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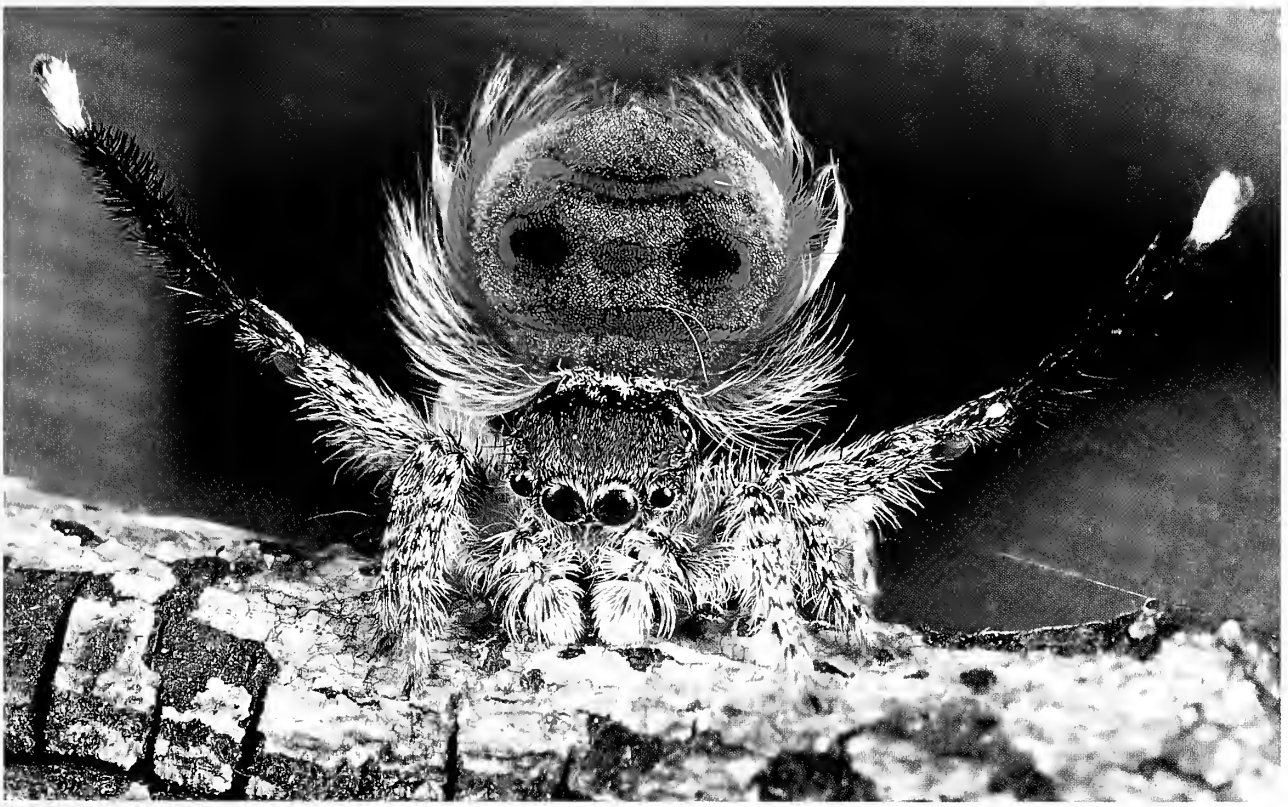

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Cover photo: A displaying male Coastal Peacock Spider, *Maratus speciosus* (Salticidae), from coastal dune habitats near Perth in Western Australia. Photo by Jurgen Otto.

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REVIEW

New sequencing technologies, the development of genomics tools, and their applications in evolutionary arachnology

Michael S. Brewer[1,2], **Darko D. Cotoras**[3], **Peter J. P. Croucher**[1,2] and **Rosemary G. Gillespie**[1,2]: [1]Department of Environmental Science, Policy, and Management, University of California, Berkeley, California 94720 USA. E-mail: michaelsbrewer@gmail.com; [2]Essig Museum of Entomology, University of California, Berkeley, California 94720 USA; [3]Department of Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. Molecular genetic tools have been a boon to arachnologists for decades and used to study many unique aspects of arachnid biology including genomics, phylogenetics, population genetics, and biogeography. These tools have evolved over time and now provide myriad methods for exploring evolutionary questions. Early tools, while still useful under the proper circumstances, are giving way to a new generation of DNA sequencing technologies. These new platforms yield impressive amounts of data at a fraction of the cost of traditional techniques. Herein, we discuss the history and future of molecular evolutionary arachnology in terms of available genetic/genomic tools and their potential applications, strengths, weaknesses, and relative costs. Next-generation sequencing (NGS) platforms are varied in their methods and potential uses, making high-throughput sequencing studies focusing on a wide array of questions tractable. To date, relatively few studies have employed NGS technologies using arachnids, but many could benefit from using them. Because no model species exist within the class Arachnida, we have a limited understanding of arachnid genomics. With the ever-advancing nature of sequencing technologies and bioinformatics, arachnologists can relatively easily implement NGS studies to bridge the gaps in our understanding and open avenues for deeper and more powerful experiments. To this end, we discuss examples of applications of NGS technologies focusing on arachnid taxa. Despite the allure of acquiring massive quantities of sequence data, we should recognize the limitations of existing NGS technologies and not forsake pre-NGS methods when these technologies could adequately address our questions.

Keywords: Next-generation sequencing, genome, transcriptome, phylogeny, population genetics, genomics, adaptation, selection

TABLE OF CONTENTS

1. INTRODUCTION

Since Linnaeus and before, scientists have sought to put order into the diversity of life, the thirst for information increasing with the recognition of the role of evolutionary processes in shaping that diversity. The advent of molecular techniques in the 1980s introduced a huge diversity of novel markers for assessment of phylogenetic affinities. Moreover, with the growth of the human genome project, the potential use of vast numbers of genes across the genome was soon recognized (Jones 1991). Analytical tools were developed that could use the almost limitless data to address questions ranging from historical and recent demographic and migratory patterns to identifying signatures of recent natural selection (Nielsen 2010; Rasmussen et al. 2011; Lohmueller 2011). Here, we examine where the field of arachnology stands within the genomic revolution.

In recent years, advances in sequencing technology have led to great increases in genomic resources for many non-model species. The arthropod class Arachnida, encompassing over 100,000 nominal species classified into 12–13 traditional orders (Krantz and Walter 2009; Blick and Harvey 2011), comprises a diverse array of taxa that serve key functions in terrestrial ecosystems as important predators and decomposers. Mueh of their diversity is unique to particular arachnid taxa, including complex silk production (spiders and mites), venom composition (spiders, scorpions, and pseudoscorpions) and detoxification of plant compounds (Grbić et al. 2011), and has long been of particular interest to researchers. As an example, spiders have been used to study behavior (reviewed by Herberstein and Hebets 2013), development (e.g., Kanayama et al. 2010; Wolff and Hilbrant 2011; Mittmann and Wolff 2012), sexual selection (e.g., Kuntner et al. 2009; Su et al. 2011), genetics (reviewed by Goodacre 2013), evolutionary ecology (reviewed by Moya-Laraño et al. 2013) and biogeography (reviewed by Gillespie 2013), among other fields. However, relationships within and among arachnid taxa are, in many cases, not presently resolved (Giribet and Edgecombe 2013), and a paucity of genomic resources has hindered efforts in various fields of arachnology. Molecular teehniques have been successfully employed to investigate a number of these issues, but some problems require data at a scale heretofore unavailable to arachnologists.

Here we first briefly review how more "traditional" molecular tools have been applied to arachnid biology and then go on to discuss emerging next-generation sequencing ("NGS") applications and their potential impact within the field. Instead of deeply discussing each area of arachnology and thoroughly reviewing the literature, we provide a brief background with select citations and proceed to describe some ways in which the rapidly multiplying set of NGS tools may be used.

2. "TRADITIONAL" MARKERS OF NUCLEAR GENETIC VARIATION

Prior to genomic tools, multiple techniques were used for examining variation in nuclear DNA and hence to assess geographic and population structure, the most widely used being randomly amplified polymorphic DNA (RAPDs), restriction fragment-length polymorphisms (RFLPs), and satellite and microsatellite DNA.

2.1. Allozyme electrophoresis.—Allozyme electrophoresis has proven very useful for the analysis of geographic structure of arachnids. Some early studies focused specifically on population structure (Porter & Jakob 1990; Steiner et al. 1992; Smith & Engel 1994; Hudson & Adams 1996; Smith & Hagen 1996; Boulton et al. 1998), but allozymes have also been used to examine questions of relatedness among colonies of social spiders (Johannesen et al. 1998; Johannesen & Lubin 1999, 2001; Johannesen & Veith 2001; Evans & Goodisman 2002; Yip et al. 2012), paternity (Schafer & Uhl 2002), species boundaries and speciation (Piel & Nutt 2000; Ramirez & Chi 2004), dispersal (Pedersen & Loeschcke 2001; Schafer et al. 2001), the effects of forest fragmentation, whether natural (Vandergast et al. 2004) or manmade (Ramirez & Haakonsen 1999; Gurdebeke et al. 2000), and to estimate selection on color polymorphisms (Tso et al. 2002; Oxford 2005; Oxford & Gunnarsson 2006; Croucher et al. 2012) as well as patterns of diversification within rapidly diversifying lineages (Pons & Gillespie 2004; Baert et al. 2008; De Busschere et al. 2010).

The primary limitations of allozyme electrophoresis are: a) Organisms must generally be alive or deep-frozen before use; b) when bands co-migrate, they are assumed to be homologous; c) only a very small subset of the genetic variation at a given locus is revealed; and d) it is not possible to distinguish ancestry and descent among different alleles. The technique is inexpensive, fast, and can give insight into multiple loci and so is useful for addressing questions of geographic structure.

2.2. Satellite & microsatellite DNA.—Tandem repeats include three subclasses: satellites, minisatellites and microsatellites. Satellites range in size from 100 kb to over 1 Mb with repeat units of ca. 100–200 bp; most are located at the centromere. Minisatellites range from 1 kb to 20 kb in size with shorter repeats (9–80 bp), while microsatellites (also known as short tandem repeats, STR), are repeats of sequences less than about five base pairs in length (an arbitrary cutoff). Among spiders, satellite DNA has proven very useful in assessing relationships among species within a radiation of spiders in Hawaii; this was because the tandem-arranged units show a high intraspecific sequence identity due to concerted evolution (Pons & Gillespie 2003, 2004). As a result, the length of the branches and corresponding support were much greater for satellite DNA than for mtDNA sequence data.

Microsatellites are repeated short sequences of DNA that occur throughout the genomes of many organisms, including spiders. Because repeat units are readily added to or lost from microsatellite DNA, the sequence length of these regions evolves rapidly. Microsatellites offer a valuable pool of genetic variation that has proven very useful for looking at paternity and relatedness among spiders, including social species (Ji et al. 2004; Bilde et al. 2009; Duncan et al. 2010), as well as understanding geographic structure between closely related populations (Rutten et al. 2001; Reed et al. 2007, 2011; Krehenwinkel & Tautz 2013; Parmakelis et al. 2013). However, compared to many other fields, the development and application of microsatellites in spider ecology and evolution has been limited. A potential cause for the paucity of microsatellite studies in arachnids is the apparent difficulty in finding reliable loci. However, the low % GC (percentage of

guanine and cytosine residues in DNA sequences) in some lineages, as discussed below, could potentially play a role.

2.3. Random amplified polymorphic DNA (RAPD).—In the RAPD procedure, a single nucleotide primer (8–10 base pairs long) is used to amplify random sections of nuclear DNA, with differences in band sizes being used to provide information on relationships. The method has been used in spiders (e.g., A'Hara et al. 1998; Gurdebeke et al. 2003). However, although the approach provides a lot of variability, RAPDs suffer from poor repeatability, lack of codominance, and the possibility of non-heritable or non-homologous bands.

2.4. Restriction fragment length polymorphisms (RFLP).—For generating RFLPs, regions of nuclear DNA isolated through PCR or other means can be digested with restriction enzymes that cut samples of homologous DNA at specific four- or six-base sequences, differences arising from the locations of restriction enzyme sites. This technique could, compared to other technologies of the time, exploit an enormous amount of genetic variation. However, although used in mites (e.g., Osakabi & Sakagami 1994), it was never an important technique in other arachnid groups.

2.5. Amplified fragment length polymorphisms (AFLP).—AFLPs use restriction enzymes to digest genomic DNA, with the fragments then amplified and separated, providing markers across many loci that are highly variable and are also reproducible. Like RAPDs, however, they are also both anonymous and dominant and may produce non-homologous bands. They have been used in studies of geographic structure among spider populations (Jung et al. 2006; Lambeets et al. 2010; Croucher et al. 2011a, b), where they provided fine resolution of population differentiation and subdivision. They have also been used in assessments of inbreeding and sociality (Bilde et al. 2005).

3. SEQUENCING METHODS

3.1. Sanger sequencing of mitochondrial DNA.—Mitochondrial DNA, notably the cytochrome oxidase 1 (CO1), NADH dehydrogenase 1 (ND1) and 16S rRNA genes (Agnarsson et al. 2013) proved particularly useful in the earliest studies of biogeography and species differentiation in spiders (Gillespie et al. 1994; Hedin 1997a, b; Johannesen et al. 2002). The reason for this is simply because of the abundance of mitochondrial DNA relative to nuclear DNA, making it much easier to amplify. However, problems with mtDNA that affect recently evolving lineages include the lack of recombination, as a result of which it behaves as a single locus, making it of limited value for analytical approaches requiring multiple loci. This makes its use in species delimitation particularly problematic (Hamilton et al. 2014). Moreover, the haploid nature of mitochondrial DNA means that the marker is more sensitive to small population sizes than is nuclear DNA, and the maternal inheritance means that biases in movement between sexes cannot be recovered. For this reason, recent studies that have used mtDNA sequences have generally included various nuclear markers (e.g., Vandergast et al. 2004; Starrett & Hedin 2007; Croucher et al. 2011a & b, 2012; Satler et al. 2013). Mitochondrial DNA has been applied to questions at deeper phylogenetic levels where the microevolutionary problems mentioned above are less severe. However, the rapid evolution of the marker means that it tends to become saturated rather quickly (Brewer et al. 2013).

3.2. Sanger sequencing of nuclear DNA.—Because of the issues of amplification, the most reliable, and hence useful, nuclear genes have tended to be those that occur in multiple copies such as Histone 3, and the ribosomal 18S and 28S genes, and these have been of particular impact in the realm of phylogenetic reconstruction (reviewed by Agnarsson et al. 2013 and Giribet and Edgecombe 2013). At the population-species level, attention has focused on nuclear introns—noncoding sequences within nuclear genes, as these are not subject to the same selective constraints as exons and tend to evolve faster (Garb & Gillespie 2009; Hedin et al 2010). ITS (internal transcribed spacer) regions within the ribosomal RNA genes can frequently provide sufficient variability at shallow levels (Hormiga et al. 2003), though paralogy can often make the identification of homologous DNA difficult or impossible. Indeed, the problem of generating multilocus (nuclear) data has remained. Thus, researchers have looked increasingly toward modern, high-throughput (or "next-generation") sequencing technologies as a potential means to generate large amounts of multilocus data by increasing the amount of data per monetary cost by orders of magnitude.

3.3. Sanger sequencing versus NGS methods.—In the past, DNA sequence data were primarily collected using dideoxyribonucleotide (ddNTP) termination methods (i.e., Sanger sequencing). This approach provides long, high-quality sequences but suffers from a number of limitations (Table 1), including the ability to sequence only a single locus per reaction. In addition, reactions typically require taxon (or even population) specific oligonucleotide "primers"—short fragments of DNA (ca. 20–25 bp) of known sequence for polymerase chain reaction amplification and the sequencing reaction. Lastly, the cost of collecting data is much higher than in NGS approaches. Sanger sequencing methods are quite scalable in that one can easily obtain data for a single locus to hundreds of loci with a concomitant change in cost, but one cannot sequence massive amounts of genomic data from numerous specimens in a cost effective (in terms of time and money) manner.

In contrast to Sanger sequencing, NGS techniques provide vastly larger quantities of data much faster and for far less money. NGS approaches achieve this in two ways. The first involves ligating or attaching adaptors (synthesized DNA strands of known sequence) to the ends of fragments of target DNA. These adaptors allow identical sets of PCR or sequencing primers to be used for all the DNA fragments (a form of "shotgun" sequencing). From an operational point of view, the major differences between the various NGS approaches are in the size of the DNA fragment (the "insert") and the number of base pairs of sequence data that can be recovered from the end of the fragment. The second way that NGS approaches dramatically reduce costs is by miniaturization and parallelization—millions of sequencing reactions take place in small reaction chambers or flow cells (Shendure & Ji 2008). Consequently, high-throughput methods can sequence numerous DNA fragments concurrently and in a single reaction/run with full-length sequences typically being assembled after the fact.

Table 1.—Summary of the strengths, weaknesses, starting material and applications of select molecular data sources. Pre- and post-next-generation sequencing molecular data sources discussed here are listed. Several positives and negatives are given for each technique, along with differences in starting material required. Additionally, several historical and potential applications in arachnology are provided for each method.

Method	Raw sequence output	Cost per Mb	Positives	Negatives	Potential in arachnids
Traditional Sanger sequencing	1.9–84 Kb	$2,400	Long, high quality reads; scalability	Very high cost per Mb	Traditional phylogenetics, population genetics and studies of few genes
454 pyrosequencing	0.7 Gb	$10	Long reads	Problems with homopolymers; high cost per Mb (for next-gen technology)	Genomes, transcriptome and microbiome studies
Illumina (Solexa) sequencing	600 Gb (HiSeq 2000)	$0.05–$0.15	High output; relatively low cost; widely used and supported	Short reads	Genome, transcriptome, massively barcoded amplicon and microbiomes studies
SOLiD sequencing	120 Gb	$0.13	Highly accurate	Short reads; not as widely supported	Genome, transcriptome, epigenetic and resequencing studies
Ion Torrent sequencing	20 Mb–1 Gb	$1	Scalability	Short reads	Transcriptome and barcoded amplicon studies
MiSeq sequencing	1.5–2 Gb	$0.50	Longer reads and smaller scale than older Illumina technologies	Output too low for some applications	Transcriptome, barcoded amplicon and microbiome studies
Single molecule real time (SMRT)	400 Mb	$0.75–$1.50	Very long reads	High error rate; best combined with other technologies	Genome and transcriptome studies

3.4. NGS versatility in sequencing targets.—The "shotgun" nature of NGS, using ligated universal adaptors, gives these approaches tremendous versatility in terms of what can be sequenced. This versatility facilitates genomic data collection for organisms about which little or no prior genetic information is known. Sequenced DNA targets can therefore theoretically consist of any source of DNA from total genomic DNA for genome sequencing (see Section 5) to cDNA (derived from total RNA by reverse transcription of expressed mRNA) (Mortazavi et al. 2008) from whole organisms or specific tissues that may have experienced different "treatments" ("transcriptomics" and "differential expression"). RNAseq libraries used for transcriptome sequencing target only the transcribed portion of the genome and are therefore a type of "reduced representation library" (RRL) (Van Tassell et al. 2008), and we discuss this approach along with other RRL approaches such as Exon Capture (Bi et al. 2012) and RADseq (Miller et al. 2007) below. RRL approaches target specific loci and produces fewer unique reads per individual than whole genome libraries and on certain NGS platforms, especially Illumina (see below), may produce highly redundant amounts of data per individual. This has resulted in methodologies that multiplex numerous individuals using small, unique oligonucleotide indices (i.e., tags or barcodes). These are typically incorporated into the adaptors and allow the sequences from each individual to be post-hoc sorted computationally. Barcoding therefore permits NGS approaches to act as high-throughput variant detection and genotyping platforms (e.g., Dahl et al. 2007; Meyer et al. 2008). Barcoding also comes into its own when the DNA targets originate from amplicons generated by traditional PCR approaches. Amplicons might be derived from long-range PCR of mitochondrial genomes, for example, or from standard molecular markers such as bacterial 16S, fungal 18S, or metazoan COI. Such massive barcoding approaches with amplicon sequencing are permitting community-wide metagenomic/microbiome analyses (Amaral-Zettler et al. 2009; Gloor et al. 2010; Caporaso et al. 2012) and large-scale phylogenetic studies (see below).

4. NEXT-GENERATION TECHNOLOGY PLATFORMS

Modern, high-throughput (or "next-generation") sequencing technologies have made many questions more tractable by increasing the amount of data per monetary cost by orders of magnitude. Although a number of NGS sequencing techniques have a steep learning curve (in terms of both wet-laboratory work and bioinformatics), much can be outsourced, and myriad computational resources (many of which may be used free of charge) are readily available. Some of these techniques are widely used, while others are still more limited in their availability. All such methods have inherent strengths and weaknesses that can be leveraged to address a wide range of questions. As more molecular data are collected for arachnid taxa, these groups may begin to approach "model" organism status in terms of a foundational understanding of genetics. This will allow more in-depth studies, using complex and powerful genetic and genomic techniques, to understand the basis of arachnid-specific traits.

4.1. Second-generation NGS technologies.—The basic logic behind several NGS technologies has been reviewed in recent works (Liu et al. 2012; McCormack et al. 2013; Quail et al. 2012). Therefore, we do not delve into specifics of the approaches, instead choosing to highlight strengths and weaknesses of the platforms. Many of the points below are summarized in Tables 1 and 2.

The first mainstream high-throughput technology was the Roche 454 system. This method relies on pyrosequencing

Table 2.—Comparison of DNA sequencing technologies with an emphasis on uses in arachnology. Several aspects of common sequencing technology are compared. The raw sequence output is highly variable between platform and shows the potential scalability provided by using different sequencing platforms. The cost per one million base pairs of data between methods also differs substantially and must be considered when attempting a study requiring sequencing. To help with choosing between platforms, we provide select positives and negatives for each. Finally, some potential high-level applications in arachnology are given.

