

## A preliminary investigation of spider species richness in an eastern Slovenian broadleaf forest

Matjaž KUNTNER<sup>1</sup> and Ian H. BAXTER<sup>2</sup>

<sup>1</sup> Pod Jelšami 32, SI-1000 Ljubljana, Slovenia.

<sup>2</sup> Department of Biology, Imperial College, Silwood Park, Ascot Berks SL5 7PY, UK.

Present address: Biodiversity and Ecology Division, Department of Biology, University of Southampton, Southampton, Hampshire SO17 1BJ, UK.

**Key words:** spiders, Araneae, Slovenia, species richness, biodiversity.

### ABSTRACT

An estimate of spider species richness was generated using the methods described by Coddington *et al.* (in press). The objectives of the project were to measure local spider diversity, start a national spider species list solely for Slovenia and train 'newcomers' in spider identification and sampling. Over the six days of collecting (31 July - 5 August 1995) 1,201 specimens were gathered, 75 of which were adult, belonging to 11 families, 24 genera and 31 species. The data generated a species richness estimate of 48 - 57 for the forest at that given time. High numbers of juveniles and single species representatives were not a good distribution for the protocol statistics. However, a preliminary estimation of spider species richness was produced and may prove useful to future investigators in the Slovenian area. In addition to the richness estimate, five 'beginners' were trained in spider identification and could reliably key spiders to the family level by the end of the project.

### INTRODUCTION

In 1991, Slovenia declared independence from the Yugoslavian Republic. Since the break up, Slovenians have been keen to establish their own flora and fauna lists to which, it is intended, our data will make a small contribution. Information on indigenous spiders has mainly come from the Yugoslavian Araneae Catalogue, by Nikolić and Polenec (1981) which cites some 420 species occurring in the republic of Slovenia which was then in the same geographical borders as the independent state today. Now (1996), including contributions from the authors, around 500 species of spiders are known to be indigenous to Slovenia. The diversity of Slovenian habitats (from Alpine to sub-Mediterranean and sub-Panonian) and extent of poorly studied areas of the country suggest that the total number of species may be considerably higher.

Estimates of species richness at particular sites provide a useful means of articulating conservation data to land managers or those people in the position of making land use judgements. Ultimately, such estimates can be component parts of land management and utilisation decisions (Coddington *et al.*, in press). Invertebrate diversity is being seen as important for sustainable agriculture (Stewart 1991; Pimentel *et al.* 1992) where the role of non-target arthropods such as pollinators and natural enemies is increasingly appreciated. Taking a more holistic approach, biodiversity in general is of great concern for assessing human impact on the general state of the world's biomes today (Hawksworth & Mound 1991; Wilson 1992). To measure richness on these large scales, alpha and beta diversity measurements, especially of the ubiquitous and often ignored organisms such as spiders, can only help in completing the global picture.

Examining the diversity of spider faunas in specific areas can be useful for crops or forest land, where practical manipulations of pest abundance is a consideration. Several studies (Riechert & Lockley 1984; Riechert & Bishop 1990; Alderweireldt 1994; Provencher & Riechert 1994) have shown that a diverse assemblage of spiders reduces prey densities more effectively than a narrow spectrum of spider species. This has practical implications for agricultural practices, where pest populations may be reduced by the presence of a diverse spider fauna. Interestingly, the importance of spiders in crops has been long appreciated by farmers in China (Shepard *et al.* 1987), where harbourages of straw, which serve as wintering sites and retreats after harvesting of rice, are routinely constructed for the spiders. Spider assemblages of cultivated crops such as soybean, alfalfa, maize, citrus orchards, deciduous orchards and rice fields (Barrion & Litsinger 1995) are attracting attention as the benefits of preserving spider diversity become apparent.

A study of this type is of particular value to Slovenia as much of the spider fauna has been overlooked in this region. Additionally, the vast majority of species records refer to the ex-Yugoslavia and, thus, are out of date for the independent country of Slovenia. It also provides us with a chance to examine species richness in an area where human impact is greatly reduced compared to its close neighbouring countries. Additionally, carrying out this protocol allowed a team of Slovenian biology students to become familiar with an invertebrate group they may have overlooked in favour of more popular varieties. This estimation of species richness is intended to act as a guide to which other Slovenian forests can be compared to. Through subsequent studies of richness at the Kozje forest both seasonal and human impact effects can be monitored in the future years.

