rejecting valuable information, and as they increased sample sizes significantly, their inclusion allowed statistical analysis. In araneids and thomisids, unlike in most other spider families, color and morphology facilitate the identification of some juveniles. All together, 942 individuals were captured, 56% of them juveniles; almost half (247 individuals) have been used in the analysis.

## RESULTS

In 80 sampling effort units, a total of 661 individuals were captured, representing 26 species, 11 araneids and 15 thomisids.

**Completeness of the inventories.**—The Clench model function fits the accumulation curves well in each of the three sampling plots, with percentages of explained variation higher than 99% (Table 1 & Fig. 1). The predicted asymptote score does not differ too much from the observed species richness, the percentages of collected species oscillating around 80%. The nonparametric estimators

Table 1.—Observed species richness ( $S_{obs}$ ) and results of four species richness estimators for each habitat. The relationship between the number of sampling effort units and the number of species was fitted to the asymptotic Clench equation (Colwell & Coddington 1994) where  $a/b$  is the asymptote and  $R^2$  the percentage of explained variance. Jackknife 1 (first-order jackknife), ACE (abundance base coverage) and ICE (incidence-based coverage) are nonparametric estimators of species richness (Colwell 1997).

	Forest	Shrub	Grassland
$S_{obs}$	17	20	15
Clench	$a/b = 21.6; R^2 = 99.9$	$a/b = 25.0; R^2 = 99.4$	$a/b = 18.8; R^2 = 99.9$
Jackknife 1	19.85	26.65	17.85
ICE	18.49	27.18	16.73
ACE	17.85	25.07	17.26

used indicate that the collected species richness varies from 86%–95% for the forest plot, 74%–80% for the shrub plot, and 84%–90% for the grassland plot. These results suggest that the exhaustiveness of the sampling in each of the three habitats is similar, so sampling plot composition and richness figures are comparable. However, still more intensive

sampling should be necessary to obtain an accurate inventory in each habitat.

**Sampling method performance.**—From the three sampling plots, only one individual of *Mangora acalypha* (Walckenaer 1802) was captured by leaf litter analysis method (in the shrub plot). As this species was collected plentifully with the other sampling methods,