Data source	Positives	Negatives	Starting material	Applications in arachnids
Pre next-gen sequencing				
Allozyme electrophoresis	Ease of use; widely used in arachnology	Requires fresh or frozen material; uncertain homology	Fresh or frozen specimens	Population genetics and biogeography
Variable nucleotide tandem repeats (VNTR; satellites)	More certain homology; widely used outside of spiders (many analytical tools)	Difficult to design; may not work between even closely related taxa	gDNA	Paternity, population genetics and biogeography
Random amplified polymorphic DNA (RAPD)	Easier to implement than satellite techniques	Lack of repeatability; codominance; uncertain homology	gDNA	Population genetics and biogeography
Restriction fragment length polymorphisms (RFLP)	Ease of use	Fragments of same size may not be homologous; not widely used in arachnology	gDNA	Population genetics and biogeography
Amplified fragment length polymorphisms (AFLP)	Widely used	Anonymous and dominant; uncertain homology	gDNA	Assessments of inbreeding; population genetics and biogeography
Termination (Sanger) sequencing	Sequence data; homology easier to infer; highly scalable compared to other non-NGS techniques	Much more expensive than NGS at a per base level; taxon/ population specific primers needed	gDNA	Assessments of inbreeding, population genetics, biogeography, phylogenetics and single gene studies
Post next-gen sequencing				
Genome sequencing	Full sequence data, creates foundation for many future studies	Costly, difficult, and unnecessary for many projects	High quality, high quantity gDNA	Genome studies, genetic mapping and developing model organisms
Transcriptome sequencing	Easy to sequence and serves a wide range of projects	Costly and biased towards coding regions of genome	High quality RNA from fresh or frozen specimens	Studying coding sequencing, differential expression, identifying isoforms, evolution of gene families, functional genes, and deep phylogenomics
RAD Tags	Powerful for genetic mapping, population genetics and phylogeography	Requires large amount of high-quality DNA	High quality, high quantity gDNA	Phylogeography, population genetics, species-level phylogeny and genomic mapping
Target Capture	Targets portions of the genome for wide array of projects	Requires additional genomic information	gDNA from fresh or preserved specimens (quality depends on application)	Studies of specific regions of the genome, functional genes, population genetics, species-level phylogeny and phylogeography
Anchored enrichment	Orthology certainty and phylogenetics at various taxonomic levels	Not developed in all groups	gDNA from fresh or preserved specimens (quality depends on application)	Phylogenetics of various taxonomic depths

chemistry to obtain millions of unique reads. Roche's 454 approach provides relatively long reads (~700 bp) at a much lower cost (~$10/Mb) than traditional sequencing methods (~$2,400/Mb). The 454 sequencing technology, although expensive per base of data in comparison to other NGS methods and yielding fewer unique sequence reads, is still widely used in genome and transcriptome sequencing and metagenomics because of the relatively long length of individual reads. However, other techniques (namely Illumina technologies, see below) can now serve many of the same functions as 454 sequencing at a much lower cost while providing many more unique sequence reads.

The second NGS platform to become widely used was Applied Biosystems sequencing by oligo ligation detection (SOLiD). This method uses the ligation of short probes to the template DNA. Each probe's extension relies on two-base matches, yielding highly accurate results at a lower cost-point (~$0.13/Mb) and in higher quantities than 454 sequencing (see below). A minor downside to the SOLiD sequencing platform is that the output is in a format unlike other technologies and

requires computationally expensive algorithms to assemble. Nonetheless, this method can be used to efficiently study genomes, transcriptomes, and epigenetics (i.e., non-genetic modifications of the DNA sequence that affect expression such as methylation of CpG "islands", areas of the genome containing high frequencies of cytosine and guanine residues).

The last of the commonly used second-generation technologies, and the most frequently used NGS platform, is the Illumina system. Illumina chemistry relies on fixed flow-cell binding site oligonucleotides and complementary adaptors that also contain sequencing primer sites, and that are ligated to the DNA fragments to be sequenced. This technology yields a vast quantity of raw data (600 Gb) for relatively low cost ($0.05 – $0.15/Mb), and much effort has gone into developing novel ways of applying the method to a wide array of studies. These range from tweaking protocols to creating new algorithms and software for analyses. The main downside of the Illumina technology is that the sequence reads are relatively short. Early versions of the platform yielded reads that were only 36 bp in length, but read length as well as throughput continue to increase for all the NGS technologies, and the Illumina platform, for example, although still short in comparison to Sanger and 454 approaches, can now generate reads in excess of 150 bp. Short reads lead to complications in genome sequence assembly efforts and in community/microbiome sampling where assembly of sequences from a mixed pool of taxa is problematic. Fortunately, several approaches have been developed to address these problems including combining Illumina data with other sources, using large insert sizes for scaffolding, and overlapping reads for metagenomic amplicon sequencing (Masella et al. 2012). Therefore, Illumina sequencing is often used in genome sequencing efforts, transcriptomics, community sampling, resequencing, target enrichment, and many more techniques.

4.2. Compact personal genome sequencers.—In an attempt to down-scale high-throughput sequencing technologies to provide a more manageable amount of data for less money, "personal genome sequencers" have been developed. These machines are less expensive to buy, use, and maintain; hence individual labs may realistically own these machines for smaller scale and exploratory sequencing experiments. The first of these, the personal genome machine (PGM), was released by Ion Torrent (Rothberg et al. 2012). This platform is unique in its scalability. Sequencing takes place on individual disposable chips that can collect variable amounts of sequence data for differing levels of cost. This method is used for small genome sequencing (e.g., organellar or prokaryotic genomes) and transcriptomes. The Illumina MiSeq is similar in application to the larger Illumina platform, but at a smaller scale. Sequencing and data analysis are integrated into a single machine and can yield analyzed data in a single day. This method is commonly applied in highly multiplexed amplicon sequencing, small genome sequencing, microbial community analysis (Caporaso et al. 2012) and for the identification of transcription factors (i.e., ChiP-Seq).

4.3. Third-generation NGS technologies.—The newest high-throughput sequencing platforms, or single molecule sequencing, include two main technologies—single molecule real-time (SMRT) sequencing by Pacific Biosciences (PacBio) and the unreleased Nanopore platform (Oxford Nanopore Technologies, Oxford, UK). These methods are characterized by two main features: 1) no PCR prior to sequencing (limiting artifacts) and 2) sequences are recorded in real-time (i.e., during the polymerase reaction or depolymerization). These methods can each yield very long reads (>5 Kb and up to 13–14 Kb for PacBio) making them useful in de novo genome sequencing efforts. Short reads from the PacBio SMRT technology can be highly accurate since the platform has the ability to resequence the circularized molecule repeatedly until base confidences are high; however, long reads have a very high endemic error rate (ca. 15%). Approaches are being developed to correct the SMRT data using large quantities of accurate but short-read Illumina data (English et al. 2012; Koren et al. 2012). The Nanopore technology is currently not widely available, so much is still unknown concerning its performance. Moreover, although SMRT methods provide much longer reads than earlier NGS approaches with considerable simplification of library preparation, neither is currently well supported by common NGS bioinformatics tools.

5. ARACHNID GENOME EFFORTS

NGS technologies allow the sequencing and reconstruction or "assembly" of whole genome sequences. Accurate genome assembly in model organisms (organisms that are amenable to genetic study, have short generation times, breed in large numbers, and can inform about other organisms) has traditionally relied upon an edifice of classical genetics resources including inbred lines to minimize genetic variation, genetic linkage maps generated from laboratory crosses among inbred lines, and the sequencing and hierarchical or clone-based assembly of large 40–200 kb genome fragments called "bacterial artificial chromosomes" (BACs) large-insert libraries (Lander et al. 2001). Arachnids, like most non-model organisms, lack most of these resources. They often have long generation times and can be very small (forcing pooling of individuals and an increase in heterozygosity, making assembly difficult). Moreover, they are generally difficult to breed in captivity and, except for some mite species, no inbred lines are available, with the possible exceptions of naturally inbred social species such as the eresid *Stegodyphus mimosarum* (J.S. Bechsgaard & T. Bilde pers. comm.) and theridiid *Anelosimus eximius* (I. Agnarsson pers. comm.).

5.1. Published genomes.—The three presently available arachnid genomes are from highly derived acarine species: the two-spotted spider mite *Tetranychus urticae* (Grbić et al. 2011), the honey bee ectoparasitic mite *Varroa destructor* (Cornman et al. 2010) and the deer tick *Ixodes scapularis* (http://iscapularis.vectorbase.org). The choice of these arachnids as early targets for genome sequencing is perhaps unsurprising; *Tetranychus* and *Varroa* are of tremendous agricultural and economic importance, and *Ixodes* is of great importance as a vector of numerous livestock and human diseases including Lyme disease. In addition to its economic importance, *Tetranychus urticae* was selected as a candidate for genome sequencing as it has the smallest known genome of any arthropod at a mere 89.6 Mbp (Grbić et al. 2011), is easily cultured in the laboratory and has inbred lines available. The small *Tetranychus* genome was sequenced using traditional Sanger sequencing methods to a depth of 8.05X, resulting in 640 scaffolds: 70,778 EST sequences plus RNA-seq data (see

below) were mapped to the genome and supported 15,397 of 18,414 gene models. The genome of the ectoparasitic mite *Varroa destructor*, which has emerged as the primary pest of domestic honey bees (Cornman et al. 2010), was "surveyed" using 4.3X coverage of 454 sequence data from the DNA of 1,000 pooled mites. This 2.4 Gbp was clearly insufficient to provide a comprehensive de novo assembly of this moderately sized genome (at 294 Mbp still far bigger than most sequenced insects) and yielded 184,094 contigs (assembled contiguous but not "scaffolded" sequences) with an N50 (weighted median of contig lengths) of 2,626 bp; however, the data were sufficient to permit the prediction of 31.3 Mbp of gene sequence, information about the integration of microbes into the genome and the occurrence of single nucleotide polymorphisms (Cornman et al. 2010). Finally, the genome of the deer tick *Ixodes scapularis*, which is very large compared to *Tetranychus* and *Varroa* at 2.1 Gbp, was shotgun sequenced using Sanger sequencing to a coverage of 3–6X. Although many data on the expressed gene sequences (i.e., the transcriptome) are available in the public databases, the genome sequence remains highly fragmented (e.g., ca. 571,000 contigs with a contig N50 of 3000 bp) and has not been officially published (http://iscapularis.vectorbase.org). Of the three acarine species, *Tetranychus* provides the most complete genome reconstruction, with genome assemblies for *Varroa* and *Ixodes* remaining highly fragmented.

5.2. Genomes in progress – what have we learned?—Apart from the three acarine species discussed above, our knowledge of the nuclear DNA structure of arachnids remains extremely limited. Most knowledge about arthropods comes from insects—a reflection of biological diversity, societal impact and economic and medical importance, and the scale of the research community, among other factors. From an evolutionary and phylogenetic perspective, this bias of course does not reflect relative importance. However, efforts such as the research community-driven I5K project (http://www.arthropodgenomes.org/wiki/Main_Page) that aims to sequence 5,000 arthropod genomes over five years should redress the balance to some extent. Even so, of 787 species currently nominated for sequencing, there are 702 Hexapoda (89%), 64 Chelicerata (8%), only 20 Crustacea (2%), and 6 Myriapoda (1%) (http://www.arthropodgenomes.org/wiki/i5K_nominations). Several arachnids have been included in the pilot sequencing project of the I5K, and these are discussed below, together with our own efforts on *Theridion* (Theridiidae) and other efforts on *Stegodyphus* (Eresidae) and *Acanthoscurria* (Theraphosidae). In addition, the genome of *Limulus* has been sequenced, and a preliminary assembly is about to be publicly released (Nipam Patel pers. comm.). However, pre-NGS we have revealed much about arachnid mitochondrial genomes, and we briefly review this here before going on to examine nuclear genomes.

Mitochondrial genomes: Although knowledge of the arachnid nuclear genome remains in its infancy, several decades of research, based upon traditional PCR and Sanger sequencing, have yielded detailed knowledge of arachnid mitochondrial genomes. This work has revealed lineage-specific gene order rearrangements in Opiliones (Masta 2010) and pseudoscorpions (Ovchinnikov & Masta 2012), and most interestingly has revealed truncated mitochondrial tRNA (and rRNA) secondary structures among most arachnid lineages (Masta & Boore 2004, 2008; Masta et al. 2008; Fahrein et al 2009; Masta 2010; Ovchinnikov & Masta 2012). NGS technologies can potentially greatly increase our understanding of the sequence diversity, variation and transcriptional mechanisms among arachnid mitochondria since 1) whole mitochondrial genomes can rapidly be sequenced from many barcoded and pooled individuals using amplicon sequencing (e.g., on small scale MiSeq or IonTorrent systems) (see below); 2) mitochondrial genomes can be assembled from total genome sequence data (Iorizzo et al. 2012); and 3) RNA-seq reads (Illumina-based method of sequencing cDNA obtained via reverse transcription of mRNA extractions) can be mapped to mitochondrial genes to explore expression differences among genes and taxa and post-transcriptional modification and editing of gene sequences (Smith 2013).

Nuclear genomes: Since no non-acarine genomes have been published so far, detailed discussion of their structure is not yet possible. Our own efforts at sequencing a spider genome have focused on the Hawaiian happy face spider *Theridion grallator* and primarily have used Illumina paired end data based upon a variety of insert sizes. Initial assemblies were highly fragmented (resulting in many contigs of short length; i.e., a low "contig N50"). Although this is partly due to heterozygosity (no inbred lines are available), the main complication appears to be that this species has a low average % GC across the genome (ca. 28%) (Fig. 1). Although most arthropod genomes are somewhat "AT-rich" (e.g., the honey bee *Apis mellifera* has 34.8% GC; The Honeybee Genome Sequencing Consortium 2006), the only arthropod genome with a % GC in the range we have found is that of the pea aphid *Acyrthosiphum pisum* at 29.6% GC (The International Aphid Genomics Consortium 2010).

A potential extreme % GC bias in arachnid genomes is both intriguing and technically challenging from both an informatic and molecular biological point of view. In order to investigate this further, we have examined the assembled contigs data, where available, from the pilot runs for the I5K project (http://www.arthropodgenomes.org/wiki/Main_Page). In total we have examined the contig N50 length and % GC from two I5K sequenced spiders, *Latrodectus hesperus* and *Parasteatoda tepidariorum* (Theridiidae), and the Arizona bark scorpion *Centruroides sculturatus* (Buthidae), together with 15 other arthropods (14 insects and one copepod; ftp://ftp.hgsc.bcm.edu/I5K-pilot/), and these are plotted in Fig. 1. In addition we have included our data from *Theridion grallator* (PJPC unpubl. data) and data from *Stegodyphus mimosarum* (Eresidae: J.S. Bechsgaard & T. Bilde, pers. comm.). The scorpion and the three theridiid spiders (*L. hesperus*, *P. tepidariorum* and *T. grallator*) all have less than 30 % GC and correspondingly low contig N50 lengths. In general, the lower the % GC, the shorter the contig N50 as a simple function of decreased information content available to the assembly algorithms. Interestingly, the *P. tepidariorum* sequenced had been through five generations of inbreeding (A. McGregor pers. comm.)—apparently sufficient to reduce heterozygosity enough to substantially increase contig lengths despite a low % GC.

Alternatively, *S. mimosarum* did not exhibit an extreme % GC bias (34% GC) and as a soeial species (Mattila et al. 2012) is somewhat naturally inbred—and has a correspondingly

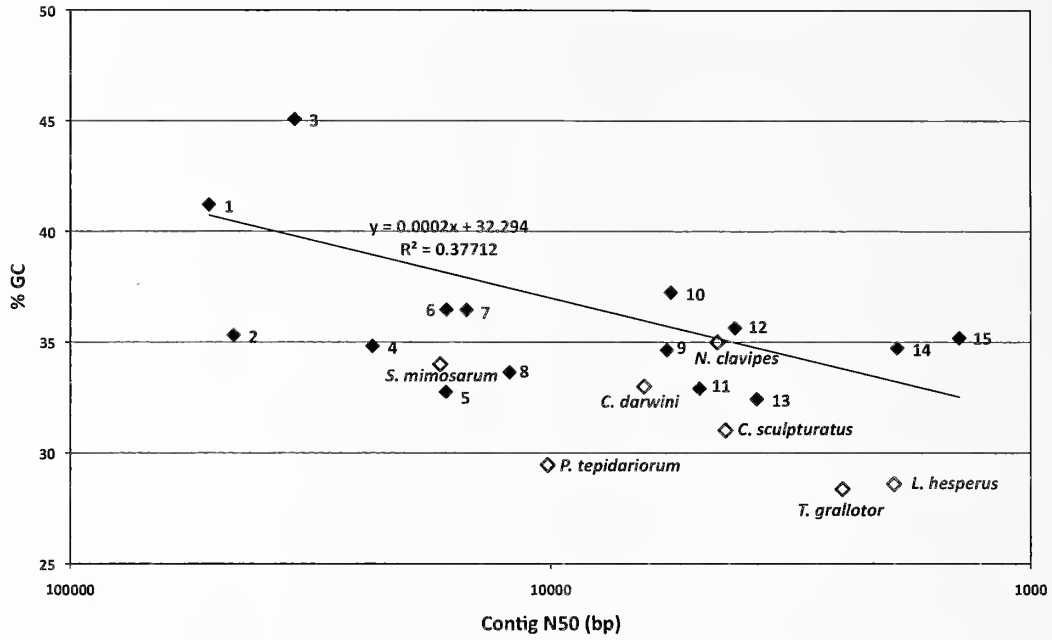

Figure 1.—Assembly contig N50 length (bp) is negatively correlated with average genome-wide %GC bias among arthropod taxa. As average %GC decreases so does the contig N50 (weighted median of contig lengths), since lower information content leads to more fragmented assemblies. Open diamonds refer to arachnid genome projects and closed diamonds refer to other arthropods. *Theridion grallator* data are calculated from the authors' own (unpublished) preliminary genome assembly and have the lowest %GC to date (28.37%). *Stegodyphus mimosarum* values from J.S. Bechsgaard (pers. comm.). *C. darwini* and *N. clavipes* values from I. Agnarsson (pers. comm.). All other values estimated from initial contig (not scaffolded) assemblies of the I5K initiative pilot genome assemblies; data and assembly parameters are therefore similar among species (ftp.hgsc.bcm.edu/I5K-pilot/). Although all arthropods show a bias toward low %GC (<32%), the theridiid spiders *T. grallator*, *L. hesperus* and *P. tepidariorum*, as well as the scorpion *C. sculpturatus*, have very low %GC. *S. mimosarum* has a more moderate bias (34% GC), and both this species and *P. tepidariorum* show the benefit of inbreeding and low-heterozygosity and have longer contig N50 lengths than the other arachnids. Additionally, the remaining non-theridiid spiders, *C. darwini* and *N. clavipes*, have moderate %GC values but low contig N50s (<10,000 bases), possibly due to heterozygosity stemming from the lack of inbreeding. The included insects are 1) *Athalia rosae* (turnip sawfly: Insecta: Hymenoptera); 2) *Ceratitis capitata* [Mediterranean fruitfly (medfly): Insecta: Diptera]; 3) *Orussus abietinus* (parasitic wood wasp: Insecta: Hymenoptera); 4) *Cimex lectularius* (bed bug: Insecta: Hemiptera); 5) *Anoplophora glabripennis* (Asian long-horned beetle: Insecta: Coleoptera); 6) *Libellula fulva* (scarce chaser: Insecta: Odonata); 7) *Helicoverpa punctigera* (Australian bollworm: Insecta: Lepidoptera); 8) *Ephemera danica* (green drake mayfly: Insecta: Ephemeroptera); 9) *Agrilus planipennis* (emerald ash borer: Insecta, Coleoptera); 10) *Copidosoma floridanum* (chalcid wasp: Insecta: Hymenoptera); 11) *Homalodisca vitripennis* (glassy-winged sharpshooter: Insecta: Hemiptera); 12) *Leptinotarsa decemlineata* (Colorado potato beetle: Insecta: Coleoptera); 13) *Eurytemora affinis* [copepod: Maxillopoda (Crustacea): Calanoida]; 14) *Limnephilus lunatus* (caddisfly: Insecta: Trichoptera); 15) *Pachypsylla venusta* (hackberry petiole gall psyllid: Insecta: Hemiptera).

much better assembly contiguity (J.S. Bechsgaard & T. Bilde pers. comm.). Furthermore, initial sequencing of the huge (6 Gbp) genome of the Brazilian white knee tarantula *Acanthoscurria geniculata* (Theraphosidae) indicates that this species has a ca. 40% GC genome content (J.S. Bechsgaard & T. Bilde pers. comm.). Until more arachnid genome sequence becomes available, the question as to how widespread %GC-bias is among arachnids will remain unclear. From the above data it may appear to be specific to theridiid spiders and *Centruroides* scorpions; however it is tempting to speculate that extreme %GC-bias may extend to other spider families and other arachnid orders. This possibility should be considered in future genome-sequencing efforts, and we note that transcriptome assembly (RNA-seq) is unlikely to be so impacted by %GC-bias, since coding regions typically do not exhibit such extreme biases.

5.3. A cautionary note.—Despite the allure of NGS technologies, some caution is needed before embarking on a project to sequence an arachnid genome. Particular questions a researcher working on a specific taxon should pose are: 1) Do we need a genome sequence? And if so, 2) how complete do we need it to be? And, perhaps more fundamentally, 3) what level of completeness can we attain without spending an unreasonable amount of resources? In reality, no genome sequence (including human) is fully complete, and de novo assembled and NGS derived genomes are even less so. De novo assembly of short-read shotgun sequence data without references, such as linkage maps or BAC libraries, remains extremely challenging. However, as the *Tetranychus*, *Varroa,* and *Ixodes* projects demonstrate, a fractured assembly may still be useful if it is contiguous enough to build valid gene models. In addition to life history and often body-size considerations (i.e., the need for pooling individuals), intrinsic features of arachnid genomes—in particular, the low % GC content in some lineages mentioned above—might raise a substantial barrier to whole genome de novo assembly projects.

Even though the cost of NGS sequencing continues to drop rapidly, depending upon the biological question, either classical genetic marker-based approaches (Section 3 above)

may be cheaper, easier, and sufficient, or NGS based alternatives to genome sequencing may be more attainable (e.g., transcriptome sequencing, and reduced representation methods). Indeed these approaches may even be best used as a means to rapidly develop numerous classical markers or identify single nucleotide polymorphisms (as discussed in Section 6). RNA-seq (the sequencing of cDNA libraries derived from extracted mRNA and hence targeting transcribed and therefore mainly coding regions—the transcriptome) is rapidly becoming the tool of choice in genomic studies. This is because RNA-seq data permits one both to build gene models rapidly and to measure "digital" gene expression among taxa and tissues; consequently the technique has many potential applications.

Although "complete" genome sequences, even fragmented ones, will yield fascinating information about genome structure (repeats, transposons, translocations, etc.), to be of greatest functional utility genomes must be annotated. While computational annotation of gene models is possible (although of course not optimized for arachnids), most annotation schemes work best when supported by sequence evidence. Again, RNA-seq and transcriptome data are of greatest utility here and thus should also be generated for the taxon whose genome is sequenced. Since RNA-seq data can be assembled de novo, for example using software Trinity (Grabherr et al. 2011), and annotated by homology searches (at least for genes where known homologs exist) (e.g., using BlastX and Blast2GO; Conesa et al. 2005), the experimenter must again ask whether a full genome sequence is required at all, and be cautious about assuming that this is a practicable route.