## MATERIAL AND METHODS

### I. Study site

The studied forest is in the Kozjansko region of eastern Slovenia, 1.5 km N of Kozje. The region around is named Kozjansko, UTM co-ordinates WM 40, at 400-440 m elevation. The study site was a southern facing mixed broadleaf forest, predominantly of coppice oak (*Luzulo-Fagetum*), with a 20-30 cm layer of leaf litter and little understory.

### II. Collection techniques

The methods were carried out according to Coddington *et al.*, (in press).

Six collectors sampled a forest area of approximately 200 x 200 m simultaneously for one hour using one of the following collection techniques: Aerial hand collecting (1), where each presumably mature spider above knee-level is collected.

Ground hand collecting (2), where spiders below knee-level are collected, including those under bark, stones, leaf litter, etc.

Beating tray (1 m<sup>2</sup>) (3) to sample spiders on vegetation.

Collecting was done in 3 hour blocks, with the sampling techniques rotated after each hour. The investigators attempted not to resample areas with the same technique (approximate visual borders were set). This method of collecting was used twice - 31 July 1995, from 10<sup>30</sup>-14<sup>30</sup> (at 26 °C), to sample the diurnal fauna, and again on the night of 1/2 August 1995, from 22<sup>30</sup>-2<sup>30</sup> (at 20 °C), for the nocturnal fauna.

One sample unit equalled one hour of intensive sampling by one collector using one of the first three collecting techniques described above. These were equally represented. Thus, 18 sample units were collected at day time (6 aerials, 6 grounds and 6 beating) and 18 sample units at night (as above).

Unlike the Coddington *et al.* (in press) methods, this study did not include a Tullgren-funnel analysis. Instead, an attempt to broaden the spectrum of habitats sampled was made by including pitfall traps (4). Four traps containing acetic acid were deposited at one location within the forest from 31 July to 5 August 1995. The contents were duly identified and included in the protocol statistics. We evaluated this additional technique as equal to a one hour sample unit.

The total number of one hour sample units collected was thus 37. We followed the reference protocol (Coddington *et al.*, in press) in counting only adult specimens as observed species. The protocol was designed this way because identifying juvenile spiders to the species level is in most cases impossible.

### III. Species richness estimate

The CHAO 1 (Chao 1984) and jack-knife (Heltshe & Forrester 1983) estimators were used to generate an estimate of species richness.

CHAO 1:

$$S_1^* = S_0 + \frac{a^2}{2b}$$

Where:

$S_1^*$  = estimation of the true number of species

$S_0$  = observed number of species in the sample

$a$  = number of species that are represented by only a single individual in the sample (singletons)

$b$  = number of species represented by exactly two individuals in the sample (doubletons)

The variance is:

$$\text{var}(S_1^*) = b \left[ \left( \frac{a/b}{4} \right)^4 + \left( \frac{a/b}{b} \right)^3 + \left( \frac{a/b}{2} \right)^2 \right]$$

jack-knife:

$$S_2^* = S_0 + L \left( \frac{n-1}{n} \right)$$

Where:

$S_2^*$  and  $S_0$  = as above

$L$  = number of species which appear in only 1 sample unit

$n$  = number of sample units

The variance is:

$$\text{var}(S_2^*) = \frac{n-1}{n} \left( \sum_0^{S_0} j^2 \cdot f_j - \frac{L^2}{n} \right)$$

Where:

$f_j$  = the number of sample units containing exactly  $j$  of the  $L$  unique species.

## RESULTS

In 37 sample units 1,201 spiders were collected, of which 75 individuals were adult. These belonged to 11 families, at least 24 genera and 31 species. 'Intensity' of the sample, that is the ratio of adult individuals to species was 2.4:1. The percent of juvenile spiders in the sample was 93.8 %. The percentage of singletons among adults was 51.6 %. Table 1 shows the list of observed species (with adult individuals) and the number of individuals according to collecting techniques. In Tab. 2 numbers of all collected

individuals, adult individuals and species are compared according to the collecting techniques and the time of sampling. The systematics is according to Platnick (1993).