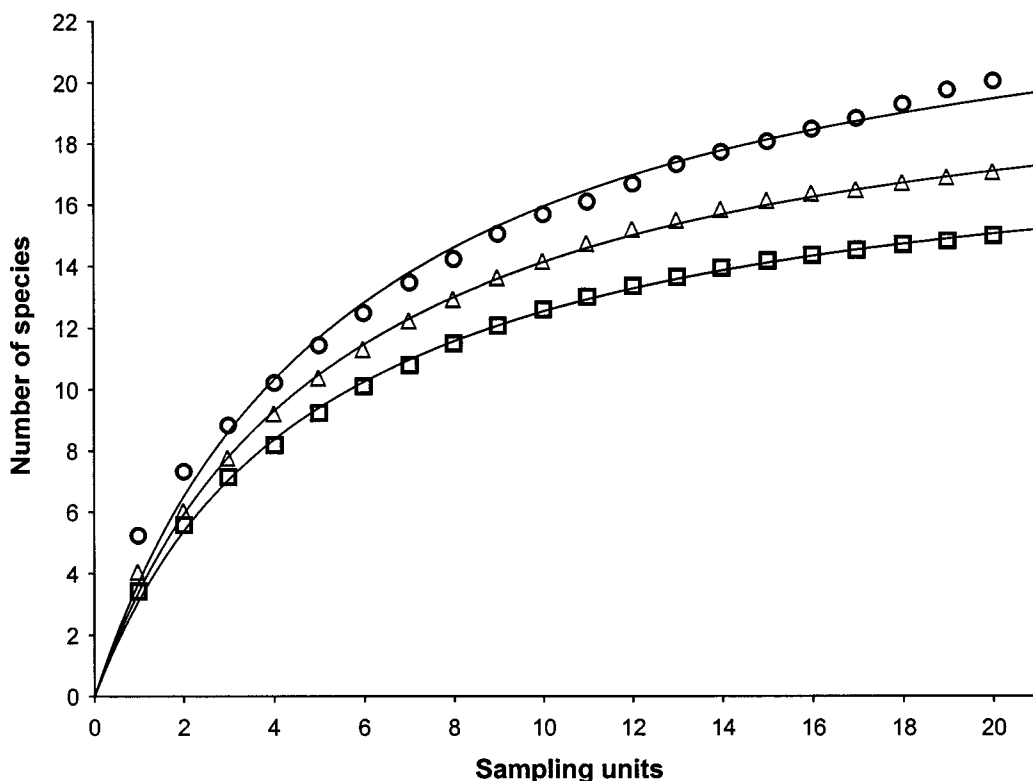


Figure 1.—Species accumulation curves for the three sampling plots with Clench function fitted: □ grassland; ○ shrub; △ forest. The cumulative number of species found at different numbers of sampling effort units was randomized 500 times using the EstimateS 5.0.1 software (Colwell 1997):

Table 2.—Total number of individuals ( $n$ ), mean number of individuals ( $\pm$  SE) per sampling unit ( $N_{\text{MEAN}}$ ), total number of species ( $S$ ), mean number of species ( $\pm$  SE) per sampling unit ( $S_{\text{MEAN}}$ ), and number of unique species ( $S_{\text{UNI}}$ ) for each sampling plot and each sampling method.

	Sampling Plot		
	Forest	Shrub	Grassland
$n$	205	348	108
$N_{\text{MEAN}}$	$2.07 \pm 0.36$	$3.48 \pm 0.56$	$1.57 \pm 0.5$
$S$	17	20	15
$S_{\text{MEAN}}$	$0.92 \pm 0.14$	$1.5 \pm 0.17$	$0.72 \pm 0.1$

	Sampling Method				
	Pitfall	Sweeping	Beating	BVS	AVS
$n$	25	442	90	13	91
$N_{\text{MEAN}}$	$0.41 \pm 0.14$	$8.08 \pm 1.06$	$1.6 \pm 0.23$	$0.22 \pm 0.09$	$1.55 \pm 0.28$
$S$	5	17	15	7	9
$S_{\text{MEAN}}$	$0.3 \pm 0.08$	$2.7 \pm 0.24$	$1.08 \pm 0.14$	$0.18 \pm 0.07$	$0.98 \pm 0.14$
$S_{\text{UNI}}$	2	5	3	0	0

the results of this technique are not considered. Both the mean number of collected species ( $F_{(4,285)} = 58.5$ ;  $P < 0.0001$ ) and the mean number of individuals ( $F_{(4,285)} = 79.9$ ;  $P < 0.0001$ ) differ statistically from one sampling method to another. Both in the case of species richness and for the number of individuals, all pairwise comparisons between sampling methods are significant by a posteriori Tukey HSD test, except in the case of pitfall traps and BVS, and beating and AVS (see Table 2). Sweeping, the technique which captured the greatest number of species and individuals, with araneids making up 47% of the species and 68% of the individuals collected, is also the method that captured more species not captured by any other sampling method (unique species, two araneids and three thomisids). Pitfall traps and BVS are the methods that captured the smallest number of species and individuals, but while BVS did not yield unique species, pitfall traps did capture two unique species. With pitfall traps, only thomisids of the genera *Xysticus* and *Ozyptila* were captured. In the case of the BVS, araneids make up 57% of the species and 62% of the individuals. With regard to the other sampling methods, beating and AVS yield the same number of individuals, though the total number of species is larger for the former. In beating, araneids make up 47% of the species and 43% of the individuals; using AVS araneid, captures were more frequent, accounting for 78% of species and 89% of individuals.

AVS did not yield any unique species, while beating produced three unique thomisids.

By an iterative procedure the sampling methods were ranked sequentially, for each habitat, according to contribution to total species richness in this habitat. Both in the forest and shrub, sweeping is the method that yielded more species, followed by beating and pitfall traps. Together, these three methods captured all the observed species in these habitats. In grassland, where a broader combination of methods is necessary to obtain a reliable inventory (Table 3), beating captured more species, while sweeping, AVS and pitfall traps or BVS seem to be indispensable.