6. APPLICATIONS OF NGS TECHNOLOGIES IN ARACHNOLOGY

The number of possible applications using NGS technologies is vast and continues to grow. Here we provide examples of their use, most of which do not require the sequencing of entire genomes. There are many more potential applications than those discussed below, and, as new platforms and bioinformatic tools are developed, new avenues of research will open.

6.1. Functional genomics: adaptation & selection.—Biologists frequently seek to elucidate the relationship between environmental parameters and organismal diversity. The potential for detailing the genetic response of an organism to changes in the biotic and abiotic environment are now in plain sight with the availability of vast quantities of DNA sequence that can be generated by NGS technologies, in particular through the "assembly" of whole genome sequences. A review by Stapley et al. (2010) discusses the potential of high-throughput technologies in studies of adaptation. Whether focusing on coding gene sequences, differential expression of transcripts, identifying genomic regions experiencing linkage disequilibrium (LD), or quantitative trait locus (QTL) mapping to detect genomic regions under selection, established methods using high-throughput data exist.

Measures of selection: When studying protein-coding loci, the most common method for measuring selection involves the ratio of nonsynonymous to synonymous changes (dN/dS or ω). The resulting value potentially indicates the mode of selection acting on the gene: $\omega = 1$ (neutral selection), $\omega < 1$ (stabilizing selection) and $\omega > 1$ (positive selection). By employing a likelihood ratio test, *P*-values can be obtained to differentiate between neutral and directional selection in pairwise comparisons. Additionally, comparisons of ω between branches in a multi-species/population phylogeny are possible to identify genes or residues evolving differently or similarly between clades. Inherently, ω-based tests for selection require coding data and are best served by transcriptomic data. Commonly used tools for analysis of these data include PAML (Yang 2007) and HyPhy (Pond et al. 2005). Some studies have employed ω tests in arthropods (e.g., Averof 2002; Porter et al. 2006; Viljakainen et al. 2009; Fort et al. 2011), recently including spiders (Brewer et al. in review; Yim et al. in prep).

To collect the data necessary for investigating selection in coding sequences, RNAseq libraries are often generated. To obtain the most nearly unique sequence possible in a single run, the resulting cDNA libraries can be normalized by removing excessive copies of highly expressed transcripts to "equalize" the numbers with respect to the more poorly-expressed transcripts (Zhulidov et al. 2005), but normalizing is not essential. In addition to retrieving sequences, non-normalized RNAseq libraries provide information concerning the expression levels of transcripts. In order to leverage this information, specimens must be treated to control all variables so that the sources of differential expression (DE) can be identified. Methods to analyze expression data using RNA-seq data include the R packages "edgeR" (Robinson et al. 2009) and "DEseq" (Anders & Huber 2010). Differences in expression of transcripts between populations or species indicate the evolution of coding loci involved in the expression of a gene or non-coding regions of the genome that affect the transcription (i.e., promoters, enhancers, and suppressors). These methods are currently being employed in Hawaiian *Tetragnatha* spiders to study differences in venom composition between a lineage that builds webs compared to one that does not build webs (Brewer et al. in review), building on earlier work that used protein gel electrophoresis patterns to show coarse differences between these lineages (Binford 2001). With NGS techniques, we are now able to explore the individual genes and relative changes in expression levels.

Selection can also be examined using LD approaches, although this is necessarily limited to taxa where full genomes are available. By mapping SNPs to a reference genome, data obtained using reduced representation techniques (e.g., RAD-seq) can be used to detect regions of the genome under strong LD. This method has been used to identify regions of the genomes of stickleback populations that are resistant to introgression of outside genes (Hohenlohe et al. 2010). Unfortunately, RAD-seq methods require high quality and high quantity gDNA, which is often limited in small organisms such as many spiders, even when freshly collected (Cotoras unpubl. data).

Molecular basis for adaptation: Perhaps the most important applications of NGS technologies in arachnids relate to silk and venoms, two aspects of the biology of these organisms that provide an almost endless variety of questions relating to gene function. Both silks and venoms comprise complex combinations of highly-derived, and often highly repetitive,

proteins that serve myriad functions within and between taxonomic groups. Both are linked to major ecological shifts and evolutionary modifications in a number of clades. The evolution of the forms and functions of spider silks has great potential in evolutionary studies, as well as bioengineering applications (Blackledge 2012; Garb 2013). Tools such as SMRT and Nanopore, with their long reads, could help to alleviate assembly issues associated with the highly repetitive elements and allow more detailed exploration of the diversity of spider silks at the genomic level. Venoms also vary greatly across the Arachnida and are found in several orders (e.g., Araneae, Scorpiones, and Pseudoscorpiones). Beyond differential expression analyses, such as that described above, characterization of venom cocktails and their molecular evolution is lacking in most groups. Most work done so far has focused on medically relevant species such as those in the spider genera *Latrodectus* (e.g., Garb & Hayashi 2013) and *Loxoceles* (e.g., Zobel-Thropp et al. 2013) and the scorpion genus *Centruroides* (e.g., Valdez-Velázquez et al. 2013). In an applied context, these compounds have vast potential in pharmacology and as pest control substances (reviewed by King and Hardy 2013). Moreover, as mentioned above, these compounds may also provide insights into the factors underlying adaptation and how selection acts at the transcriptional level (Binford 2001).

6.2. Phylogenetics.—To date, most molecular studies of arachnids have sought to ascertain relationships between taxa. Until recently, assessment of the phylogenetic affinities of an organism required PCR amplification with degenerate primers followed by amplicon sequencing to study loci in distantly related taxa. The weakness of this approach is that rather few loci can be examined, limiting the resolution of the Tree of Life. Thus, the internal phylogeny of the subphylum Chelicerata, class Arachnida, and lower taxonomic levels has remained unresolved despite numerous efforts to ascertain the relationships between taxa, including molecular phylogenetic studies (recently reviewed in Agnarsson et al. 2013; Giribet and Edgecombe 2013). Mitochondrial sequences have been the most common data source. However, for the reasons mentioned above (3.1), mitogenomic sequence data may not be appropriate for reconstructing deep arthropod relationships (Brewer et al. 2013). For example, although the Euchelicerata (Xiphosura + Chelicerata) is almost unambiguously recovered using nuclear loci, datasets using mitochondrial genomic data often fail to support this relationship (Masta et al. 2009; Rota-Stabelli 2010).

Within the Arachnida, most molecular phylogenetic studies have focused on spiders, including the relationships within the subclasses Mygalomorphae (Hedin and Bond 2006; Bond et al., 2012) and Araneomorphae (Blackledge et al. 2009). Molecular phylogenetic analyses within other orders exist, including the Opiliones (Hedin et al. 2010; Hedin et al. 2012; Burns et al. 2013), Acari (Klompen et al. 2007; Dabert et al. 2010; Pepato et al. 2010), Scorpiones (Salomone et al. 2007; Borges et al. 2010; Prendini & Esposito 2010) and Amblypygi (Esposito et al. in review). Representing a small sampling of published works, all of these studies except Hedin et al. (2012) use traditional Sanger sequencing approaches. Even at these lower taxonomic levels, nuclear molecular markers with appropriate phylogenetic signal are lacking, and primer combinations for PCR often do not transfer between arachnid groups, especially for species/population-level appropriate loci.

High-throughput sequencing technologies provide a means to collect vast amounts of molecular data for many taxa in a timely manner and are currently used in various ways in phylogenetics (see McCormack et al. 2013 and Rocha et al. 2013). The potential use of some NGS technologies in spider systematics was recently discussed by Agnarsson et al. (2013) and in Opiliones by Hedin et al. (2012). As for most non-model organisms, the most common NGS data sources for deep phylogenetics in arachnids are transcriptomes (Agnarsson et al. 2013) and information generated from bait capture techniques (for all taxonomic levels) such as anchored enrichment (Lemmon et al. 2012). These approaches do not require full genome sequences, which is especially useful given the potential difficulties with arachnid genome efforts mentioned above; moreover, the data generated provide loci that are relatively easy to assign orthology and can be used at deep taxonomic levels. Tools for the assignment of orthology include HaMStR (Ebersberger et al. 2009), OrthoDB (Waterhouse et al. 2012) and AGALMA (Dunn et al. 2013), while PhyDesign (López-Giráldez and Townsend 2011) can be used to investigate the phylogenetic signal of a locus across an ultrametric tree. Recent molecular models of evolution (e.g., CAT, Lartillot and Phillippe 2004) and algorithms for phylogeny reconstruction (e.g., Phylobayes, Lartillot et al. 2009; RAxML, Stamatakis 2006; and Fasttree 2, Price et al. 2010) have made phylogenomic studies much more tractable. However, these analyses still can take weeks of computation time, require large amounts of computer memory, and demand a somewhat deep understanding of bioinformatics.

6.3. Population genetics & phylogeography.—NGS approaches have been widely celebrated for their potential in providing large numbers of markers across the genome, which is essential for population genetic and phylogeographic studies. Since the per base cost is much lower than for Sanger sequencing, it has become economical to apply NGS techniques to generate traditional markers [e.g., microsatellites in a tetragnathid species (Parmakelis et al. 2013)].

Among the most useful tools for population genetics, and phylogenetics for that matter, are those based on reduced representation libraries (RRLs), which attempt to recover a small, random (i.e., unlinked) snapshot of the total genome. As a result of focusing on a small sample of the genome, the cost of sequencing a single individual is greatly reduced and yet RRL methods can still identify many thousands of usable single nucleotide polymorphisms (SNPs).

RADseq is a popular method for genome-wide marker analysis because it reduces the complexity of the genome by sub-sampling at certain restriction sites, assumed to be homologous among taxa/specimens, to generate a single nucleotide polymorphism (SNP) data set. The approach is much like RFLPs and AFLPs, except that, instead of separating the fragments on a gel to recover a DNA fingerprint, they are sequenced (Davey et al. 2011). This approach can provide several SNPs from each fragment, multiplying the amount of data obtained from a single run. A recent modification of this technique uses a double-digestion and yields an increase in efficiency and a reduction in cost

(Peterson et al. 2012). However, for many arachnid groups, the RADseq method has requirements that may limit its use. First, a large amount of high molecular weight DNA is required (>2 micrograms per sample). Such high quality DNA is essential in order to generate fragments that result only from the restriction enzyme digest (i.e., library adaptors are not ligated to the end of randomly sheared/degraded fragments). Moreover, a high starting concentration of DNA is necessary, since the protocol involves many steps that result in the loss of DNA—typically only 7–15% of the starting material will be recovered. The issue of DNA quality can be resolved by preserving samples in 95% ethanol at -80 C, RNAlater at −80 C, or by using fresh specimens. Standard extraction kits using ion-exchange columns or salt precipitation should work well without causing undue shearing of the DNA. Ultimately, DNA yield depends on the organism and although large-bodied arachnids (e.g., many mygalomorphs, scorpions, amblypigids, or solfugids) may yield sufficient DNA, smaller taxa may require specimens to be "pooled" together, thus losing individual-level data (Emerson et al. 2010).

An alternative RRL approach to RADseq is to use bait capture methods, such as Exon Capture, which, in contrast to RADseq, requires starting material to be randomly fragmented (Bi et al., 2012). The basic approach is to sequence the transcriptome of one individual and use those sequences to design small, overlapping probes that are then attached to a capture array ("chip") or beads. The protocol starts with either naturally (i.e., degraded or historical) or intentionally fragmented DNA, which is used to prepare DNA libraries following a standard NGS protocol (e.g., Meyer & Kircher 2010). These libraries are barcoded for each individual and used in a hybridization experiment similar to a microarray. The number of individuals that can be multiplexed in these experiments depends on the target size in base pairs (i.e., the number of bases printed on the chip), the desired depth of coverage and the availability of barcodes. The number of single barcodes commercially available is currently 96, but by using eight double barcodes this can be increased to 768. This theoretically allows the parallel sequencing of thousands of loci in hundreds of individuals at the same time. One advantage of exon capture for non-model organisms is that the sequences for the array are obtained directly from a transcriptome and do not require a previously sequenced genome. Moreover, the protocol is suitable for historical museum samples, since it explicitly requires randomly fragmented DNA, whieh is often the natural state for museum-derived material.

There are two main limitations to the Exon Capture approach. The first is that starting costs are high (reagents and specialized equipment not common in most laboratories), though they can be minimized by sharing among research groups. Second, as in most NGS applications, sophisticated expertise in bioinformatics is needed to manage the large and complex data sets. Fortunately, user-friendly programs and tools are becoming increasingly available for post-NGS processing and analyses. Since exon capture targets exons, most of the captured variation will correspond to synonymous mutations in coding genes, allowing insights into population variability. However, because genomic DNA is captured, some of the non-coding flanking regions (e.g., untranslated regions, introns) will also be recovered.

7. CONCLUSION

Arachnids have a rich history of molecular studies focusing on many aspects of their biology. To date, few of these have made use of recent advances in sequencing technology, but, as we have outlined above, many future projects should benefit from the use of next-generation sequencing platforms. These technologies are diverse in their methods and applications, and promising advances are on the horizon. However, it is important to realize the strengths and weakness of NGS tools and to embrace traditional techniques when more appropriate.

Although it is easy to be seduced by the amount of data that can be generated by sequencing an entire genome, this is often not necessary. In many cases, studies using transcriptomes or reduced representation techniques can collect incredible amounts of useful data to address any number of questions. Regardless of the study, the number of potential avenues to gather molecular data is large in terms of strategy and scale. As arachnologists continue to amass novel data from diverse lineages, our ability to identify loci, in terms of function and homology, will increase and open more research opportunities. The unique biology and evolutionary history of arachnids, coupled with technological and bioinformatic advances, will provide research opportunities for years to come.

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A new spider (Araneae: Haplogynae: Plectreuridae) from the Cretaceous Fossil-Lagerstätte of El Montsec, Spain

Paul A. Selden: Paleontological Institute and Department of Geology, University of Kansas, 1475 Jayhawk Boulevard, Lawrence, Kansas 66045, USA, and Natural History Museum, Cromwell Road, London SW7 5BD, UK. E-mail: selden@ku.edu

Abstract. Two specimens of a new spider from the Cretaceous (ca. 129 Ma) El Montsec Fossil-Lagerstätte, northeastern Spain, are described as *Montsecarachne amicus* gen. et sp. nov. and referred to the extant haplogyne family Plectreuridae. Plectreurids are found today in only in the southwestern USA, Mexico, Central America and Cuba, but fossils show a more widespread distribution in Eurasia. The new species adds an additional stratigraphic and biogeographical record. The paleobiogeographical history of plectreurids is discussed, and it is concluded that the most likely scenario is that the family was more widespread in the past, and has suffered extinction over much of its range, resulting in the present distribution. Possibly, extant plectreurids represent a living remnant of a more diverse group of haplogynes that were widespread in the Mesozoic.

Keywords: Mesozoic, paleobiogeography, Pholeoidea

Spiders from the Cretaceous Fossil-Lagerstätte (locality of exceptional fossil preservation) of El Montsec, Spain, were among the earliest Mesozoic Araneae to be described (Selden 1989, 1990). In these works, both araneoid and deinopoid orbweavers were recognized, superfamilies that belong to the Entelegynae Simon 1893 of the infraorder Araneomorphae Smith 1902. Within this infraorder, the Haplogynae Simon 1893 are considered more primitive spiders, and the new species described here belongs to that group. The two specimens, conspecific adult males, come from the lithographic limestones of earliest Barremian age (ca. 129 Ma), from the locality of La Cabrua in the Sierra de Montsec, northeast Spain (Selden & Nudds 2012).

The fossils show distinctive characters, which allow them to be placed in the modern haplogyne superfamily Pholcoidea Koch 1850. This superfamily includes three families with the sister-group relationship: Pholcidae Koch 1850 (Diguetidae Pickard-Cambridge 1899, Plectreuridae Simon 1893) (Dunlop & Penney 2011). Among these, the fossil species most closely resembles the relatively plesiomorphic haplogyne family Plectreuridae. The fossils are placed in a new genus and speeies, *Montsecarachne amicus* gen. et sp. nov., in Plectreuridae.

Today, plectreurids are restricted in their distribution to southwestern North America, Central America and the Caribbean, but fossils show a more widespread distribution in Eurasia. The new species described here, from the Cretaceous of Spain, adds an additional biogeographical record. It is possible that extant Plectreuridae represent a living remnant of a group of haplogynes which were widespread in the Mesozoic.

STRATIGRAPHY AND PALAEOECOLOGY

The specimens come from the outcrop known as La Cabrua, which is an old quarry on the track between Rúbies and Santa Maria de Meià, just above the Sant Sebastia hermitage, in the Sierra de Montsec mountain range (Bataller et al. 1953). They were collected during an expedition from the Institut d'Estudis Ilerdencs, Lleida, Spain.

The lithographic limestones of El Montsec were mapped as a subunit of the Calcaires à Charophytes du Montsech (Peybernès & Oertli 1972). Marine fossils such as rudist bivalves and foraminiferans occur beneath and above the Calcaires à Charophytes du Montsech. Studies on regional stratigraphy, charophytes, and fossil assemblages have shown that the age of the El Montsec lithographic limestones is earliest Barremian (Gomez et al. 2002). The lithographic limestones are rhythmically laminated carbonate mudstones without any sign of currents or emersion features such as raindrop marks or mud cracks. The exceptional preservation of soft tissues, skeletons undisturbed by scavengers or decomposers and random orientation of fossils, all point to deep water, probably an anoxic lake bottom. The rhythmic laminations are typical of lacustrine conditions and reflect seasonal changes in the lake environment. Although the lake was close to the sea, there is no evidence of a permanent connection; e.g., in the form of marine fossils (De Gibert et al. 2000).

METHODS

The fossils are preserved on thin slabs of pale buff-grey limestone. Grains are not visible in the rock, and the hackly fracture and vitreous appearance under high-power microscopy suggest crystallization from a lime mud. Calcite-filled cracks cross the specimens. The spiders are preserved as pieces of cuticle on, and slightly within, the bedding surface. The cuticle is brittle and brown: thicker parts are deep brown and the thinnest cuticle pale buff. The cuticle is presumed to be preserved as kerogen (Gupta et al. 2008). The best-preserved parts are visible through a thin layer of translucent limestone, but their morphological details are hazy due to the presence of the overlying matrix. In such instanees, 2–4% hydrochloric acid was used, sparingly and with care, to remove the matrix and thus to reveal fine structural details. The specimens are fairly complete, with part and counterpart available for both.

The specimens were studied and photographed under 70% ethanol (to enhance contrast) using a Leica MZ16 stereomicroscope, and photographed using a Canon EOS 5D MkIII

digital camera attached to the microscope and DSLR Assistant software (www.kaasoft.com) on an Apple MacBook Pro computer. Photographs were manipulated using Adobe Photoshop software, and final drawings were made from the photographs using Adobe Illustrator. All measurements are in millimeters and were made from the drawings using Photoshop's analysis tools. Measurements of paired organs are means of left and right of part and counterpart; i.e., maximally four measurements if all are preserved.

Leg formula (e.g., 1423) indicates the length of each leg relative to the others, from longest to shortest. Abbreviations: car carapace, ch chelicera, cx coxa, fe femur, L length, lb labium, mt metatarsus, mx maxilla, op opisthosoma, pa patella, Pd pedipalp, sp spinnerets, st sternum, ta tarsus, ti tibia, W width.

MORPHOLOGICAL INTERPRETATION

The two specimens are both adult males with identical pedipalp morphology; they differ slightly in size (the holotype is slightly larger), but otherwise any other morphological differences can be explained by preservation, so they are considered to be conspecific. Like other spider specimens from this locality, and others from similar Mesozoic lacustrine deposits (e.g., the Daohugou Lagerstätte of China (Selden & Huang 2010), only cuticular structures are preserved, dorsal and ventral superimposed. After splitting the rock, part and counterpart may bear mainly dorsal or ventral structures, but may show structures from both surfaces superimposed, or some structures split between part and counterpart. For example, LC-2936 B and LC-3780 B show mostly dorsal features of the body (Figs. 1C, 3C), while their counterparts, LC-2936 A and LC-3780 A (Figs. 1A, 3A), show predominantly ventral body features. Nevertheless, chelicerae, pedipalps, and legs occur on all specimens, in some cases ventral, others dorsal, and in places both sides superimposed. As with most matrix-preserved fossil spiders, eyes are very difficult to discern on the carapace. Commonly in fossil spiders, the chelicerae splay apart during compression (e.g., in the palpimanoids from Daohugou: Selden et al. 2008). This phenomenon is not seen in the Montsec specimens, however, which show a distinct overlap in the basal half; this suggests cheliceral fusion, which is common in haplogyne spiders.

A peculiar feature of LC-2936 is an elongate piece of setose cuticle running along the length of the tibia of leg 4 (on the right in the part, LC-2936 A). The cuticle is identical in pattern to that of the podomere and most likely belongs to the animal. Since it is an adult male, which would not undergo post-adult molting, the fossil represents a carcass, and so this feature can best be explained as part of the podomere disrupted post mortem, possibly by decay or scavenging.

SYSTEMATIC PALEONTOLOGY

Order Araneae Clerck 1757
Suborder Opisthothelae Pocock 1892
Infraorder Araneomorphae Smith 1902
Haplogynae Simon 1893

Remarks.—A number of features identify this species as a member of the Haplogynae (see Table 1), including the distinctive, pyriform pedipalpal bulb and embolus; swollen male pedipalpal tibia; basally fused chelicerae and convergent pedipalpal endites (maxillae). Also, the general habitus, with legs of subequal length and low carapace, is haplogyne in appearance.

Superfamily Pholcoidea Koch 1850
Family Plectreuridae Simon 1893

Remarks.—The characters of swollen male pedipalpal tibia, chelicerae fused basally, and convergent (parallel) maxillae are indicative of the pholcoid–scytodoid branch of the Haplogynae (Platnick et al. 1991; Griswold et al. 2012; table 1). Plectreurid characters are unfused labium and sternum (also shared with Periegopidae), and legs bearing macrosetae. The distinctive tarsal bristles (Figs. 3E,F) are not unique to plectreurids among haplogynes (e.g., figures in Labarque & Ramírez 2012), but very similar bristles were described and figured by Gertsch (1958, fig. 9). The new fossils show particular resemblance to the extant *Plectreurys castanea* Group, in having a short embolus, and the Jurassic *Eoplectreurys gertschi* Selden & Huang 2010 in lacking onychium, subsegmented tarsus and cheliceral stridulatory ridges.