The estimates of the true spider species richness in the surveyed forest were:

CHAO 1	S* = 56.6	(SD = 13.4)
jackknife	S* = 48.5	(SD = 4.8)

Tab. 1. Species and numbers of adult spiders collected in the forest near Kozje. Collecting methods and time of day are indicated (D = day, N = night), numbers and sex of individuals (m = male, f = female), and the status of 'rare' species (a = singletons, b = doubletons, L = species unique to one sample unit).

taxon	collecting methods						status	
	aerial		ground		beating			traps
	D	N	D	N	D	N		
<b>Agelenidae</b> <i>Histopona torpida</i>			1 m				2 m	
<b>Amaurobiidae</b> <i>Coelotes</i> sp. A				1 f				a, L
<b>Araneidae</b> <i>Nuctenea umbratica</i> <i>Zilla diodia</i>		2 f		1 m		2 f	1 f	
<b>Clubionidae</b> <i>Cheiracanthium</i> sp. A <i>Clubiona marmorata</i>							1 f	a, L a, L
<b>Dysderidae</b> <i>Dysdera ninnii</i> <i>Dysdera</i> sp. A (gr. <i>longirostris</i> ) <i>Harpactea hombergi</i>				2 f	1 f		1 f	a, L a, L
<b>Gnaphosidae</b> <i>Zelotes</i> sp. A					1 f			a, L
<b>Linyphiidae</b> <i>Linyphia triangularis</i>	3 m	2 m				1 m	1 m	
Linyphiidae sp. A						2 m		b, L
Linyphiidae sp. B						1 f		a, L
Linyphiidae sp. C						1 m		a, L

Tab. 1 cont.

taxon	collecting methods							status
	aerial		ground		beating		traps	
	D	N	D	N	D	N		
Linyphiidae sp. D							1 m	a, L
Linyphiidae sp. E				1 f				a, L
<b>Lycosidae</b>								
<i>Pardosa lugubris</i>			4 f				3 f	
<i>Trochosa</i> sp. A			1 f	1 f				b
<b>Salticidae</b>								
<i>Marpissa muscosa</i>	3 f			1 f				
<b>Segestriidae</b>								
<i>Segestria senoculata</i>	1 f							a, L
<b>Theridiidae</b>								
<i>Achaearanea lunata</i>		1 f				1 f		b
<i>Achaearanea</i> sp. A					1 f			a, L
<i>Dipoena melanogaster</i>	1 f	1 f						b
<i>Enoplognatha ovata</i>		3 f				1 f		
<i>Episinus maculipes</i>		9 f			1 f	1 f		
<i>Episinus truncatus</i>			1 f		3 f			
Theridiidae sp. X					2 f			b, L
<i>Theridion</i> cf. <i>pallens</i>		1 f						a, L
<i>Theridion</i> sp. A						1 f		a, L
<i>Theridion tinctum</i>						1 f		a, L
<b>Uloboridae</b>								
<i>Hyptiotes paradoxus</i>						1 m		a, L
<b>TOTAL</b>	<b>8</b>	<b>19</b>	<b>10</b>	<b>7</b>	<b>14</b>	<b>9</b>	<b>8</b>	

**DISCUSSION**

The fieldwork data generates a species richness estimate of 48-57 for the forest at that given time. However, the sample quality makes it difficult for this species richness estimate to mean anything of real substance. Firstly, an extremely high proportion of juveniles (more than 93 % compared to 75 % in Coddington's reference protocol) suggests that this study was too early in the season for any meaningful result to be obtained. Secondly, as a direct result of high juvenile numbers, a lop-sided singleton to doubleton ratio (i.e. more single than double species representatives) produced an inaccurately high species richness estimate. The somewhat high calculations of variance reinforce the fact that the data needs much more replication over a several month period before the data will be truly representative of the local fauna.

Tab. 2. Numbers of individuals, adults and species of adult spiders according to the collecting method and time of day. 'Other' means unidentified juvenile spiders collected by using the first three collecting methods (see text).