**Sampling method-habitat interaction.**—The mean number of species per sampling unit ( $F_{(2,285)} = 15.14$ ;  $P < 0.001$ ), as well as the mean number of individuals ( $F_{(2,285)} = 15.73$ ;  $P < 0.001$ ), differs significantly between sampling plots. According to a posteriori Tukey HSD test, only in the shrub sampling plot is the species richness and number of individuals significantly greater than in the other two sampling plots (Table 2). However, sampling method and habitat interaction significantly affect both the mean number of species ( $F_{(8,285)} = 6.6$ ;  $P < 0.0001$ ) and the mean number of individuals per sampling unit ( $F_{(8,285)} = 9.6$ ;  $P < 0.0001$ ), indicating that the performance of the various sampling methods depends on the habitat.

The results of a posteriori Tukey HSD test highlight the significantly different interaction

Table 3.—Results of a complementarity procedure in which the inventories of each sampling method were sequentially selected for each habitat according to its contribution to the species richness.

Habitat	Iteration	Sampling method	Number of species	Accumulated species
Forest	1	Sweeping	12	12
	2	Beating	4	16
	3	Pitfall	1	17
Shrub	1	Sweeping	13	13
	2	Beating	4	17
	3	Pitfall	3	20
Grassland	1	Beating	8	8
	2	Sweeping	4	12
	3	AVS	2	14
	4	Pitfall or BVS	1	15

terms. The scheme generated for the mean number of species and individuals is quite similar (Fig. 2). There is not a significant between-habitat variation in the number of individuals or species collected by pitfall-traps, BVS or beating. The AVS method collected a significantly greater number of species and individuals in shrub and grassland than in forest (Fig. 2), only in the grasslands did it capture more species and individuals than BVS and pitfall traps; its captures equalled those of beating in the three habitats. Likewise, sweeping method captures also varied with habitat; the mean number of species and individuals captured in grasslands was significantly smaller than in the other two habitats (Fig. 2). Indeed, the sweeping method captured more species and individuals in forest and shrub, while in grassland its performance was similar to that of beating or AVS.

#### DISCUSSION

Methods differ greatly in the number of species and individuals caught, and collecting method performance depends on vegetation structure. Sweeping is a standard item in an arachnologists fieldwork due to its ease of use and effectiveness (Buffington & Redak 1998). It was the most efficient sampling method in forest and shrub sampling plots, and sweeping yielded more species and individuals. However, in the grassland sampling plot, the extreme shortness of the grass and the presence of thorny shrub patches limited its use; AVS and beating there produced equal value of mean individuals and species richness. While other authors have also noticed the reduced usefulness of sweeping in certain habitats

(Churchill & Arthur 1999), as sweeping was found here to yield unique species in the three sampling plots, it must continue to be fundamental to sampling protocol.

Because beating and AVS work on similar vegetation habitats, they sample the same part of the spider community. However, while beating yielded unique species in the three habitats, AVS only did so in the grassland sampling plot, where araneids were concentrated in shrub patches and therefore easily spotted. Furthermore, AVS, a sampling method biased towards big and flashy spiders, yielded a greater proportion of araneids. It can be noticed that where vegetation structure makes visual search difficult, i.e. in the forest sampling plot, AVS is less efficient and beating yielded more (although not statistically significant) species and individuals. Beating must be added to the sampling protocol, along with AVS in habitats with such a vegetation structure that the visual detection of individuals is easy.