Genus ***Montsecarachne*** new genus

Diagnosis.—Distinguished from all other plectreurids, except those belonging to the *Plectreurys castanea* Group (Gertsch 1958), by its short embolus; all except *Kibramoa* Chamberlin 1924 and *Palaeoplectreurys* Wunderlich 2004 by its lack of a spur on the tibia of leg 1 of the male; from *Kibramoa* by the robust femur, as long as the carapace; and from *Palaeoplectreurys* by the pedipalp and the presence of additional spines.

Etymology.—After the type locality, El Montsec, and the Greek ἀράχνη (L. *arachne*), a spider.

Type species.—*Montsecarachne amicorum* n. sp. (monotypic).

Montsecarachne amicorum new species
Figs. 1–3

Etymology.—Latin *amicus*, a friend, in honor of the Amics de la Paleontologia (Friends of Paleontology), who began the series of organized excavations at the quarry of La Cabrua in the 1970s and discovered so many of the exciting fossils from the Fossil-Lagerstätte of El Montsec (Selden & Nudds 2012).

Type series.—Holotype adult male, LC-3780 IEI A,B (part and counterpart); paratype LC-2936 IEI A,B (part and counterpart) adult male; from lithographic limestones within the Calcaires à Charophytes du Montsech, of Cretaceous (earliest Barremian) age, in the quarry of La Cabrua, Sierra de Montsec, northeast Spain; deposited in the Institut d'Estudis Ilerdencs, Lleida, Spain.

Diagnosis.—As for the genus.

Description.—Holotype and paratype, adult males. Body thickly clothed in fine setae. Macrosetae on legs, especially distally. Carapace subcircular, very slightly longer than wide, narrowed somewhat anteriorly; opisthosoma suboval, ca. 1.25× longer than wide (Figs. 1, 3). Sternum suboval, ca. 1.15× longer than wide, gently scalloped around coxae; labium approximately pyriform, slightly longer than wide, separate from sternum; maxillae longer than wide, convergent and meeting in front of labium (Fig. 3A,B). Chelicerae parallel, elongate, porrect, apparently conjoined in basal half; fang short, curved; small tooth on paturon adjacent to fang tip

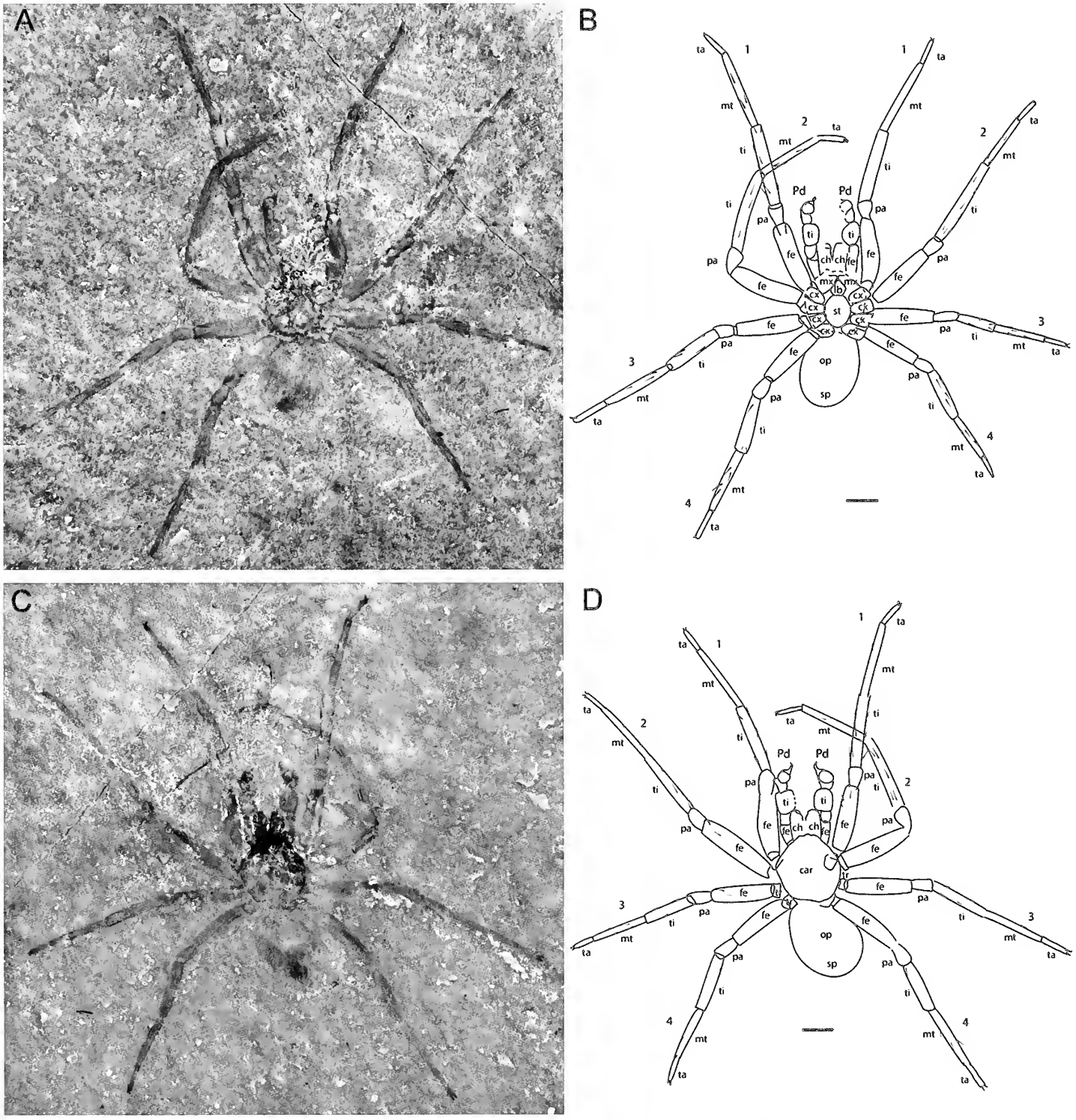

Figure 1.—*Montsecarachne amicorum* gen. et sp. nov.: A. photograph of holotype part LC-3780 IEI A; B. explanatory drawing of A; C. photograph of holotype counterpart LC-3780 IEI B; D. explanatory drawing of C. Scale bars = 1 mm.

(Fig. 2A). Pedipalp approximately equal in length to leg 1 femur + patella; tibia swollen, bulb subspherical, embolus narrows rapidly, from wide base, in short spiral to sharp pointed apex. Leg formula 2134; legs subequal in length. Long podomeres (femora, tibiae and metatarsi) approximately equal in length (Figs. 1, 3). Leg 1 femur slightly curved, approximately equal in length to carapace length. Few, thin macrosetae on femora; distal femur of leg 1 with two strong, curved macrosetae mesiodistally, another in midline, adjacent to swollen pedipalp tibia (Fig. 1C,D). No macrosetae on patellae; tibiae and metatarsi with numerous, stronger macrosetae along their lengths; macrosetae at distal joints of metatarsi. Tarsi without macrosetae but with strong bristles distally, especially two or three large ones dorsally at distal

Table 1.—Comparison of morphological features of *Montsecarachne anticorum* gen. et sp. nov. with other Plectreuridae and selected haplogyne families. See Ubick (2005), Labarque & Ramírez (2012), and Griswold et al. (2012) for details. Notes: [1]mostly, [2]blade-like morphology, [3]flexibly according to Ubick (2005).

	Montsecarachne	*Eoplectreurys*	*Palaeo-plectreurys*	*Plectreurys tristis* Group	*Plectreurys castanea* Group	*Kibramoa*	*Diguetidae*	Pholcidae	Scytodidae	Drymusidae	Sicariidae	Periegopidae
Swollen ♂ pedipalp tibia	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓[1]	✓	✓
Chelicerae fused basally	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Maxillae convergent	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stridulatory file (some ♀ only)	✗	✗	?	✗	✗	✗	✗	✗	✗	✗	✗	✗
Sternum elongate	✗	✗	✗	✗	✗	✗	✓	✓	✓	✗	✓	✗
Labium broad/ subtriangular (✗), or elongate (✓)	✗	✗	✗	✗	✗	✗	✓[2]	✗	✗	✗	✗	✗
Subsegmented tarsus 1	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tarsal claws 3 (✓), 2 (✗)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓[3]	✓
Onychium present	✗	✗	?	✓	✓	✓	✓	✓	✓	✗	✗	✗
Long, thin embolus	✗	✓	✓	✓	✗	✓	✓	✗	✓	✗	✗	✗
Labium & sternum fused	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Legs with macrosetae	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗
Fe 1 robust, curved ≤ carapace	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗
Tibial spur on ♂ tibia 1	✗	✓	✗	✓	✓	✗	✗	✗	✗	✗	✗	✗
Other spines on ♂ tibia 1	✓	✓	✗	✓	✓	✓	✗	✗	✗	✗	✗	✗

end above claws (Fig. 3E, F). Tarsal paired claws pectinate, small unpaired claw present (Fig. 3E, F). Calamistrum absent. Spinnerets subterminal on opisthosoma; short, one pair much larger than the others (Figs. 1A, 3A).

Measurements of LC-3780 IEI: body length (inc. ch) 5.36; car L 2.13, W 1.99, ratio 1.07; op L 2.62, W 1.93, ratio 1.36; st L 1.01, W 0.86, ratio 1.17; lb L 0.46, W 0.46; mx L 0.65, W 0.50; ch L 0.89. Podomere lengths: Pd fe 0.75, pa 0.40, ti 0.73, ta 0.46, bulb L 0.51, W 0.38, total 2.60; leg 1 cx 0.66, fe 2.11, pa 0.72, ti 2.41, mt 2.42, ta 0.90, total fe–ta 8.56; leg 2 cx 0.73, tr 0.19, fe 2.32, pa 0.81, ti 2.54, mt 2.07, ta 0.89, total fe–ta 8.62; leg 3 cx 0.62, tr 0.20, fe 2.02, pa 0.77, ti 1.68, mt 1.94, ta 0.95, total L fe–ta 7.35; leg 4 cx 0.69, tr 0.22, fe 2.03, pa 0.76, ti 1.77, mt 1.85, ta 0.94, total L fe–ta 7.34.

Measurements of LC-2936 IEI: body length (inc. ch) 5.01; car L 2.04, W 2.02, ratio 1.01; op L 2.30, W 2.02, ratio 1.14; st L 1.14, W 0.96, ratio 1.19; lb L 0.48, W 0.34; mx L 0.68, W 0.47; ch L 0.72. Podomere lengths: Pd fe 0.71, pa 0.37, ti 0.77, ta L 0.34, bulb L 0.66, W 0.40, total 2.59; leg 1 tr 0.21, fe 2.04, pa 0.84, ti 2.16, mt 1.90, ta 1.01, total fe–ta 5.41; leg 2 tr 0.23, fe 2.36, pa 0.66, ti 2.13, mt 2.16, ta 1.01, total fe–ta 5.66; leg 3 tr 0.23, fe 2.00, pa 0.58, ti 1.64, mt 1.89, ta 0.95; total fe–ta 5.34; leg 4 tr 0.25, fe 1.87, pa 0.64, ti 1.82, mt 1.51, ta 0.99, total fe–ta 4.35.

DISCUSSION

Most fossil spiders, including Haplogynae, are known from Cenozoic ambers. However, since haplogynes are generally regarded as plesiomorphic among araneomorphs, they would be expected to occur in older strata. Few Mesozoic haplogynes are known, and almost all of them are from Cretaceous Myanmar amber, described by Wunderlich (2008, 2012). Among Pholcoidea, pholcids are known only from Cenozoic ambers and Diguetidae have no fossil record, but plectreurids have been described from the Jurassic (Selden & Huang 2010) as well as Eocene Baltic (Wunderlich 2004) and Miocene Dominican (Penney 2009) ambers. Some authors have considered Plectreuridae to be among the more plesiomorphic of the haplogyne spiders; in the introduction to his revision of the family, Gertsch (1958, p. 1) stated: "The primitive hunters of the family Plectreuridae are among the most generalized of all the haplogyne ecribellate spiders", and Král et al. (2006), in their study of karyotypy among basal spider clades, showed that plectreurids exhibit the most plesiomorphic state. On the other hand, phylogenetic analyses by Platnick et al. (1991) and Ramírez (2000) placed plectreurids fairly high within the haplogyne clade. A recent phylogenetic analysis, which sampled the majority of spider families, placed plectreurids lower down the haplogyne branch, adjacent to Hypochilidae Marx 1888 and Filistatidae Ausserer 1867 (Agnarsson et al. 2013). Within the Plectreuridae, Montsecarachne resembles most closely the Jurassic *Eoplectreurys* Selden & Huang 2010, from which it differs in having a short embolus, lacking tibial spurs on leg 1 of the male, and fewer macrosetae at the distal joint of the metatarsus. In the first character, *Montsecarachne* resembles the extant *Plectreurys tristis* Group of Gertsch (1958) and, in the second, the living genus *Kibramoa*. The lack of a clasping spur on the tibia of leg 1 in the male, which is present in the living *Plectreurys* and the Jurassic Eoplectreurys, but absent in the Recent *Kibramoa*, is replaced

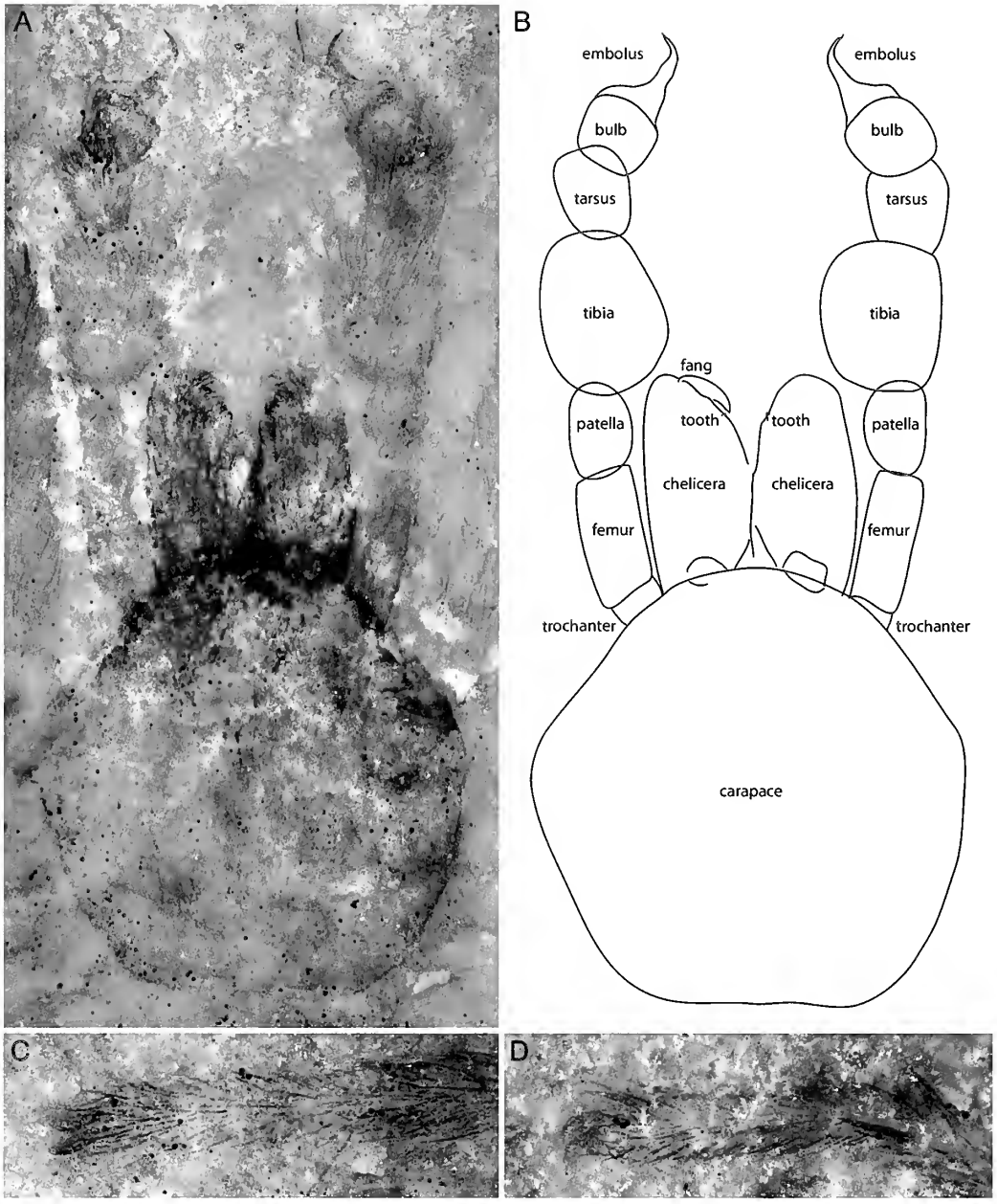

Figure 2.—*Montsecarachne annicorum* gen. et sp. nov. holotype counterpart LC-3780 IEI B: A. detail of carapace, chelicerae, and pedipalps; B. explanatory drawing of A; C. tarsus of left leg 3, note pectinate paired claws; D. tarsus of right leg 2, note strong bristles dorsal to paired claws and distal macroseta on metatarsus. Scale bars = 0.5 mm.

functionally by the strong, curved macrosetae at the distal end of femur 1 in *Montsecarachne* (Figs. 1C, 3C). The femur of leg 1 of *Montsecarachne* is slightly curved and robust, and approximately equal in length to the length of the carapace; this character state resembles that in most plectreurids except *Kibramoa* (femur 1 slender, much longer than carapace length: Gertsch 1958). Interestingly, both of the Mesozoic plectreurids have rather short carapaces in comparison with all other plectreurid species. No phylogenetic analysis has been performed on the Plectreuridae, so it is impossible to know the relationships between any of the genera and species, fossil and extant, of the family.

Biogeography.—Plectreurids are distributed today only in the southwestern USA, Mexico, Central America and Cuba (Alayón & Víquez 2011), but fossils are known from Miocene Dominican Republic amber (Penney 2009), from Eocene Baltie amber (Wunderlich 2004) and from the Jurassie of China (Selden & Huang 2010). The presence of a plectreurid in Dominican amber is unsurprising, given their present-day distribution; indeed, it might be expected that a living specimen will turn up in the Recent fauna of Hispaniola (Penney 2009). The presence of a plectreurid in middle Eocene Baltic amber, however, suggests that the family either migrated from Eurasia to its present area of endemism, or that it was once more widespread and its range has contracted. The existence of plectreurids in the early Cretaceous of Spain and the mid-Jurassic of the North China Block (Selden & Huang 2010) suggests that the family had a more widespread

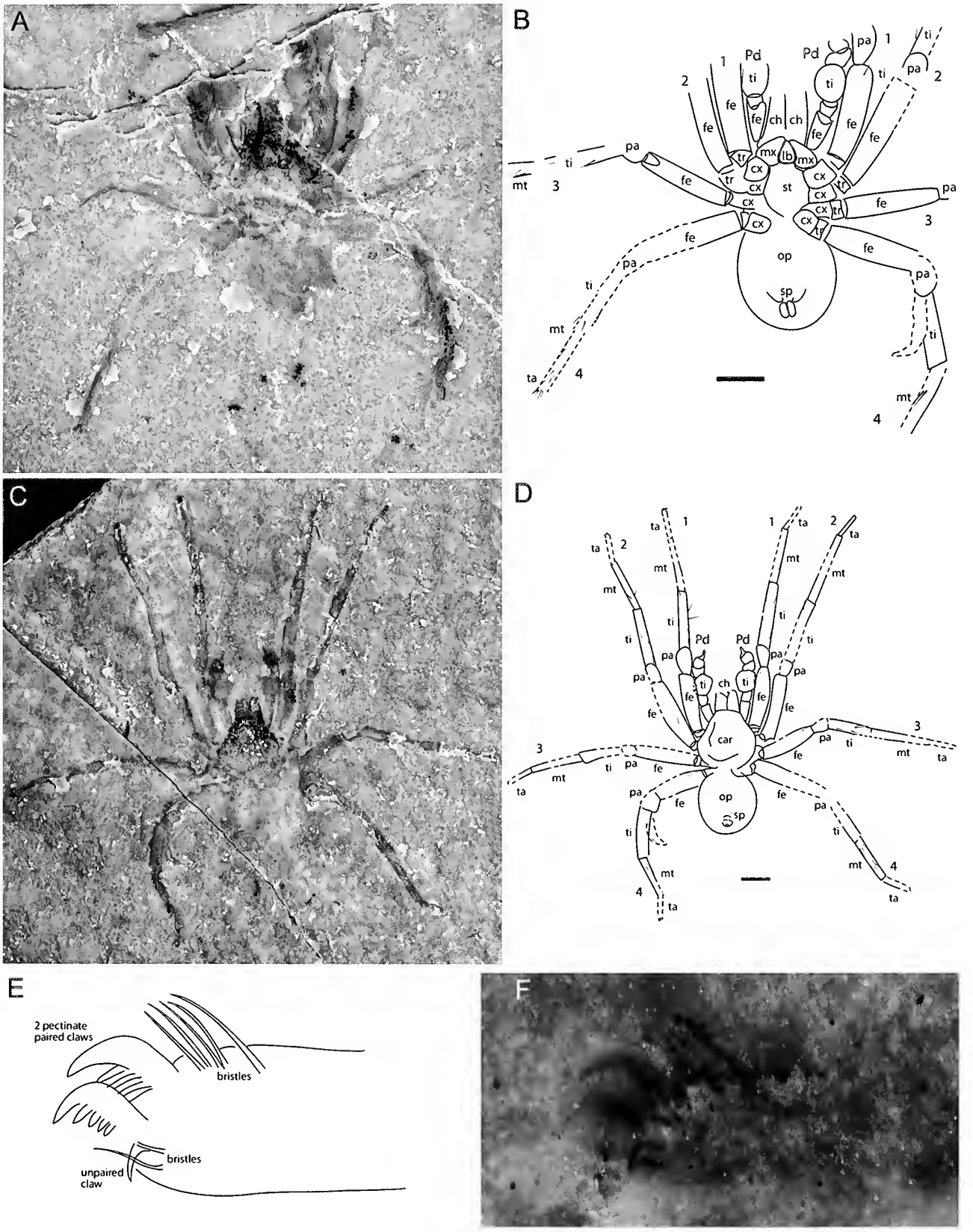

Figure 3.—*Montsecarachne anicorum* gen. et sp. nov.: A. photograph of paratype part LC-2936 IEI A; B. explanatory drawing of A; C. photograph of paratype counterpart LC-2936 IEI B; D. explanatory drawing of C; E. explanatory drawing of F; F. detail of tarsal claws of left leg 4. Scale bars = 1 mm.

distribution in the past; alternatively, its distribution could have migrated from the Eastern Palearctic region to the Western Palearctic, and then to the Western Nearctic, though this scenario seems less likely.