	No. of sample units	No. of individuals	No. of adults	Mean No. of adults per sample unit	% of total adults	No. of species	Mean No. of species per sample unit	% of total species
AERIAL								
day	6	103	8	1.3	10.7	4	0.7	12.9
night	6	272	19	3.2	25.3	7	1.2	22.6
subtotal	12	375	27	2.3	36.0	11	0.9	35.5
GROUND								
day	6	78	10	1.7	13.3	6	1.0	19.4
night	6	84	7	1.2	9.3	7	1.2	22.6
subtotal	12	162	17	1.4	22.7	13	1.1	41.9
BEATING								
day	6	302	14	2.3	18.7	9	1.5	29.0
night	6	217	9	1.5	12.0	9	1.5	29.0
subtotal	12	519	23	1.9	30.7	18	1.5	58.1
TRAPS	1	25	8	8.0	10.7	5	5.0	16.1
OTHER	/	120	/	/	/	/	/	/
<b>TOTAL</b>	<b>37</b>	<b>1201</b>	<b>75</b>	<b>2.0</b>	<b>100</b>	<b>31</b>	<b>0.8</b>	<b>100</b>

Another reason for a high proportion of juveniles collected might be the difference in collectors' effectiveness (Tab. 3). The number of species collected per sample unit varies from 2.67 in the most experienced collector to 0.25 in the inexperienced one. As most of the collectors were inexperienced, many of the cryptic species may have been overlooked in favour of the large numbers of small, but highly visible, immature specimens. This resulted in many juvenile individuals of common species collected (which are not included in the protocol) and a high proportion of singletons among adults. In similar studies in the future the use of additional collecting techniques like sweep-net, Tullgren-funnel analysis and searching for cryptic fauna should prove useful (Coddington pers. comm.).

Examining all spiders collected during the protocol (including juveniles) gave the number of different species to stand at 53. As many of the immature specimens could be identified to genus only, it is, however, more likely that the true number of species stands at closer to 60. Thus it is speculated that the true number of spider species active in the surveyed forest at the time of the study and accessible to the methods used was higher than the estimators show. The jack-knife estimator, in particular, seems rather low for an established broadleaf forest of this density during the summer months.

An important part of this study was to evaluate the use of this protocol as a method of sampling Slovenian faunas. If favourable, the authors intended that the protocol would be repeated in other regions of Slovenia in order to make country wide comparisons. Coddington *et al.* (1991) designed this type of experiment with tropical faunas in mind, where quick, reliable and cheap surveys could be completed with the minimum of the specialist sampling knowledge. Although Slovenia does not strictly fit this description, it was believed that some sort of quantifiable method of collecting would be far more representative of the poorly studied Slovenian spider fauna than more traditional collecting techniques. An added bonus of following this protocol being that subsequent studies in Slovenia would use the same sampling strategy and give investigators a useful insight of species distribution.

Tab. 3. Collectors' effectiveness.

Collector	No. of sample units	No. of adults collected	No. of species	No. of adults per sample unit	No. of species per sample unit
1. Kuntner	6	25	16	4.17	2.67
2. Baxter	6	7	6	1.17	1.00
3. Polak	6	20	11	3.33	1.83
4. Antauer	5	4	3	0.80	0.60
5. Kostanjšek	5	2	2	0.40	0.40
6. Fišer	4	8	8	2.00	2.00
7. Lokovšek	4	1	1	0.25	0.25

Despite the obvious anomalies in this data the sampling protocol did indeed produce some interesting results. There appears to be little significant difference in nocturnal and diurnal collections, however simply by collecting at a time of night traditional sampling techniques would ignore allows for a more complete picture of the sampling fauna to be produced. Through the hourly rotation of collecting techniques amongst the investigators (aerial, ground and beating) differences in an individual's ability to locate spiders is, to a certain extent, covered up. It was noticed that the investigators improved greatly at collecting within a very short period of time. This is yet another reason why replication over a several month period would improve the validity of these results.

An attempt to quantifiably sample spiders is always going to be difficult where resources are limited and, in the temperate zone, the mature season is uncharacteristically late. However, in using this protocol and also collecting with standard methods in the vicinity of the forest, six previously unrecorded species for Slovenia were collected (Kuntner 1996), and five 'beginners' to arachnology were introduced to a fauna and method of sampling they

probably would never have encountered. In addition to this, the results will serve as a yardstick for other investigators in Slovenia and surroundings to work by. Perhaps with increased randomisation of the collecting sites, the implementation of more sampling techniques and, of course, replication, the protocol will be used again in Slovenia.