Although its efficiency was quite low in our study, pitfall trapping, one of the most frequently used methods to sample surface-active terrestrial arthropod communities, is essential for sampling that part of the community (i.e., genera *Xysticus* and *Ozyptila*, which comprise more than the 70% of the Iberian thomisid fauna). Indeed, all the pitfall captures in the three sampling plots belong to these two genera. As already noted by other authors (Churchill 1993; Standen 2000), the captures of this sampling method were biased in favor of adult individuals, facilitating the identification of the specimens and helping in

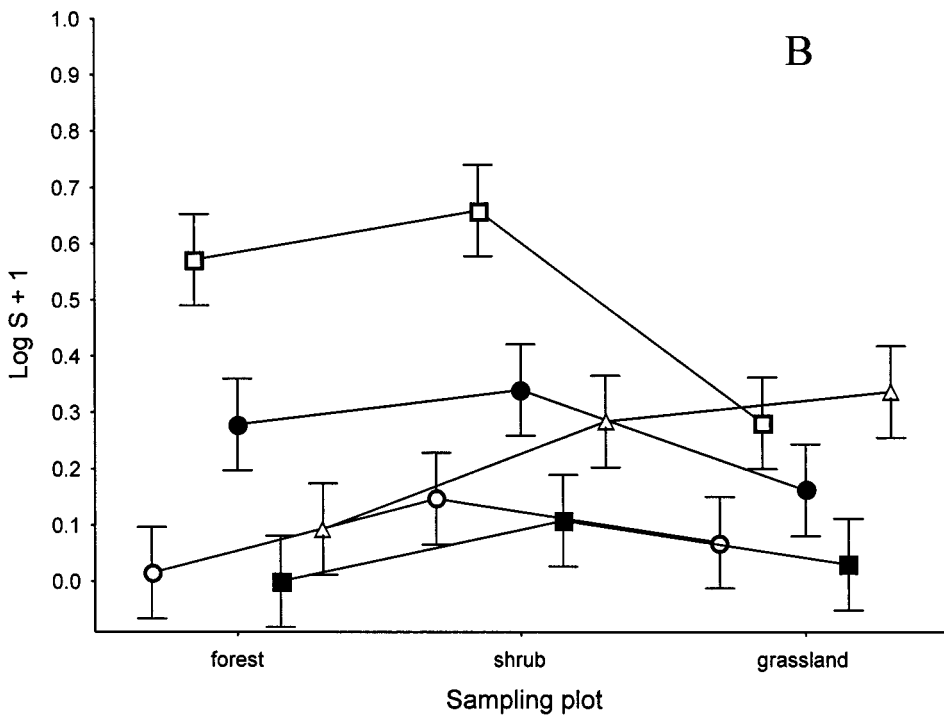
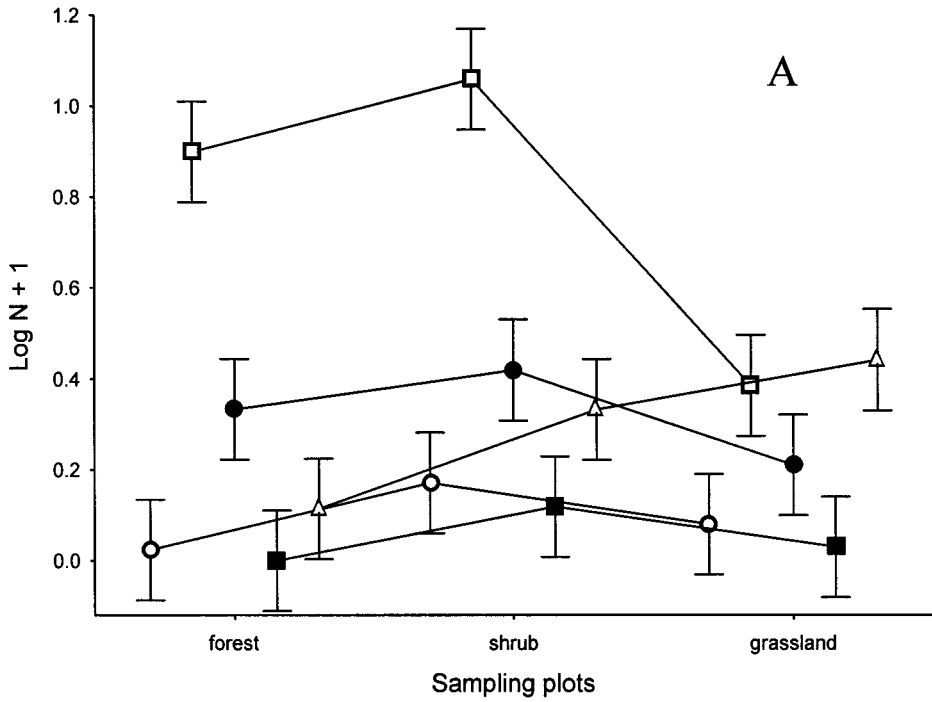