Sanmartín et al. (2001) provided a concise description of the reconstructions of the history of the Holarctic region from the Mesozoic to the present day. By mid-Jurassic times (ca. 165 Ma), the North China Block, on which the oldest known plectreurid, *Eoplectreurys*, was living by a volcanic lake (Selden & Huang 2010), was close enough to eastern Asia for biota to exchange between these continents (Metcalfe 2009). The climate at the locality at this time was warm temperate (Ren et al. 2010). However, America and Asia were widely separated by a polar ocean in the mid-Jurassic, though some dispersal may have been possible from Eurasia to North America either directly, or by island-hopping via the British Isles block (Sanmartín et al. 2001). By early Cretaceous times, *Montsecarachne* was living near a lake in what is now northern Spain, which was also part of Eurasia. This locality was in the same warm temperate zone as occupied by *Eoplectreurys* (Boucot & Scotese 2012). Dispersal of plectreurids had been possible from eastern to western Eurasia through this period, though interrupted much of the time by the presence of the central Asian Turgai Sea (Cox & Moore 2010). Also at this time, dispersal was possible from western Eurasia to North America, via the same route through the British Isles across the incipient Atlantic Ocean but, by the later Cretaceous, this route became impassable. In the later Cretaceous, a seaway developed dividing western North America (at that time linked to Asia by the Beringian land bridge) from eastern North America, which was becoming more isolated from Europe as the Atlantic Ocean continued to open northwards. By the end of the Cretaceous, the mid-continental seaway dividing North America had regressed and the continent became one again.

After the opening of the North Atlantic in late Cretaceous times, terrestrial connections between Europe and North America persisted across possible North Atlantic land bridges until at least the early Eocene (ca. 50 Ma). The Thulean Bridge is supposed to have connected southern Europe to Greenland via Scotland, Iceland, and the Faeroes; Greenland was then connected with eastern North America through the Queen Elizabeth Islands. As the climate improved after the end-Cretaceous extinction event and subsequent nuclear winter, the climate warmed through the early Cenozoic, allowing an exchange of temperate biota until the breaking of the Thulean Bridge in the early Eocene (ca. 50 Ma). It was during the earliest Eocene, when the combination of the availability of the Thulean Bridge and the short-lived Paleocene–Eocene Thermal Maximum climatic event, which saw temperatures rise sharply, that mammals were able to interchange between Eurasia and North America (Jones 2011), and also likely caused a turnover in mammal faunas (Gingerich 2006). This was also a time when plectreurids could have migrated to North America. Another possible North Atlantic land bridge is the so-called de Geer Land Bridge, connecting Greenland to Eurasia via Svalbard, though this is likely never to have been a complete bridge, rather a stepping-stone, and may have been too northerly and cold for thermophilic biota to have utilized (Jones 2011).

Another available land bridge between Eurasia and North America was Beringia. Eastern Asia and western North America became connected by land across the Bering Sea in the mid-Cretaceous (ca. 100 Ma), and they remained joined until the Pliocene. However, dispersal by this route appears to have been less common than via the North Atlantic land bridges for many plants and animals, with some exceptions (Condamine et al. 2012). In the early Cenozoic, as the temperature rose, boreal and, later, boreotropical forest developed across Beringia, facilitating biotic interchange until the Eocene–Oligocene transition event (EOT) (Hren et al. 2013), when temperatures decreased rapidly, rarely to return to their previous levels. At the end of the Eocene, shortly after *Palaeoplectreurys* was living in the Baltic amber forest (Wunderlich 2004), the North Atlantic land bridges were no longer available and the EOT had created a cold climate in Beringia; so, if plectreurids had not spread to North America before this time, then later opportunities became fewer and less likely.

Selden & Huang (2010) discussed the possible center of origin of the plectreurids, which was most likely on Eurasia. Dispersal to the North American continent could have occurred in the Jurassic, or later in the early Eocene. The present distribution of plectreurids is most likely the result of extinction across the large part of their range. Moreover, they appear to have changed their habitat preference somewhat, in that now they occur principally in arid environments. However, although the records from Cuba (Alayón García 2003) and Central America (Alayón & Víquez 2011) are not from obvious arid habitats, the specimens were found in arid habitats within otherwise humid environments. The Cenozoic amber plectreurids come from humid forest environments, though it is possible that they, too, were living in arid situations within these environments. The Mesozoic plectreurids come from perilacustrine environments, which could include xeric habitats.

CONCLUSION

The new genus *Montsecarachne* can be accommodated within the modern family Plectreuridae; it adds to the diversity within that family and adds an additional stratigraphic and biogeographical record. Plectreurids are restricted today to arid habitats in the southwestern USA, Mexico, Central America and Cuba, but Mesozoic and Cenozoic fossils show a more widespread distribution in Eurasia. The most likely paleobiogeographic history of the family is that it was more widespread in the past and has suffered extinction over much of its range, resulting in the present distribution. It is possible that the extant plectreurids represent a living remnant of a greater diversity of haplogynes, which were widespread during the Mesozoic.

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Sex ratio bias caused by endosymbiont infection in the dwarf spider *Oedothorax retusus*

Bram Vanthournout[1,2], **Viki Vandomme**[1] and **Frederik Hendrickx**[3]: [1]Terrestrial Ecology Unit, Department of Biology, Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium. E-mail: bram.vanthournout@hotmail.com; [2]Ecology and Genetics, Department of Bioscience, Aarhus University, Ny Munkegade 116, 8000 Aarhus, Denmark; [3]Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium

Abstract. Spiders exhibit a remarkable variety of reproductive phenotypes such as induced parthenogenesis and reproductive skew in primary sex ratio. However, observations of distorted sex ratios are mainly inferred from field catches of adult individuals, whereas detailed information on clutch primary sex ratio and sex ratio inheritance, resulting from multiple generations of laboratory rearing, is scarce. One of the potential causes of sex ratio variation is infection with maternally inherited endosymbiont bacteria that alter a mother's offspring sex ratio to increase their own fitness. Although studies show that spiders are infected with several endosymbiont species, it was only recently discovered that endosymbiont bacteria can cause a female sex ratio bias in this order. To explore the distribution of biased sex ratios and endosymbiont infection patterns, we investigated sex ratio variation and bacterial presence in *Oedothorax retusus* Westring 1851. Significant sex ratio variation was detected in six matrilines originating from wild-caught females, one of which consistently showed a female bias in offspring production. Congruent with a bacterial effect, the sex ratio bias showed a clear maternal inheritance, and treatment with antibiotics reversed the sex ratio to equal numbers of males and females. Female-biased clutches were found to exhibit a significantly lower number of hatched spiderlings than unbiased clutches, suggesting the occurrence of male-killing. All matrilines showed infection with the *Cardinium* endosymbiont, while two matrilines, including the female biased one, were additionally infected with *Wolbachia* and *Rickettsia*. These findings indicate that bacterial endosymbionts are responsible for the sex ratio variation in this species, and suggest that effects of endosymbiont bacteria in the order Araneae could be more widespread than previously assumed.

Keywords: Sex ratio distortion, solitary spider, endosymbiont bacteria, male-killing, *Wolbachia*

Spiders display a diversity of reproductive phenotypes, such as parthenogenesis and primary sex ratio distortion [sex ratio at the time of fertilization (overview in Goodacre et al. 2006; Martin & Goodacre 2009)]. Biases in offspring sex ratios have been most extensively studied in social spider species through both collections in the field and laboratory-based studies (Aviles & Maddison 1991; Rowell & Main 1992; Aviles et al. 2000; Lubin & Bilde 2007). However, in solitary species, existence of biased sex ratios is mainly inferred from population studies where the absence or low numbers of males could indicate either parthenogenesis and sex ratio distortion or differential mortality of one sex (Vollrath & Parker 1992; Baert & Jocque 1993; Levi 1996). Detailed information on primary sex ratio distortion in solitary spiders based on laboratory rearing is scarce (but see Gunnarsson & Andersson 1996; Gunnarsson et al. 2004; Vanthournout et al. 2011).

One of the mechanisms that can cause a distorted sex ratio is infection with endosymbiont bacteria. Endosymbiotic bacteria are maternally inherited microorganisms that may cause a variety of reproductive alterations in their hosts. Due to their almost exclusively maternal inheritance, induction of parthenogenesis, feminization, and male-killing, which all bias the offspring sex ratio towards females, and the occurrence of cytoplasmic incompatibility result in an increase of infected females in the population (Werren & Beukeboom 1998; Stouthamer et al. 1999; Charlat et al. 2003; Werren et al. 2008; Engelstadter & Hurst 2009). Owing to their obvious effects on host ecology and reproductive biology, endosymbiont bacteria have received increasing attention, and the combination of screening studies and meta-analyses provides mounting evidence that these endosymbionts are more widespread than previously thought (Goodacre et al. 2006; Duron et al. 2008a; Hilgenboecker et al. 2008). However, understanding of the phenotypic effects of a large number of such endosymbiont infections remains poorly investigated, particularly in the Araneae.

Although several studies show that spider species exhibit a high diversity and prevalence of endosymbiont species known to influence their hosts' reproductive biology (Goodacre et al. 2006; Baldo et al. 2008; Duron et al. 2008a, b; Martin & Goodacre 2009; Yun et al. 2011), their potential effects are verified in few cases. No effect of endosymbiont infection on spider host reproduction could be characterized in *Holocnemus pluchei* Scopoli 1763 (Stefanini & Duron 2012). In *Pityohyphantes phrygianus* C.L. Koch 1836, Gunnarsson et al. (2009) suggested that endosymbionts play a role in influencing offspring sex ratio, and recently Vanthournout et al. (2011) showed that the endosymbiont bacterium *Wolbachia* is a causative agent of a female-biased sex ratio distortion in the dwarf spider *Oedothorax gibbosus* Blackwall 1841.

Despite these documented cases, it remains at present unknown whether susceptibility to endosymbionts is confined to only a very limited number of spider species, or whether the effect is more widespread. To test this, we investigated the occurrence of sex ratio variation and presenee of cytoplasmic sex ratio-distorting elements in a related species *Oedothorax retusus* Westring 1851 (Araneae: Linyphiidae: Erigoninae). This palearctic dwarf spider is found in a variety of habitats, usually in mosses, grasses and undergrowth, and is known to be infected with several endosymbiont species (Goodacre et al. 2006). Precise data on clutch sex ratio are currently lacking, however, making this a suitable candidate for examination.

In this study we investigate the diversity and prevalence of the endosymbiont community in *O. retusus* using endosymbiont-specific PCR assays. To quantify the potential effects of each endosymbiont on clutch sex ratio, we combined pedigree data resulting from several generations of laboratory rearing and results from a broad-spectrum antibiotic treatment. Furthermore, the phylogenetic position of the identified endosymbiont species was determined and compared with those found in *Oedothorax gibbosus*.

METHODS

1) Field collection, rearing conditions and breeding design.—Six matrilines were set up in the laboratory by collecting adult females by hand catches at Damvallei (Belgium) in the summer of 2010. We placed females individually in plastic vials of 5 cm diameter and 2.5 cm height with plaster bottoms. Moistening the plaster with tap water kept humidity levels at 100%. A piece of moss was added to allow the construction of a functional web. We provided fruit flies (*Drosophila* sp.) in overabundance and checked food and humidity levels several times a week. Vials were placed in a climate chamber with a constant temperature of 20°C and a light-dark regime of 16L–8D. Females were allowed to deposit up to three egg sacs before being preserved in ethanol. Offspring were reared individually as described above, except that juvenile spiders received collembolans as a food source until the third molt. After the final molt, we determined the sex of the spiders by visual inspection using a stereomicroscope. This allowed assessment of the tertiary sex ratio (number of adult male offspring/total number of adult offspring).

Adult females were mated with unrelated males (n = 22 females, F1 generation) to investigate the inheritance pattern of the sex ratio trait and for the application of antibiotics (see 2). We reared offspring under standard conditions and again determined tertiary sex ratio. Adult female offspring were further mated with unrelated males to increase sample size and to investigate the underlying mechanism (n = 19 females, F2 generation; see 4).

2) Antibiotic treatment.—It has been previously shown that application of antibiotics is effective in eliminating endosymbionts in spiders (Goodacre et al. 2009; Vanthournout et al. 2011). To test whether administering antibiotics restores an equal sex ratio (Morimoto et al. 2006; Gotoh et al. 2007), we exposed F1 females from the distorted line (M1: Table 2) to the broad-spectrum antibiotic, tetracycline. After reaching adulthood, six haphazardly chosen F1 females were treated by moistening the plaster on the bottom of the vial with the antibiotic solution (0.1%, w/v; 0.002 M). After approximately seven days, females were allowed to copulate with first-generation unrelated males. Offspring were reared individually as described above with the continuous use of an antibiotic solution. We used other F1 females from the distorted matriline as a control treatment (n = 6 females). Sex ratio and survival of the clutches were determined and compared between the treatments with a generalized linear mixed model (proc GLIMMIX in SAS v. 9.1.2). To account for dependence in sex ratio among mothers, mother ID was included as a random effect.

3) Endosymbiont detection and phylogenetic relationship.—We investigated the infection status of wild-caught females by means of a PCR assay for five endosymbionts: *Wolbachia*, *Rickettsia*, *Cardinium*, *Spiroplasma* and *Arsenophonus*. All potentially cause reproductive alterations in arthropods. Since one female died before being stored in ethanol, three of her daughters were used in the PCR assay to determine maternal infection status. All three daughters gave consistent results for every endosymbiont tested.

Whole spiders were used for DNA extraction using the Nucleospin Tissue kit (©Machery Nagel) following the manufacturer's recommended protocol. We used primers for five endosymbiont bacteria: *Wolbachia*, *Rickettsia*, *Cardinium*, *Spiroplasma* and *Arsenophonus* (Table 1). PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing (for temperature, see Table 1), 30 sec, extension (72°C, 90 sec) and a final extension at 72°C for 5 min. Electrophoresis was performed on a 1.5% agarose gel. Gels were stained in a solution of GELRED for approximately 15 min. Bands were visualized by UV-fluorescence.

As *Cardinium* tested positive for all individuals, we were able to confirm the reliability of the PCR detection of bacterial infection; hence, no further positive control was necessary. Prey items can be ruled out as a potential source of *Wolbachia* contamination in the samples, as it has previously been shown that our fruit fly and springtail breeding stocks were uninfected (Vanthournout et al. 2011). To test the significance of a relationship between endosymbiont infection and sex ratio, we used a generalized linear mixed model (Proc GLIMMIX in SAS v.9.1.2.) with endosymbiont presence/absence as a fixed effect. Dependence in sex ratio among matrilines was accounted for by including matriline identity as a random factor. The estimates obtained from the sex ratios for infected and uninfected females were compared with an even sex ratio using a t-test. Differences in sex ratio between matrilines were analyzed using a chi-square test (RxC).

In order to check primer specificity and to investigate strain diversity, we sequenced the PCR products using the BigDye v.1.1 Terminator Sequencing mix and ran them on an ABI 3710 automated sequencer. For *Wolbachia,* only the *wsp* gene was sequenced. We used BLAST searches to identify the closest relatives of the endosymbiont sequences obtained. The ClustalW algorithm implemented in MEGA5 (Tamura et al. 2011) was used to align the sequences obtained with those from other endosymbionts available in Genbank, which mainly originate from the studies reported in Rowley et al. (2004), Goodacre et al. (2006), Duron et al. (2008a) and Wang et al. (2010). We then compared the phylogenetic position of the endosymbiont *wsp* (*Wolbachia*), 16S (*Cardinium*) and *citrate* (*Rickettsia*) gene sequences with those found in spiders and other host species. For the *wsp* phylogeny, *Wolbachia* supergroup delimitation was used as reported in Rowley et al. (2004) and Goodacre et al. (2006). We constructed a p-distance-based neighbor joining tree as implemented in MEGA 5 (Tamura et al. 2011). Bootstrap percentage support was calculated for the nodes by generating 10,000 bootstrap values.

4) Mechanism.—Infection with male-killing endosymbionts typically lowers the clutch size to about one half, compared to clutches produced by uninfected females. Feminization and parthenogenesis induction do not influence clutch size. Based

Table 1.—Primers used for detection of five endosymbiont genera.

Endosymbiont	Primer	Gene	Annealing temperature	Reference
Wolbachia	WSP81F WSP691R	*wsp*	54°C	Braig et al. 1998
	16Swolb99F 16Swolb99R	16S rRNA		Oneill et al. 1992
Rickettsia	RICS741F RCIT1197R	*citrate*	54°C	Davis et al. 1998 Majerus et al. 2000
Cardinium	CLO-F1 CLO-R1	16S rRNA	54°C	Gotoh et al. 2007
Spiroplasma	SP-ITS-J04 SP-ITS-N55	Spacer region between 16S rRNA and 23S rRNA	52°C	Majerus et al. 1999
Arsenophonus nasoniae	ArsF, ArsF3 ArsR2	16S rRNA	52°C	Duron et al. 2008a

on this difference, we can discriminate among these mechanisms using two different approaches: testing for correlations between number of offspring and sex ratio and censusing the number of offspring as eggs and at hatching.

First, we tested for a correlation between number of adult offspring and egg sac sex ratio among *Wolbachial Rickettsia*-infected (M1, M2; Table 1) and among uninfected (M3–M6: Table 1) females by means of a Pearson correlation on all clutches, weighted for number of adult offspring. This was further explored by investigating the relationship between the egg sac sex ratio and the infection status of the mother, total number of adult offspring and their interaction using a generalized linear mixed model (Proc GLIMMIX in SAS v. 9.1.2). Dependence in sex ratio among mothers was taken into account by adding the identity of the mother as a random effect. Moreover, if feminization occurs in this species, females producing a biased clutch should produce more female offspring than females producing offspring in equal numbers of males and females. Therefore, we compared the number of female offspring produced by *Wolbachial Rickettsia*-infected and uninfected females by means of a generalized linear mixed model (Proc GLIMMIX in SAS v. 9.1.2). To account for dependence in sex ratio among matrilines, matriline ID was included as a random effect.

Second, we determined the number of offspring at two different census times: at the egg stage and at hatching from the egg sac. Females that were used to produce the second-generation offspring were allowed to oviposit up to three egg sacs before being stored in ethanol. The spiderlings from the first egg sac were allowed to emerge, and offspring were reared to adulthood to determine the total number of emerged spiderlings and tertiary sex ratio. The second and third egg sacs were stored in ethanol six days after oviposition to allow sufficient development of the eggs. Afterward, the proportion of fertile eggs to the total number of eggs produced was determined. Using a generalized linear mixed model (proc GLIMMIX in SAS v.9.1.2), we compared the total number of emerged spiderlings and number of eggs produced for the *Wolbachial Rickettsia* infection status of the mother, census time (before hatching versus after hatching), and their two-way interaction. Identity of the mother was included as a random effect to correct for dependence between clutches.

RESULTS

1) Sex ratio variation among maternal lines.—A highly significant difference in average clutch sex ratio was detected among matrilines (Table 2: $df = 5$, $X^2 = 65.6$, $P < 0.0001$). This difference was primarily attributed to a single matriline, in which the wild-caught female and the daughter offspring of at least two subsequent generations produced significantly female-biased offspring. In the other five matrilines, equal numbers of males and females were produced, even in subsequent generations (M2–6: Table 2). Given that daughters of the M1 line were in many cases crossed with males from the other lines, persistence of the sex ratio distortion over several generations strongly suggests a maternal inheritance (M1:

Table 2.—Sex ratio data and endosymbiont infection status grouped by matriline.

Matriline	Endosymbiont infection status			Number of crosses	Number of males	Total number[1]	Sex ratio	*P*-value[2]
	Cardinium	*Rickettsia*	*Wolbachia*					
M1	+	+	+	16	123	434	0.29	<0.0001
M2	+	+	+	5	62	135	0.46	0.38
M3	+	-	-	6	102	208	0.49	0.8
M4	+	-	-	5	73	165	0.44	0.16
M5	+	-	-	4	88	156	0.56	0.13
M6	+	-	-	5	123	225	0.55	0.18
Tetracycline treatment								
M1				6	71	165	0.43	0.09

[1] Denotes the sum of the number of adult males and females.
[2] Denotes the probability value of difference from an even sex ratio as calculated by a binomial test.

Table 2). Conversely, three females from the undistorted lines mated with three male offspring of M1 produced sex ratios that were not significantly different from 0.5 (mean ± SE: 0.54 ± 0.05, $P = 0.22$). Therefore, since no effect of males was observed in the reciprocal crosses, this demonstrates that the sex ratio distortion is not heritable through males and confirms the exclusive maternal inheritance.

2) Antibiotic treatment.—Treatment of female offspring of the distorted matriline with antibiotics significantly affected the tertiary sex ratio ($F_{1,\ 10} = 6.46$, $P = 0.03$). Untreated females produced a significantly female-biased sex ratio (mean ± SE: 0.21 ± 0.05, $t_{10} = -4.47$, $P = 0.0012$), while tetracycline treatment returned the sex ratio to an equal proportion of males and females (mean ± SE: 0.43 ± 0.07, $t_{9.92} = -0.97$, $P = 0.4$). Applying antibiotics did not influence offspring survival, as no difference was found ($F_{1,1} = 2.6$, $P = 0.4$) in survival between tetracycline-treated (mean ± SE: 0.93 ± 0.02) and control offspring (mean ± SE: 0.97 ± 0.01).

3) Endosymbiont detection and phylogenetic relationship.—Screening of six individual females showed infection with up to three different endosymbionts known to cause reproductive alterations in arthropods. All females were infected with *Cardinium*, while two females were infected with both *Wolbachia* and *Rickettsia*. Over all generations, *Wolbachia*/*Rickettsia* infection status had a significant effect on the sex ratio produced by a female ($F_{1,35} = 4.62$; $P = 0.04$), with females infected with *Wolbachia* and *Rickettsia* producing a significantly more distorted sex ratio than uninfected females (mean ± SE: 0.35 ± 0.06, $t_{35} = -2.46$, $P = 0.02$; 0.51 ± 0.04, $t_{35} = 0.25$, $P = 0.8$, respectively).