### **Acknowledgements**

We thank Dr. Jonathan A. Coddington and Dr. Frederick A. Coyle who gave valuable advice and donated literature, Dr. Boris Sket and France Velkovich (Department of Biology, University of Ljubljana, Slovenia) for the loan of laboratory equipment and for logistic support. Thanks are due to Gregor Antauer, Cene Fišer, Rok Kostanjšek, Tjaša Lokovšek and Slavko Polak for their help on the field, and to Dr. Ivan Kos and Andrej Blejcek for their comments on the results.

### **REFERENCES**

- Alderweireldt M. 1994. Habitat manipulations increasing spider densities in agroecosystems: possibilities for biological control? *J. Appl. Ent.*, **118**: 10-16.
- Barrion A. T. & Litsinger J. A. 1995. *Riceland Spiders of South and Southeast Asia*. CAB International.
- Chao A. 1984. Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, **11**: 265-270.
- Coddington J. A., Griswold C., Davila D., Penaranda E. & Larcher S. 1991. Designing and testing sampling protocols to estimate biodiversity in tropical systems. In: Dudley E. C. (ed.), *The unity of evolutionary biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology*. Dioscorides Press, Portland, Oregon, Vol. 1, pp: 44-60.
- Coddington J. A., Young L. H. & Coyle F. A. In press. Estimating spider species richness in a Southern Appalachian cove hardwood forest. *J. Arachnol.*
- Colwell R. K. & Coddington J. A. 1994. Estimating terrestrial biodiversity through extrapolation. *Phil. Trans. R. Soc. Lond.*, **B, 345**: 101-118.
- Hawksworth D. L. & Mound L. A. 1991. Biodiversity databases: the crucial significance of collections. In: Hawksworth D. L. (ed.), *Proceedings of the first workshop on the ecological foundations of Sustainable Agriculture*. CAB International.
- Heltshel J. & Forrester N. E. 1983. Estimating species richness using the jackknife procedure. *Biometrics*, **39**: 1-11.
- Kuntner M. 1996. Prispevek k poznavanju favne pajkov Kozjanskega, vzhodna Slovenija (Arachnida: Araneae) Contribution to the

- knowledge of the spider fauna of the Kozjansko region, eastern Slovenia (Arachnida: Araneae). In: Bedjanič M. (ed.), Raziskovalni tabor študentov biologije Kozje'95. Zveza organizacij za tehnično kulturo, Gibanje znanost mladini, Ljubljana, pp. 49-60.
- Nikolić F. & Polenec A. 1981. *Catalogus faunae Jugoslaviae*, III/4. Aranea. SAZU, Ljubljana.
- Platnick N. I. 1993. *Advances in Spider Taxonomy 1988-1991. With Synonymies and Transfers 1940-1980*. New York Entomological Society and the American Museum of Natural History, New York.
- Pimentel D., Stachow U., Takacs D. A., Brubaker H. W., Dumas A. R., Meaney J. J., O'Neil J. A. S., Onsi D. E. & Corzilius D. B. 1992. Conserving biological diversity in agricultural/forestry systems. *BioScience*, **42**(5): 254-362.
- Provencher L. & Riechert S. E. 1994. Model and field test of prey control effects by spider assemblages. *Environm. Ent.*, **23**:1-17.
- Riechert S. E. & Bishop L. 1990. Prey control by an assemblage of generalist predators: Spiders in garden test systems. *Ecology*, **71**(4): 1441-1450.
- Riechert S. E. & Lockley T. 1984. Spiders as biological control agents. *Ann. Rev. Ent.*, **29**: 299-320.
- Shepard B. M., Barrion A. T. & Litsinger J. A. 1987. *Helpful Insects, Spiders and Pathogens*. International Rice Research Institute, Manila.
- Stewart W. 1991. The importance to sustainable agriculture of biodiversity among invertebrates and microorganisms. In: Hawksworth D. L. (ed.), *Biodiversity of Microorganisms and Invertebrates: its role in sustainable agriculture*. Proceedings of the first workshop on the ecological foundations of sustainable agriculture. CAB International.
- Wilson E. O. 1992. *The Diversity of Life*. Belknap, Harvard.