Figure 2.—Variation in the mean number of individuals (log of N + 1; ± 95% confidence interval) per sample (A) and mean number of species (log of S + 1; ± 95% confidence interval) per sample (B) between the three studied habitats or sampling plots. □ = sweeping; ● = beating; △ = AVS; ■ = BVS; ○ = pitfall trapping.

the inventory work. As BVS samples the same part of the community as pitfall traps do and does not contribute unique species, it can be done without. Thus, only pitfall trapping must be included in the sampling protocol.

Because the aim of this sampling protocol is the estimation of species richness, visual search could be more efficient if centered on new species, ignoring the common ones (Dobyns 1997; Churchill & Arthur 1999). The paucity of species and individuals captured by pitfall trapping suggests that the inventory would have been more effective if greater sampling effort were allocated to this method. Brennan et al. (1999) found that the larger the pitfall trap diameter, the greater the number of species captured. Work et al. (2002) pointed out that larger traps were more effective in the characterization of rare elements of an epigeal fauna. They also recommended combining large traps with smaller ones in order to sample a greater range of microhabitats. However, it is difficult to judge if the protocol would have been improved by changing the pitfall trap design or by trying another method that samples this epigeal fauna more accurately.

For none of the three sampling sites does the observed species accumulation curve reach an asymptote, although it seems that the simpler the vegetation structure, the smaller the curve-asymptote separation, and the smaller the difference between  $S_{obs}$  and the Clench model estimation from the nonparametric estimator values. Tight clustering of these three nonparametric estimators was also found by Toti et al. (2000), suggesting that they either estimate the same real value or are biased similarly. Other researchers working with the entire spider fauna (Coddington et al. 1996; Dobyns 1997; Toti et al. 2000; Sørensen et al. 2002) have also failed to produce asymptotic species accumulation curves. However, according to the estimations obtained, the three inventories sampled around 80% of spider fauna, indicating that it is possible to estimate the probable number of species in a 1 km<sup>2</sup> plot. The percentages of completeness are quite similar to those found by other authors in temperate forests (Dobyns 1997, 89%; Sørensen et al. 2002, 86–89%).

Our study is just a spring “snapshot” of the entire annual spider species richness of three sampling plots in different habitats. Spider assemblages, dynamic during the season, change

in species composition. Thus, results depend on the time of sampling (Churchill & Arthur 1999; Riecken 1999). Nevertheless, estimating species richness accurately at a given time carries weight because sampling designs for annual studies depend on it (Coddington et al. 1996; Sørensen et al. 2002). Determining the proportion of the entire annual spider fauna that is represented in the spring sample is an objective of work currently being carried out.

Spider life history and behavioral diversity pose a challenge to the development of a precise and cost-effective sampling program (Costello & Daane 1997). Studies that have tried to take in the entire range of spider fauna have found that even intensive sampling does not reflect the whole of species richness (Coddington et al. 1996; Toti et al. 2000; Sørensen et al. 2002). So, Sørensen et al. (2002) suggest that long-term monitoring programs should focus on single, or few, families, or a single feeding guild, and use a few standardized and practical sampling methods. Our study has focused on two abundant spider families, Araneidae and Thomisidae, and has shown that a particular combination of sampling methods in each habitat is required to optimize efficacy and minimize effort. Sweeping, beating, pitfall traps and AVS in specific locations yield a reliable inventory of these two spider taxa in a 1 km<sup>2</sup> plot. Given how imperative a more detailed knowledge of Iberian spiders is, additional studies should be carried out in order to develop standardized sampling protocols for other spider families and/or guilds.

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