For the two females testing positive for *Wolbachia* infection, both the *wsp* and *Wolbachia*-specific 16S rDNA primer gave consistent results. Sequencing of the *wsp* primer revealed no individual variation. BLAST searches revealed high similarity with available *Wolbachia* sequences (E-values < 1e-199). The *wsp* sequence [Genbank: JN889706] was most similar, with sequences from the spiders *Cybaeus penedentatus* Bennet 2009 [Genbank: GQ480746], *Araneus diadematus* Clerck 1757 [Genbank: DQ231505] and *Pityohyphantes phrygianus* C.L. Koch 1836 [Genbank: DQ231504], and clustered with high support within supergroup B (Fig. 1: neighbor joining tree of *Wolbachia* sequences).

The females with *Wolbachia* infection also tested positive for *Rickettsia*. BLAST searches showed homology with previously published *Rickettsia* sequences (E-values < 1e-199). The *Rickettsia* sequence [Genbank: JN889707] showed high similarity with the sequences of the spiders *Oedothorax gibbosus* [Genbank: HQ286289], *Hylyphantes graminicola* Sundevall 1830 [Genbank: DQ231487] and a *Theridiidae* sp. [Genbank: DQ231486] (Fig. 2: neighbor joining tree of *Rickettsia* sequences).

The *Cardinium* endosymbiont was found in all of the females tested. Alignment of the sequences obtained revealed no individual variation, and BLAST searches yielded high similarity with available *Cardinium* sequences (E-values < 1e-199). Sequences [Genbank: JN889705] were closely related to the *Cardinium* sequence of the spider *Holocnemus pluchei* Scopoli 1763 [Genbank: EU333930] and clustered with high support together with the sequence of the spider *Oedothorax gibbosus* [Genbank: HQ286292] (Fig. 3: neighbor joining tree of *Cardinium* sequences). We detected bands for *Arsenophonus* in the two females infected with *Wolbachia* and *Rickettsia*. However, sequencing and BLAST searches revealed that these were amplifications of *Rickettsia* and thus constituted false positives.

4) Mechanism.—We found a significant relationship between the number of adult offspring and egg sac sex ratio in *Wolbachia*/*Rickettsia* infected females, with a significantly lower proportion of males in smaller egg sacs (weighted Pearson correlation: $r = 0.57$, $P = 0.005$: Fig. 4). For uninfected females, no such correlation could be detected (weighted Pearson correlation: $r = 0.02$, $P = 0.95$: Fig. 4). There was a significant effect found for the total number of adult offspring ($F_{1,11} = 7.05$, $P = 0.02$) and *Wolbachia*/*Rickettsia* infection ($F_{1,11} = 13.02$, $P = 0.004$) on the egg-sac sex ratio. Moreover, when *Wolbachia*/*Rickettsia*-infected mothers produced a high number of offspring, the egg-sac sex ratio was not biased; if a lower number was produced, the egg sac sex ratio became significantly female biased, suggesting the occurrence of male-killing. This was not observed in *Wolbachia*/*Rickettsia* uninfected mothers ($F_{1,11} = 6.60$, $P = 0.03$).

There was no significant effect of infection status of the female on the total number of female offspring ($F_{1,3.3} = 0.02$, $P = 0.9$, mean ± SE: 11.9 ± 1.7 and mean ± SE: 12.2 ± 1.3 for infected and uninfected females, respectively). The total number of spiderlings was smaller than the total number of eggs produced, irrespective of the *Wolbachia*/*Rickettsia* infection status of the mother ($F_{1,31} = 47.58$, $P < 0.0001$). However, significantly fewer spiderlings emerged when the female was infected with *Wolbachia* and *Rickettsia* (mean ± SE: 19.4 ± 1.8) than did uninfected mothers (mean ± SE: 32.8 ± 3.2, $F_{1,31} = 15.32$, $P = 0.0005$: Fig. 5). *Wolbachia*/*Rickettsia* infection status significantly lowered the effect over the total number of spiderlings and number of eggs ($F_{1,13.41} = 6.76$, $P = 0.02$). Again, for this subset of mothers (F2 generation, see 1) a significant effect was found for *Wolbachia*/*Rickettsia* infection status on the offspring sex ratio ($F_{1,13} = 11.67$, $P = 0.005$). *Wolbachia* and *Rickettsia*-infected mothers produced a significant female-biased sex ratio (mean ± SE: 0.30 ± 0.04, $t_{13} = -5.08$, $P = 0.0002$), while uninfected mothers produced an even sex ratio (mean ± SE: 0.48 ± 0.04, $t_{6.78} = -0.65$, $P = 0.54$).

DISCUSSION

In this study, we report the presence of a maternally inherited, sex-ratio-distorting bacterium in the solitary dwarf spider *Oedothorax retusus*. This is deduced from several lines of evidence: 1) one matriline produced significantly female-biased offspring sex ratios, and several generations of outcrossed laboratory rearing did not diminish the biased sex ratio, 2) administering antibiotics to females of this distorted matriline resulted in equalized sex ratios and 3) three endosymbionts known to cause sex-ratio biases in their hosts were found: *Wolbachia, Rickettsia* and *Cardinium*. Differential mortality of the sexes during juvenile development is unlikely to contribute to the sex ratio distortion, as average juvenile survival is generally high (92%), and some highly distorted clutches had almost 100% juvenile survival. All females were infected with *Cardinium*, while only two females were infected with *Wolbachia* and *Rickettsia*. Since 4 of the 5 undistorted

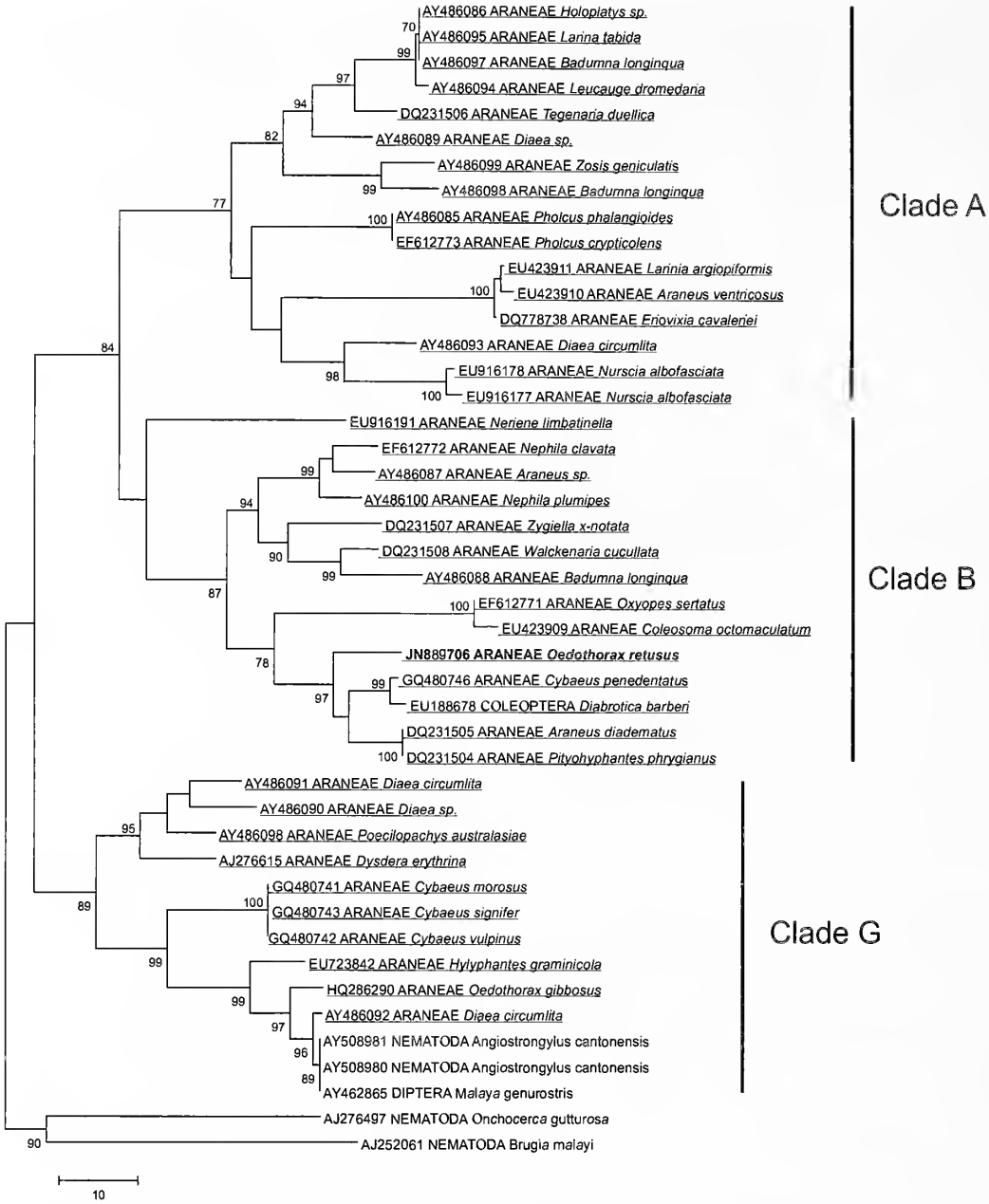

Figure 1.—Phylogenetic position of *Wolbachia wsp* sequence of *Oedothorax retusus* [GenBank: JN889706]. Terminal taxa represent host species. A p-distance based neighbor joining tree was constructed as implemented in MEGA 5 (Tamura et al. 2011) on a subset of *Wolbachia wsp* sequences available at GenBank, with indication of the major *Wolbachia* supergroups (as reported in Rowley et al. 2004; Goodacre et al. 2006). Percentage bootstrap support was calculated for the nodes. Genbank accession numbers are given in front of the taxonomic group to which the host species belongs. Sequences that originate from spider hosts are underlined. *Oedothorax retusus* is shown in bold.

lines were not infected, and a significant relationship was found between *Wolbachial Rickettsia* infection and occurrence of the sex ratio bias within this matriline, infection with these endosymbionts is the most plausible causative agent of the sex ratio distortion.

However, the relationship between bacterial presence and sex ratio effect is not completely clear-cut. A significant difference in sex ratio was found between M1 and M2, both *Wolbachia* and *Rickettsia* infected matrilines (Table 1: $df = 2$; $X^2 = 14.51$; $P < 0.0002$). This variable pattern of bacterial expression of sex ratio distortion could be due to differences in endosymbiont density (Breeuwer and Werren 1993; Hurst et al. 2000; Bordenstein et al. 2006). Alternatively, the presence of host suppressor genes could produce variation in the effect on the sex ratio. Such suppressor genes are expected to evolve in the framework of general sex ratio theory. The discovery of such genes in butterflies and ladybirds indeed provides empirical confirmation (Hornett et al. 2006; Majerus and Majerus 2010a). Performing planned crosses to investigate the precise mode of action of a proposed suppressor gene will be necessary to demonstrate their presence in this species (Majerus and Majerus 2010a).

The combination of these factors does not allow us to identify the actual causal agent for sex ratio distortion in this

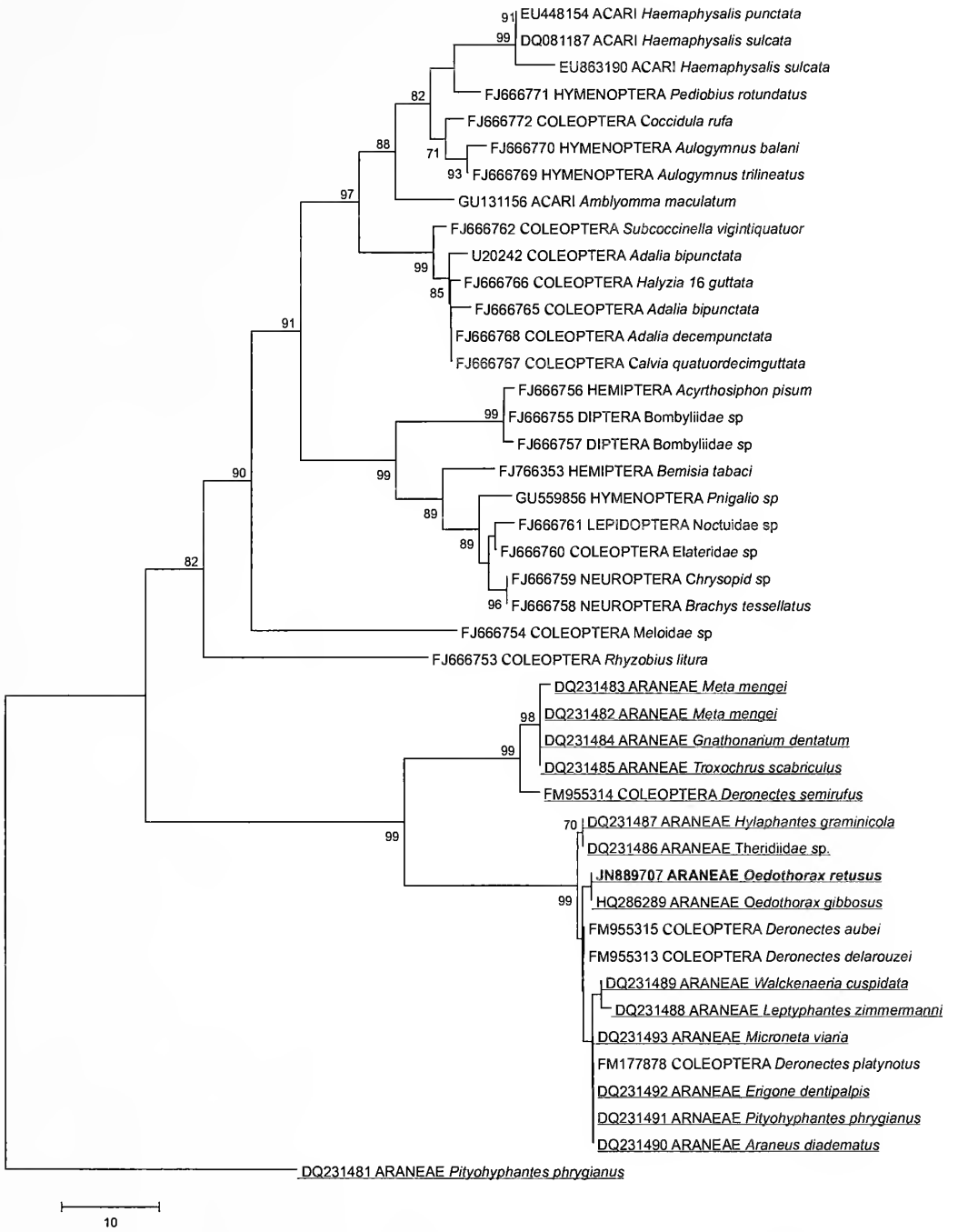

Figure 2.—Phylogenetic position of the *Rickettsia* (partial *citrate* sequence) endosymbiont of *Oedothorax retusus* [GenBank: JN889707]. Terminal taxa represent host species. A p-distance based neighbor joining tree was constructed as implemented in MEGA 5 (Tamura et al. 2011) on a subset of *Rickettsia* sequences available at GenBank. Percentage bootstrap support was calculated for the nodes. Genbank accession numbers are given in front of the taxonomic group to which the host species belongs. Sequences that originate from spider hosts are underlined. *Oedothorax retusus* is shown in bold.

spider. A first indication of the identity of the sex ratio distorter as well as a possible explanation for the apparently variable sex ratio effect can be obtained by analyzing the different densities of *Wolbachia* and *Rickettsia*, using quantitative PCR (Goto *et al.* 2006). Obtaining females singly infected with either endosymbiont could lead to more conclusive evidence on the exact roles of each endosymbiont and their possible interactions. This might be realized by increasing the number of field-caught females if natural variation is present between females infected with either *Wolbachia* or *Rickettsia*. Extending the current study by increasing the sample size of collected females and by including multiple populations would equally allow for an accurate assessment of the occurrence of the sex ratio bias in natural populations.

Alternatively, treatment of doubly infected females with low doses of antibiotics (Sasaki et al. 2005) or transfection of endosymbionts (Sasaki et al. 2002) can establish such single

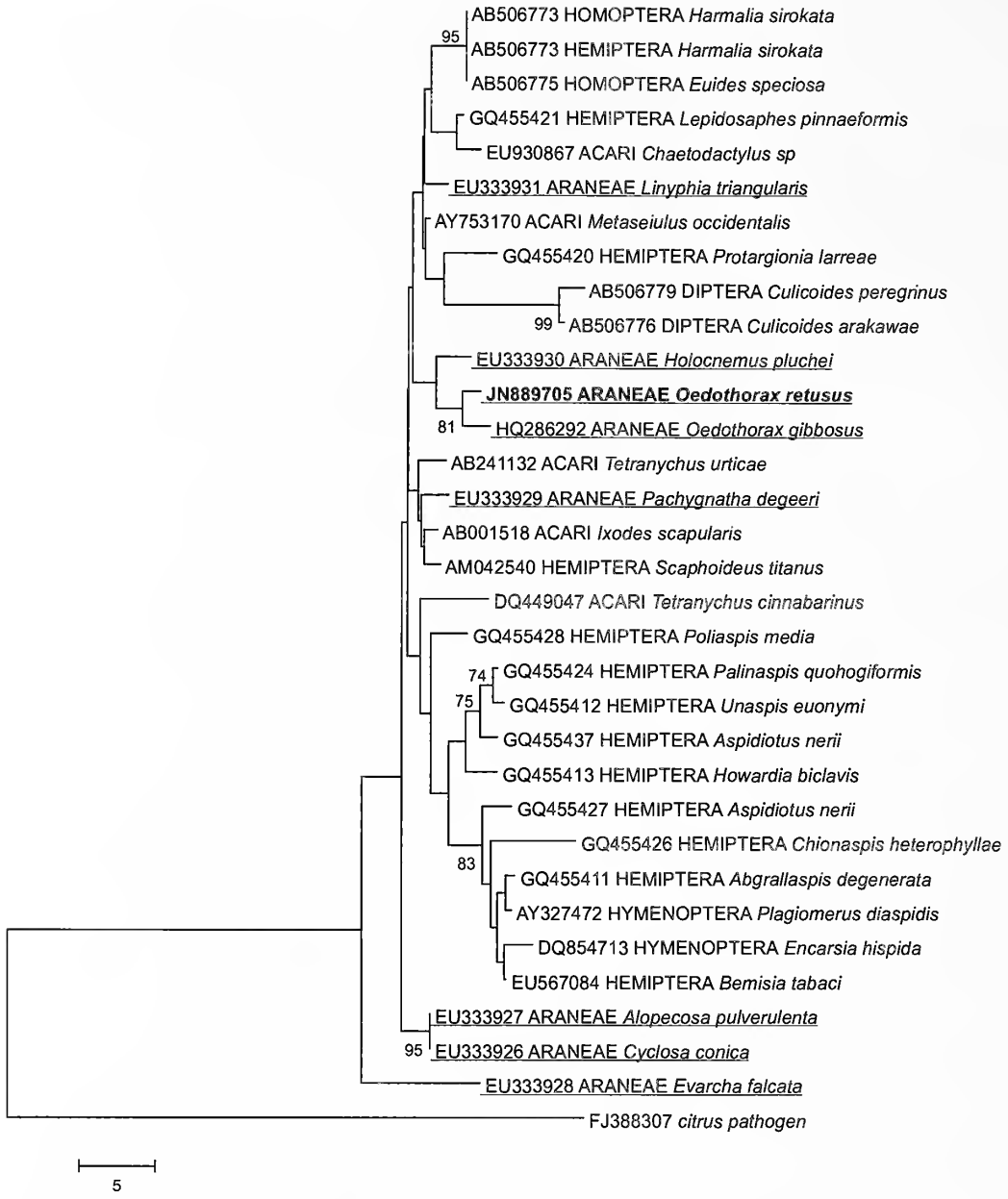

Figure 3.—Phylogenetic position of the *Cardinium* (16S rRNA gene) endosymbiont of *Oedothorax retusus* [GenBank: JN889705]. Terminal taxa represent host species. A p-distance based neighbor joining tree was constructed as implemented in MEGA 5 (Tamura et al. 2011) on a subset of *Cardinium* sequences available at GenBank. Percentage bootstrap support was calculated for the nodes. Genbank accession numbers are given in front of the taxonomic group to which the host species belongs. Sequences that originate from spider hosts are underlined. *Oedothorax retusus* is shown in bold.

infections. Also, we cannot exclude the possibility that the observed sex ratio bias is caused by an as yet unidentified endosymbiont distorter. A next generation sequencing approach would allow us to obtain a broad assessment of the endosymbiont diversity in this spider species (Andreotti et al. 2011; Hirsch et al. 2012).

The strong correlation between number of adult offspring and egg-sac sex ratio supports the hypothesis that the killing of males is the most plausible mechanism of sex ratio distortion. Feminization is highly unlikely, as infected and uninfected females produced equal numbers of female offspring. The occurrence of male-killing is supported by comparing the total number of spiderlings with the total number of eggs produced. This is the most favorable approach for directly linking the number of emerged spiderlings, egg number, and corresponding offspring sex ratio of one female, since development of eggs within the egg sac hampers visual inspection of egg development and hatching rates. Moreover, hatching from the egg and first molt of the spiderlings occur inside the egg sac. This causes a time lag between hatching of the eggs and emergence of the spiderlings from the egg sac, resulting in the inability to single out any undeveloped eggs. As expected, spiderling number is significantly smaller than egg number for both infected and uninfected mothers. This can be caused by mortality during egg hatching and early juvenile cannibalism occurring inside the egg sac. However,

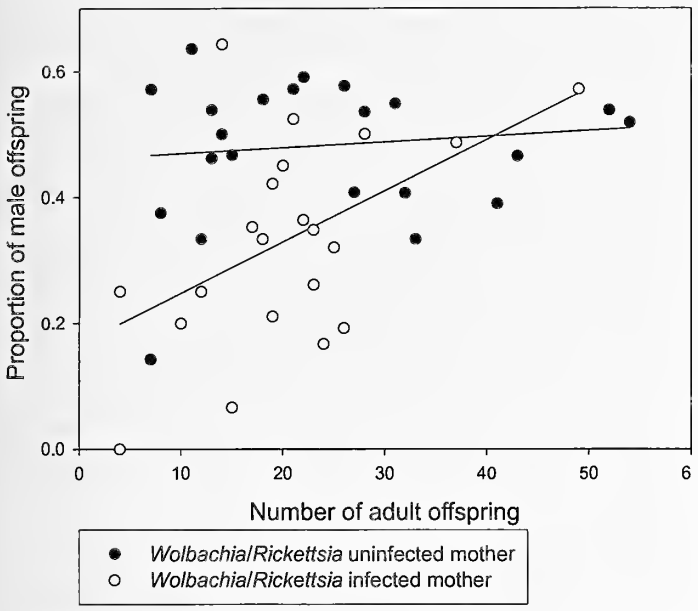

Figure 4.—Relationship between number of adult offspring and proportion of male offspring in the egg sac. Open circles: *Wolbachia* and *Ricketssia*-infeeted females, filled circles: *Wolbachia* and *Ricketssia*-uninfected females. The solid line visualizes the linear correlation.

significantly fewer spiderlings emerge from egg sacs produced by *Wolbachia* and *Rickettsia*-infected females. The reduction in offspring being produced correlates with the bias toward female offspring in these clutches, suggesting that the offspring that do not emerge from the egg sac are predominantly males. This is again strong evidence for the occurrence of male-killing. Because almost all eggs showed signs of embryonic

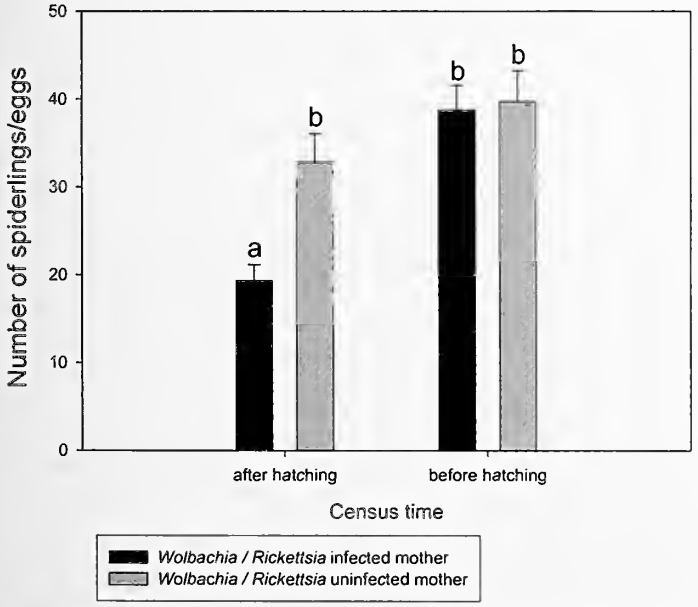

Figure 5.—Clutch size number produced by *Wolbachia* and *Rickettsia* infected (black bars) and uninfected (grey bars) females in first egg sacs (after hatching, number of spiderlings) and second and third egg sacs (before hatching, number of eggs). Bars with the same letter annotation indicate values that are not significantly different.

differentiation (95.7%, $n = 349$) in the egg sacs of infected mothers, male-killing is most likely occurring late in embryonic development or during hatching.

As the sister species *O. gibbosus* is infected with similar genera of endosymbionts, establishing the phylogenetic position of the 16S sequences could present valid information on the relatedness of the endosymbionts. This reveals a close relatedness between the *Cardinium* endosymbionts infecting both species. This is also the case for the *citrate* gene sequence in *Rickettsia*, which is closely related to the sequence of the *Rickettsia* endosymbiont of *O. gibbosus*. In contrast, a clear dissimilarity is found between the *wsp* sequences of *Wolbachia*. The *O. retusus wsp* sequence clusters within supergroup B, while the *wsp* sequence of *O. gibbosus* clusters within supergroup G. Therefore, these data suggest that *Cardinium* and *Rickettsia* infection predates the divergence of these species, followed by independent invasions of different strains of *Wolbachia* in the two species. However, to gain more insight into the routes of infection in the different species and the relatedness between endosymbionts in the genus *Oedothorax*, a more detailed analysis by applying a multilocus comparison (Baldo et al. 2006) would be most suitable.

Although our research is based on a small sample size, the similarity of these results to a prior study of endosymbionts of *Oedothorax gibbosus* is striking (Vanthournout et al. 2011). For the populations investigated, both species seem to be fixed for the *Cardinium* endosymbiont, while the *Wolbachia* infection shows a more variable pattern with approximately half of the individuals infected. The infection pattern of the *Rickettsia* endosymbiont is different for the two species; *O. gibbosus* seems to be fixed, while *O. retusus* shows infection for half of the individuals.

The effect of male-killing endosymbionts in several species of ladybirds shows high variation in the production of offspring sex ratios, ranging from all-female broods to the production of significant numbers of males, (Hurst et al. 1992; Majerus & Majerus 2010b). This is similar to the male-killing effect in both *O. retusus* and *O. gibbosus*, with infected females showing a high variation in sex ratio among clutches, which is of the same order of magnitude [*O. gibbosus*: 0.36 ± 0.04 (Vanthournout et al. 2011); *O. retusus*: 0.35 ± 0.06].

In contrast, for the butterfly species *Hypolimnas bolina* and *Acraea encedon*, infection with male-killers exhibits a higher level of penetrance with the production of only all female broods (Jiggins et al. 2001; Dyson et al. 2002). The evolutionary significance of this difference in pattern of expression of sex ratio distortion remains to be investigated.

Our findings suggest that the phenotypic effects of endosymbiont bacteria on reproductive characteristics could be more widespread in the Araneae order. This confirms the use of a bacterial model as one possible mechanism of different reproductive phenotypes found in many spider species. Further studies into the effects in other spider taxa are necessary to determine their general susceptibility to endosymbiont bacteria and the effects on their hosts' ecology and evolution.

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Vertical stratification of spider assemblages in two conifer plantations in central Japan

Hiroki Oguri[1], **Tomohiro Yoshida**[2,5], **Akihiro Nakamura**[3], **Masashi Soga**[4] and **Naoki Hijii**[1]: [1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan; [2]Field Science Center, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan; [3]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, P. R. China; [4]Department of Forest Science, Graduate School of Agriculture, Hokkaido University, Sapporo 080-8589, Japan

Abstract. We compared the structure of spider assemblages between the upper and lower canopy layers, and between the canopy and forest floor, in plantations of evergreen cedar (*Cryptomeria japonica*) and deciduous larch (*Larix kaempferi*). The estimated number of species was similar between the upper and lower canopy layers (49.0 *vs* 45.1) in *C. japonica*, but was noticeably smaller in the upper canopy layer (11.3) than in the lower layer (36.9) in *L. kaempferi*. Arboreal spider assemblages in the canopy differed significantly between the upper and lower layers in both *C. japonica* and *L. kaempferi* stands, based on an abundance-based measure. However, based on an incidence-based measure, they only differed significantly between layers in the *L. kaempferi* stand. The spider assemblages also differed distinctly between the canopy and the forest floor in both stands. Wandering spiders and orb-web builders were dominant in the canopy, while space-web builders dominated the forest floor in the *C. japonica* stand. In the *L. kaempferi* stand, wandering spiders dominated both the canopy and the forest floor. Our results suggested that spider assemblages in conifer plantations were distinctive among strata because of differences in such factors as resource quality (i.e., living or dead foliage) and association with adjacent layers along the vertical gradient of the forests.

Keywords: Community composition, forest canopy, forest floor, functional groups

Vertical stratification in forests both above ground and at ground level is attributed to the variability of the three-dimensional spatial arrangement of trees and other structural elements (Ishii et al. 2004). Forest canopies provide various food resources such as leaves, fruits, and seeds and diversified microhabitats based on the structural complexity of foliage and twigs, resulting in a high abundance and diversity of arthropods (Lawton 1983; Basset et al. 2003). The forest floor also contains a mixture of organic resources such as leaf litter, fungi, and dead wood, with a continuous stratum packed into a thin layer (Lavelle & Spain 2005). Spiders (Arachnida: Araneae) are one of the most prevalent groups of predatory arthropods in species diversity and biomass, both in the canopy and on the forest floor (Moulder & Reichle 1972; Basset 1991; Wise 1993). These groups occupy a highly diversified set of habitats, ranging from various plants to the soil itself, construct a variety of web structures (or no web for many forest-floor species) and exhibit broad feeding behaviors.

The canopy and forest floor have different architectures derived from the substrates that exist in each stratum, which could be a determining factor of the structure of spider assemblages. Previous studies have revealed that the foliage structural complexity of the canopy and vegetation, such as foliage density and number of leaves and branchlets, affected spider species composition (Gunnarsson 1988, 1990; Sundberg & Gunnarsson 1994; Halaj et al. 2000; De Souza & Martins 2005; Corcuera et al. 2008). Likewise, the structural complexity of forest-floor litter and understory vegetation, such as litter depth, litter shape, interstitial space/volume, and ground cover by plants, can influence spider assemblages on the forest floor (e.g., Uetz 1975, 1979; Bultman & Uetz 1982; Docherty & Leather 1997; Bultman & Dewitt 2008). The canopy and forest-floor strata can provide different microhabitats for arthropods, presumably leading to different spider assemblages among the strata.

Even-aged and monoculture forest plantations usually have simple architecture compared to natural forests, and thus they are good model systems for examining the effects of the vertical structure of forests on biological communities. Japanese cedar *Cryptomeria japonica* D. Don and Japanese larch *Larix kaempferi* [Lamb.] Carrière, two endemic coniferous species in Japan, are general tree plantation species that provide different microhabitats for forest arthropods. For example, the seasonal stability of microhabitats differs between the two species: *C. japonica* is an evergreen species, whereas *L. kaempferi* is deciduous. *Cryptomeria japonica* trees usually have a large amount of dead foliage attached to their trunks in the lower part of the canopy (Yoshida & Hijii 2006), whereas in *L. kaempferi* forests, most of the foliage is alive in both the upper and lower layers until the period of leaf fall in late autumn (from October to November: Miyaura & Hozumi 1988). The structural complexity of foliage also differs greatly; thicker, harder, and more complex foliage forming needle-like leaves in *C. japonica*, compared to the soft and clumped needles of *L. kaempferi*. These differences in spatial and temporal traits between habitats and between tree species should affect the composition of spider assemblages in the canopy and on the forest floor.

In the present study, we investigated the community structures of arboreal and ground-dwelling spiders in *C. japonica* and *L. kaempferi* plantations to test two hypotheses: 1) compositions of arboreal spider species differ between the upper (living foliage) and lower (dead foliage) layers of the *C. japonica* canopy, but not of *L. kaempferi* due to its similarities

[5]Corresponding author. E-mail: yoshitom@cc.tuat.ac.jp

between upper and lower layers; and 2) spiders have different community structures in the canopy and on the forest floor due to the difference of habitat resources, such as elongate foliage and accumulated litter.

METHODS

Study site.—The study was carried out in a 38-year-old (as of 2008) *C. japonica* plantation and a 15-year-old *L. kaempferi* plantation in the Experimental Forest of Nagoya University, in central Japan (35°11′N, 137°33′E; 980 to 1000 m a.s.l.). Annual rainfall at this site averages 2100 mm, and the mean annual air temperature is 9.7 °C (2008). Both stands are embedded in a forested area and are more than 1000 m from each other. Tree height and height at the lower edge of the canopy are 24 m and 7 m in the *C. japonica* stand, and 10 m and 2 m in the *L. kaempferi* stand, respectively. In the *C. japonica* stand, the canopy is almost closed due to more densely packed and elongated, thickened branches in the upper layer and large numbers of dead leaves and branches remaining attached to the trunk of each tree in the lower layer (Yoshida & Hijii 2006). Thus the lower layer is similar in overall architecture to the upper layer. In the *L. kaempferi* stand, the canopy is more open due to less crowding of branches mixed in with some young broadleaf trees in the understory. The eanopy of eaeh stand was divided into upper and lower layers at the following points: in *C. japonica* at the uppermost height of dead leaves and branches attached to the tree stems (ca. 15 m above the ground), and in *L. kaempferi* at half the length of the canopy (ca. 6 m above the ground). The vertical lengths of the upper and lower layers of the canopy are 9 m and 8 m in *C. japonica*, 4 m and 4 m in *L. kaempferi*, respectively. Five trees of each species were selected for sampling of the spider assemblages. The sampled *C. japonica* trees were located near a 20-m tower and had a mean height (± SD) of 22.6 ± 0.4 m and a mean diameter at breast height of 23.9 ± 1.8 cm. The *L. kaempferi* trees sampled have a mean height of 9.1 ± 0.9 m and mean diameter of 10.1 ± 1.4 cm. The average thickness of the litter layer on the forest floor is less in the *C. japonica* stand (0.9 ± 0.4 cm) than in the *L. kaempferi* stand (3.4 ± 0.8 cm). However, many dead branches with foliage had accumulated on the ground in some parts of the *C. japonica* stand, increasing the local thickness of the litter layer (~ 6.8 cm) and thus causing a greater habitat heterogeneity on the forest floor than in the *L. kaempferi* stand (T. Yoshida, unpubl. data).

Spider sampling.—Spiders were collected from three habitats (upper and lower layers of canopies, and forest floor) in each tree stand at one-month intervals from 10 July to 19 December 2008. We accessed the canopies by using a 20-m tower in the *C. japonica* stand and by climbing on a connectable tube ladder on the trunks of *L. kaempferi* trees. Three branches in each layer were randomly selected for spider collection. Spiders were dislodged by beating the branches with a 1.8-m bamboo stick and were trapped with a fine net (0.2-mm mesh size; 60 cm in aperture diameter of a round frame). The spiders were quickly collected with a vacuum sampler and preserved in 70% ethanol. Spiders on the forest floor were collected using pitfall traps, which consisted of 400-cm^3 plastic cups with openings 7.5 cm in diameter. Each trap contained 100 ml of water, with small amounts of detergent to prevent the animals from floating, and one to two grams of sorbic acid for preservation. Ten traps were set at least 5 m apart from each other in a transect on the forest floor of each stand, the openings level with the ground surface (not with the top of the litter layer), and collected after a week.

Spiders were first sorted to genus, and then morphospecies or identified to described species according to the keys and descriptions of Chikuni (1989) and Ono (2009). We recorded the number of individuals in each species for each habitat, eaeh forest stand, and each month. Voucher speeimens were deposited in the Laboratory of Forest Protection, Nagoya University, Japan. Using the information in Shinkai (2006) and Ono (2009), we divided these species into four functional groups, which included the guilds reported by Halaj et al. (1998, 2000), Hatley & MacMahon (1980), Uetz et al. (1999) and Cardoso et al. (2011): 1) space-web builders, including hackled-band weavers (Dictynidae), sheet-web weavers (Cybaeidae, Agelenidae, and Linyphiidae) and cobweb spiders (Theridiidae); 2) orb-web weavers (Uloboridae, Araneidae and Tetragnathidae); 3) wandering spiders, including jumping spiders (Salticidae), ambushers (Thomisidae) and running spiders (Philodromidae and Lycosidae), nocturnal hunters (Clubionidae, Anyphaenidae and Gnaphosidae), and a part of Theridiidae and Araneidae that have wandering foraging strategies (Shinkai 2006); and 4) edaphic spiders (Antrodiaetidae).

Data analysis.—We excluded juveniles and unidentified individuals prior to the analyses. Spiders eollected from each habitat (i.e., upper canopy, lower canopy and forest floor from both tree species) were pooled for each month; hence, all analyses were based on six monthly samples within each habitat. We quantified the diversity of spider assemblages in each habitat using EstimateS 8.2 (Colwell 2009). With a bootstrap estimator, we randomized the data 100 times and calculated the estimated number of species (S_{est}). Using EstimateS, we also calculated 95% confidence intervals of the observed species richness (using MaoTau function).

We used permutational multivariate analysis of variance (PERMANOVA: Anderson et al. 2008) to assess the effects of forest stand (*C. japonica* and *L. kaempferi*), layer (upper and lower canopy), sampling month and their interactions on canopy spider assemblages. Likewise, we investigated the effects of stand and month on the forest floor spider assemblages. The design of the analysis is analogous to a repeated measures ANOVA, where we treated the effect of monthly variation as a random effect factor and the differences in stand and layer as fixed factors. Although PERMANOVA was developed primarily for multivariate analysis, univariate analysis is possible using Euclidean distances, which yield Fisher's traditional univariate F statistic (Anderson et al. 2008). Type III sums of squares were used to calculate F statistics (pseudo-F statistics: Anderson et al. 2008). P values were calculated using 4999 permutations of residuals under a reduced model. Post-hoc pair-wise analyses were conducted for some of the variables by calculating t statistics and P values using 4999 permutations of the data (available within PERMANOVA routine: Anderson et al. 2008). We also used non-metric multidimensional scaling (NMDS) ordinations, available in PRIMER6 (Clarke & Gorley 2006), to visually represent the species compositions

Table 1.—Species richness of spiders in the upper (UC) and lower canopies (LC) and on the forest floors (FF) of the *Cryptomeria japonica* (CJ) and *Larix kaempferi* (LK) stands.

		CJ			LK		
		UC	LC	FF	UC	LC	FF
Number of individuals[1]	n	832	889	39	662	888	299
Number of observed species	S_{obs}	43	40	16	11	34	27
Number of estimated species	S_{est}	49	45.1	19.5	11.3	36.9	31.5

[1] Excluding juvenile and unidentified individuals.

of canopy spiders in the upper and lower layers of the *C. japonica* and *L. kaempferi* stands. We did not use NMDS ordinations for the forest floor spiders because we collected very few individuals in the *C. japonica* stand (Table 1). Ordinations were conducted based on the abundance-based (Bray-Curtis index) and incidence-based (Sørensen index) similarity measures, with 25 restarts.

RESULTS

Spider assemblages in the canopy.—In total, we collected 3,609 individuals (excluding 51 juveniles and unidentified individuals), representing 100 species and morphospecies from both the canopy and forest floor during the study period (Appendix 1). We collected 43 (with 95% confidence interval of ± 8.7) and 40 ± 8.8 spider species in the upper and lower canopy layers of the *C. japonica* stand and 11 ± 0 and 34 ± 3.9 species in the *L. kaempferi* stand, respectively (Table 1). The estimated number of arboreal species was similar between in the upper (49.0 species) and lower canopy layers (45.1 species) in *C. japonica*, but was noticeably smaller in the upper canopy layer (11.3 species) than in the lower layer (36.9 species) in *L. kaempferi* (Table 1). With the exception of the upper layer of *L. kaempferi*, the estimated species richness fell within 95% confidence intervals of the observed number of species.

Species richness of arboreal spiders was significantly influenced by stand and layer, although their interaction effect was also significant (Table 2). Post-hoc pair-wise comparisons showed that species richness was significantly greater in the lower than in the upper layer in *L. kaempferi* ($t = 0.24$, $P < 0.05$), but not *C. japonica* ($t = 0.24$, $P = 0.81$). Unlike species richness, monthly variation was the only (but highly significant) factor influencing spider abundance (Table 2). The densities of spiders in both canopy layers peaked from August to October in both stands and then tended to decrease toward December (Fig. 1a, b).

Among the functional groups, wandering spiders were a significantly more abundant and species-rich group than orb-web weavers and space-web builders in the canopies of both the *C. japonica* and *L. kaempferi* stands (PERMANOVA, pseudo-$F = 12.8$, $P < 0.001$ for abundance; pseudo-$F = 12.6$, $P < 0.001$ for species richness: see Fig. 2a, b). Orb-web weavers were the second most abundant group in the *C. japonica* canopy, whereas space-web builders were much more abundant than orb-web weavers in the *L. kaempferi* canopy. The proportions of orb-web weavers were lower in abundance but higher in species richness in the lower canopy of the *L. kaempferi* stand (Fig. 2a, b). Statistical tests showed that the stand had a significant influence on proportional abundances of space-web builders and orb-web weavers, whereas the layer was only

Table 2.—Summary results of PERMANOVA, showing pseudo-F values and degrees of freedom (df) of stand, layer, month and their interaction effects on spiders collected from the canopy. Spiders were analyzed using species composition (assemblage), total abundance, species richness and three major functional groups, based on the abundance-based (upper half of the table) and incidence-based (lower) data. Functional groups were analyzed using proportional abundance (upper) or species richness (lower) per site.

		Assemblage				Prop. abund.		Prop. abund.		Prop. abund.	
	df	(abundance-based)		Abundance		SW		OW		WS	
Stand	1	7.33	**	0.87	n.s.	13.01	*	15.73	*	3.27	n.s.
Layer	1	8.09	**	6.25	n.s.	1.82	n.s.	35.89	**	0.58	n.s.
Month	5	8	***	35.96	***	32.08	**	7.48	*	17.22	**
Stand × Layer	1	5.86	*	1.71	n.s.	1.24	n.s.	9.28	*	0.08	n.s.
Stand × Month	5	5.68	***	0.25	n.s.	16.43	**	14.09	**	11.91	**
Layer × Month	5	1.06	n.s.	0.77	n.s.	2.1	n.s.	1.22	n.s.	1.73	n.s.
Residual	5										
		Assemblage				Prop. species		Prop. species		Prop. species	
	df	(incident-based)		Species richness		SW		OW		WS	
Stand	1	7.86	**	99.46	**	0.79	n.s.	2.76	n.s.	0.06	n.s.
Layer	1	6.3	**	14.43	*	0.002	n.s.	18.1	*	19.16	*
Month	5	6.08	**	8.39	*	22.18	**	1.74	n.s.	15.67	**
Stand × Layer	1	9.35	*	7.35	*	1.93	n.s.	7.38	*	17.54	**
Stand × Month	5	5.45	**	0.23	n.s.	14.42	**	2.22	n.s.	14.76	**
Layer × Month	5	1.51	n.s.	0.63	n.s.	2.43	n.s.	0.92	n.s.	0.87	n.s.
Residual	5										

*: $P < 0.05$.
**: $P < 0.01$.
***: $P < 0.001$.
n.s.: $P > 0.05$.
SW: Space-web builders, OW: Orb-web weavers, WS: Wandering spiders.

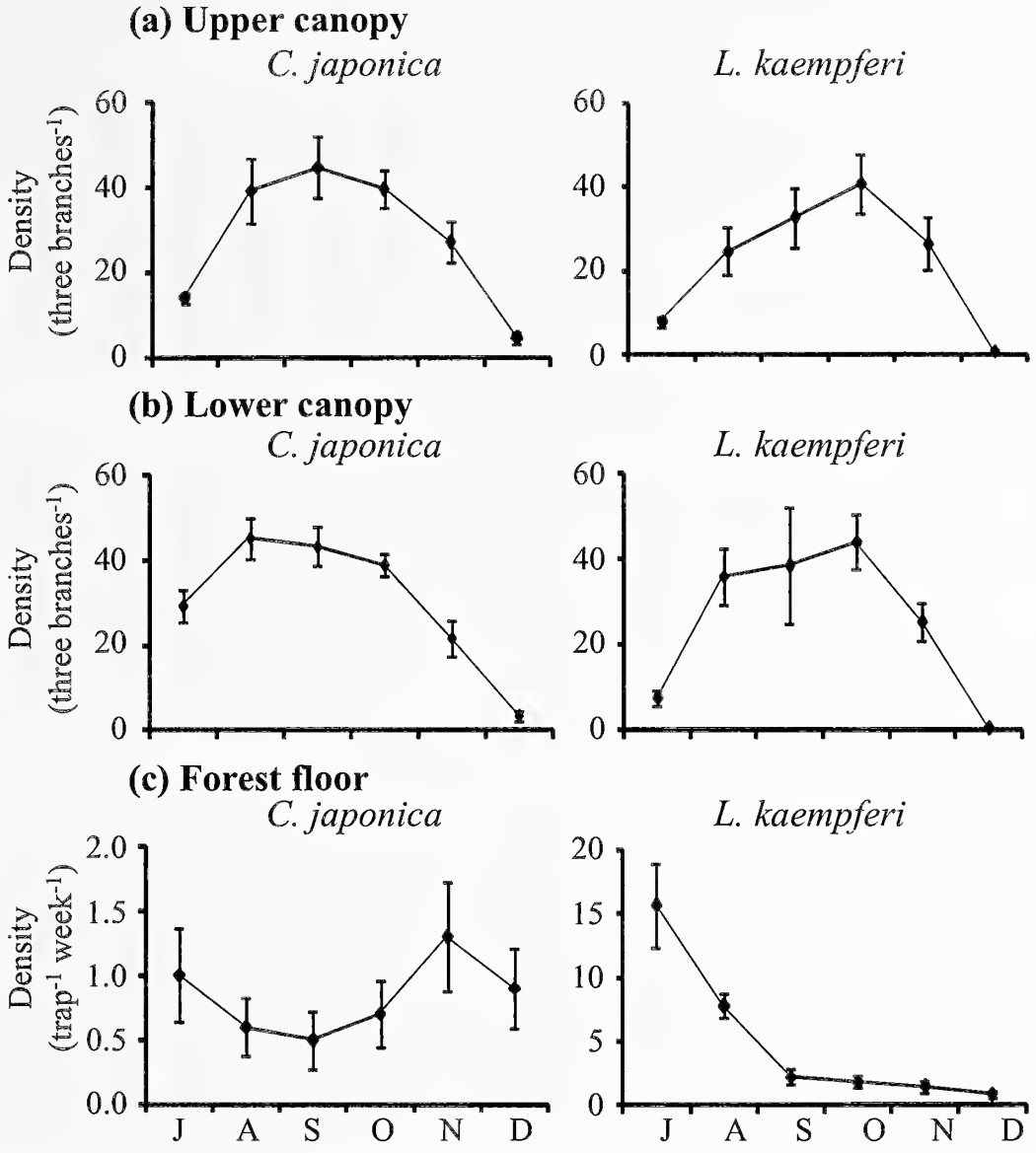

Figure 1.—Seasonal changes in the densities of spiders in the a) upper and b) lower layers of the canopy and c) on the forest floors in the *Cryptomeria japonica* and *Larix kaempferi* stands. Values represent mean ± standard error.

significant with respect to orb-web weavers (Table 2). However, as suggested by the significant interaction effect of stand and month, proportional abundances of space-web builders were higher in *L. kaempferi* than in *C. japonica*, but marked differences were observed in winter only (viz. November and December: Table 2, Fig. 2a). Likewise, proportional abundances of orb-web weavers were generally greater in *C. japonica*, but the differences were much greater in lower layers in early summer (July). Monthly variation was significant in abundances of all three functional groups; however, the differences were more pronounced within the *L. kaempferi* canopy than within *C. japonica* (Table 2, Fig. 2a). A significantly greater proportional species richness of wandering spiders was observed within the upper than the lower canopy layers in *L. kaempferi*, but similar trends were not observed in *C. japonica*, presumably due to the interaction effect between stand and layer (Table 2, Fig. 2b). Likewise, a greater proportional species richness of orb-web spiders was observed in the lower than in the upper canopy layers of *L. kaempferi*, but not in *C. japonica* (Table 2, Fig. 2b). Significant monthly variations were suggested for wandering spiders; however, due to the presence of interaction effects, such a variation was observed only in *L. kaempferi*, where the species richness declined to zero in winter. As opposed to wandering spiders, proportional species richness of space-web spiders increased in winter in the *L. kaempferi* stand (Table 2, Fig. 2b).

The community compositions of arboreal spider species according to both the abundance-based and incidence-based measures differed significantly between stands and between layers; however, there was also an interaction effect between these two factors (Table 2). NMDS ordinations and post-hoc pair-wise comparisons showed that all four treatments significantly separated species assemblages when using abundance-based Bray-Curtis measures (Fig. 3). When we used incidence-based Sørensen measures, however, spider assemblages did not differ significantly between the upper and lower canopies of the *C. japonica*.

Spider assemblages on the forest floor.—We sampled 39 individuals of 16 (with 95% confidence interval of ± 6.6)

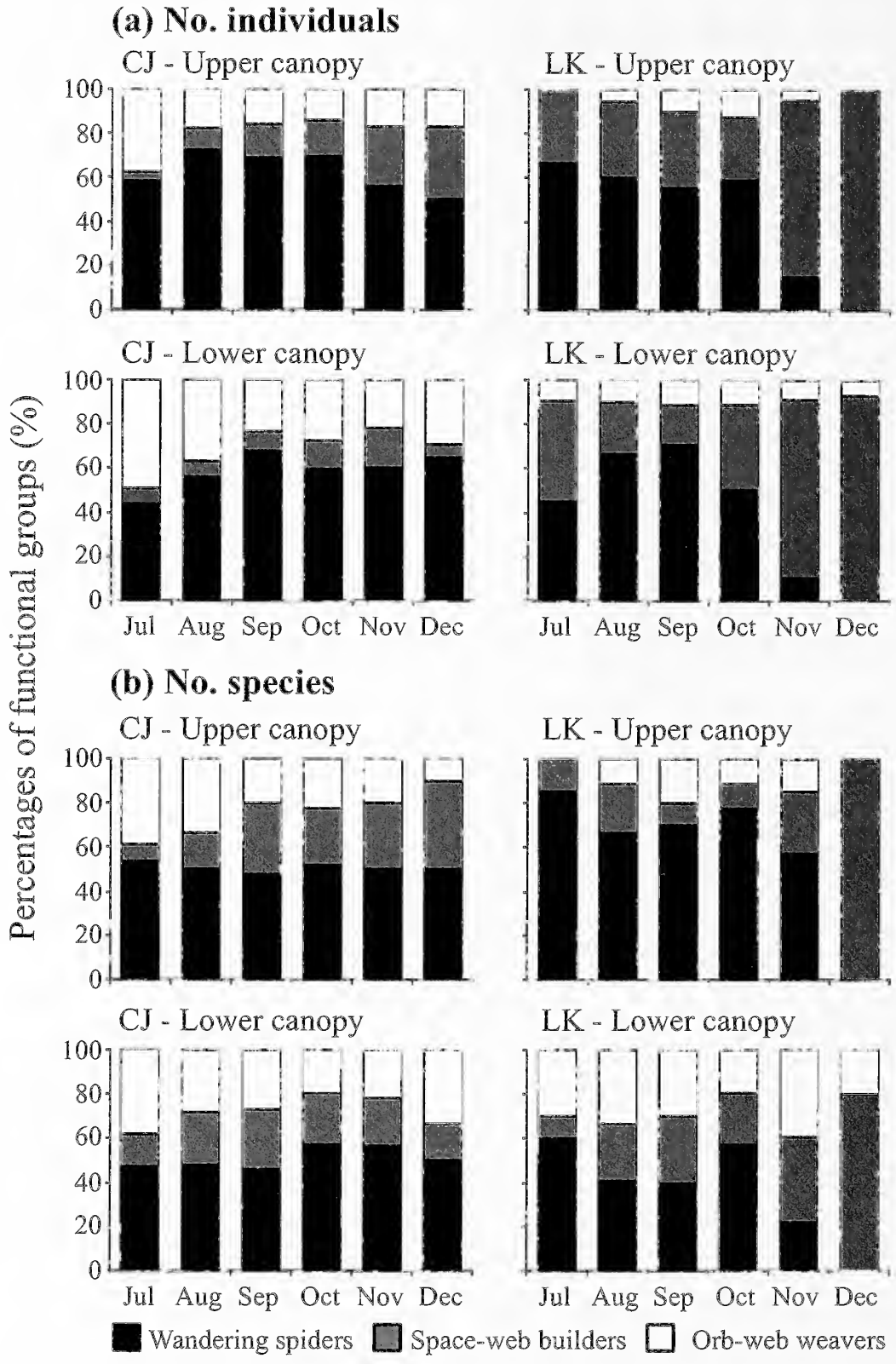

Figure 2.—Seasonal changes in the percentages (%) of a) individuals and b) species richness of functional groups in the upper and lower layers of the canopy in the *Cryptomeria japonica* (CJ) and *Larix kaempferi* (LK) stands.

species (excluding juveniles and unidentified individuals) and 299 individuals of 27 ± 5.3 species on the forest floors of the *C. japonica* and *L. kaempferi* stands, respectively (Table 1). Only three spider species were found both in the canopy and on the forest floor: *Octonoba sybotides* (Bösenberg & Strand 1906), *Tetragnatha yesoensis* S. Saito 1934 and *Pseudomicrargus latitegulatus* (Oi 1960) (Appendix 1). The estimated number of forest-floor species (19.5 species) was smaller than those in the

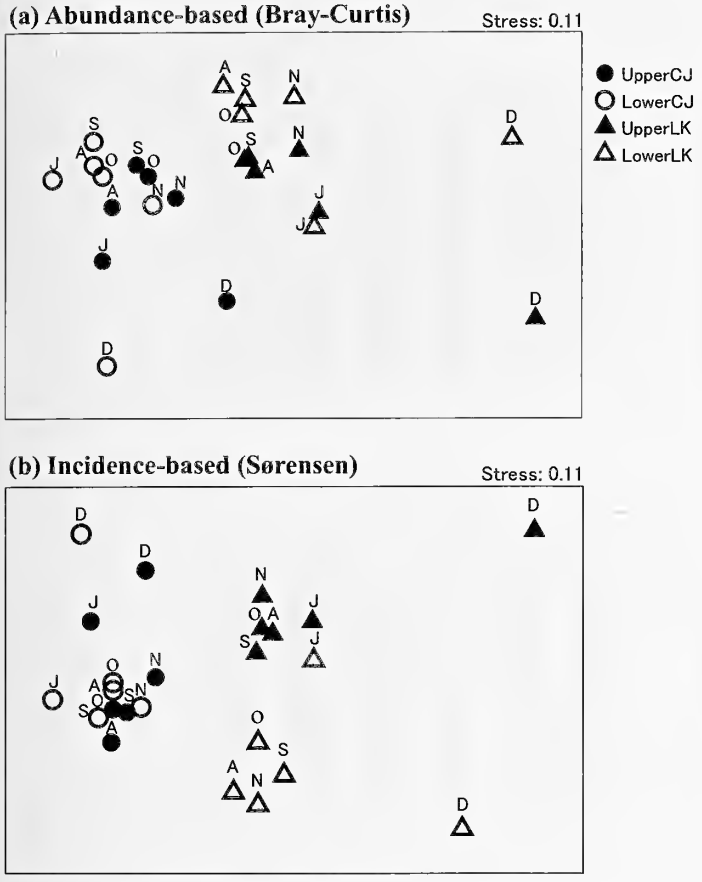

Figure 3.—Nonmetric multidimensional scaling ordination plots for spider assemblages in the upper and lower layers of the canopy in the *Cryptomeria japonica* (CJ) and *Larix kaempferi* (LK) stands according to a) abundance-based (Bray-Curtis index) and b) incidence-based (Sørensen index) similarity measures. J: July, A: August, S: September, O: October, N: November, D: December.

upper (49.0 species) and lower canopy layers (45.1 speeies) of *C. japonica*, whereas the value for the forest floor in *L. kaempferi* (31.5 species) was larger than that in the upper canopy layer (11.3 species). The active density of ground-dwelling spiders on the forest floor of the *C. japonica* stand was relatively constant across the study period, whereas the density showed a peak in July and tended to decrease toward December on the forest floor of the *L. kaempferi* stand (Fig. 1c).

The abundance of space-web builders accounted for a greater proportion than that of wandering spiders in the *C. japonica* stand, whereas the opposite was found within the *L. kaempferi* stand (Fig. 4). Few orb-web weavers and edaphic spiders were collected throughout the study period.

DISCUSSION

Our results showed that arboreal spider assemblages assessed by the abundance-based measure differed signifieantly between the upper and lower layers of the *C. japonica* and *L. kaempferi* stands, but those assessed by the incidence-based measure differed significantly between layers of the *L. kaempferi* stand only. This result may partly support the first hypothesis that different spider assemblages would be established between the upper and lower layers of *C. japonica* trees because of differences in potential resources for spider habitats between the layers (i.e., living foliage versus dead foliage). Two possible factors may be responsible for the existence of different spider assemblages within the canopy of *C. japonica* and *L. kaempferi* stands. First, arboreal spiders might prey on phytophagous arthropods in the upper canopy layer and on detritivorous microarthropods in the lower layer of *C. japonica* trees. The different composition of spider assemblages within the canopy might not depend on the physical structure of the habitats because the structural complexity was not so different between the upper layer (mainly living foliage) and lower layer (dead foliage) of the canopy in *C. japonica*. Shimazaki & Miyashita (2005) suggested that on the forest floor in *C. japonica* stands, smaller web-building spiders depend more on the prey derived from the detrital food web than do larger spiders. Although we did not perform a quantitative comparison, the evidence that detrital microarthropods (e.g., Collembola) were abundant specifically on the dead foliage of *C. japonica* (Yoshida & Hijii 2005) supports the dominance of smaller spiders in the lower layer of the canopy.

Second, the difference in spider assemblages between the canopy layers in the *L. kaempferi* stand was attributed to a large number of less abundant species (mainly orb-web weavers) in the lower layer (these species were largely absent in the upper layer). The less abundant species might colonize from understory vegetation that is next to the lower canopy layer. Although we did not collect spiders from this layer, some studies have shown that understory vegetation shared some spider species with those found on the canopy (Sørensen 2003; Larrivée & Buddle 2009; Aikens & Buddle 2012; Pinzon et al. 2013). Turnbull (1960) reported that in general spider species were stratified across the vertical structure of forests, but that they also frequently extended their distributions beyond each of their preferred strata. Pinzon et al. (2013) showed a species turnover along the vertical gradient (forest floor, understory and lower canopy) of white spruce stands. Pinzon et al. (2013) predicted that the community composition in the upper canopy was also different from other strata, and Aikens & Buddle (2012) and our result support their prediction.

Several studies have shown that the community composition of spiders differed between the canopy and forest floor, but that some spider species shared strata in coniferous (Pinzon et al. 2013), deciduous (Turnbull 1960), and montane (Sørensen 2003) forests. Pinzon et al. (2011) showed that spiders on the forest floor are more similar to those in the canopy than to those on the understory vegetation, suggesting that the two habitats could be linked by spiders moving along tree trunks. Our findings, however, indicated that the canopy habitats shared few spider species with the forest floor in the *C. japonica* and *L. kaempferi* stands. The reason is unknown, but may be attributed to the microenvironment of tree trunks (e.g., bark structure) and understory vegetation (e.g., biomass and/or architecture), which can serve as a 'habitat filter' between the canopy and forest floor.

The proportions of functional groups also differed between the canopy and forest floor. Wandering spiders were dominant in both layers in the *L. kaempferi* stand, whereas wandering spiders and orb-web builders were dominant in the canopy and space-web builders on the forest floor in the *C. japonica* stand. Although we need to be cautious about differences in sampling methods, this difference between the layers could be

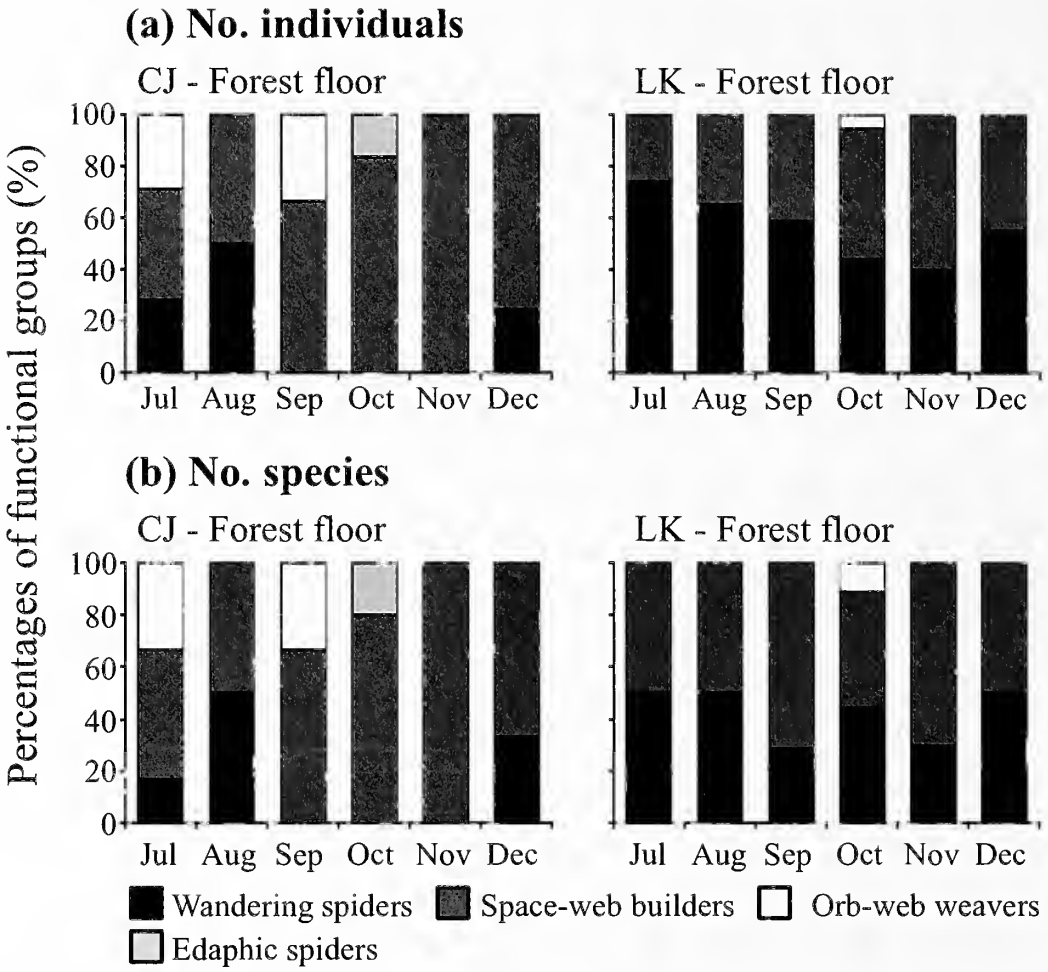

Figure 4.—Seasonal changes in the percentages (%) of a) individuals and b) species richness of functional groups on the forest floors in the *Cryptomeria japonica* (CJ) and *Larix kaempferi* (LK) stands.

related to the differences in structural complexity of the habitat substrates. Field manipulations of foliage density by Hatley & MacMahon (1980) and Halaj et al. (2000) showed that wandering spiders decreased with the removal of foliage, but increased when branches were tied up, as opposed to web-building spiders, which showed weaker responses to foliage manipulations. In our study, the relative abundances of wandering spiders in the *L. kaempferi* canopy substantially decreased in November and were almost absent in December. This would be due to the decrease in structural complexity of foliage associated with the seasonal leaf fall of *L. kaempferi* in late autumn (Miyaura & Hozumi 1988). Thus, both the canopies of *C. japonica* and *L. kaempferi* trees would provide dense foliage structures more favorable for wandering spiders than for web builders. On the forest floor of the *C. japonica* stand, branches with dead foliage made a structurally heterogeneous litter layer with much interstitial space. The structural complexity of the accumulated litter layer allowed a greater abundance of web-building spiders (Bultman & Uetz 1982), and space-web builders are known to build webs in narrow spaces, such as those formed between the needles of conifer trees (Stratton et al. 1979). Accordingly, the space-web builders might have dominated the forest floor of the *C. japonica* stand.

In conclusion, our analyses in the *C. japonica* and *L. kaempferi* stands suggest that distinctive spider assemblages were established between vertical strata, reflecting the differences in factors, such as resource quality (i.e., living- or dead foliage, accumulated litter) and association with adjacent layers, along the vertical gradient of the forests. Basset et al. (2003) noted that arthropod stratification in forests could be determined by four types of factors: abiotic factors, forest physiognomy and tree architecture, resource availability and arthropod behavior. Further quantitative approaches related to these factors are required for a comprehensive understanding of the vertical stratification and horizontal spacing of spider assemblages in forest ecosystems.

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Appendix 1.—Numbers of individuals of spiders collected from upper (UC) and lower layers (LC) of the canopy and forest floor (FF) in the *Cryptomeria japonica* (CJ) and *Larix kaempferi* (LK) stands. Juveniles and unidentified spiders (denoted by asterisks) were not included in the analyses.

Functional group		CJ			LK		
family	species	UC	LC	FF	UC	LC	FF
Edaphic spiders		**0**	**0**	**1**	**0**	**0**	**0**
Antrodiaetidae	*Antrodiaetus roretzi* (L. Koch 1878)	-	-	1	-	-	-
Space-web builders		**131**	**89**	**30**	**279**	**336**	**99**
Agelenidae	*Allochubionoides* sp.	-	-	-	-	-	1
	Coelotes personatus Nishikawa 1973	-	-	4	-	-	2
	Coelotes decolor Nishikawa 1973	-	-	1	-	-	2
	Coelotes gifuensis Nishikawa 2009	-	-	-	-	-	3
	Coelotes spp.	-	-	-	-	-	25
	Ormnicekia satoi (Nishikawa 2003), n. comb.	-	-	3	-	-	12
	Tegecoelotes corasides (Bösenberg & Strand 1906)	-	-	1	-	-	-
	Agelenidae juvenile *	2	8	8	-	-	-
Cybaeidae	*Cybaeus nipponicus* (Uyemura 1938)	-	-	2	-	-	-
	Cybaeus kirigaminensis Komatsu 1963	-	-	4	-	-	-
	Cybaeus tottoriensis Ihara 1994	-	-	1	-	-	-
	Cybaeus sp.1	-	-	8	-	-	-
	Cybaeus spp.	-	-	-	-	-	3
Dictynidae	*Lathys maculosa* (Karsch 1879)	62	38	-	-	-	-
	Lathys sexoculata Seo & Sohn 1984	1	15	-	-	-	-
Hahniidae	*Halmia corticicola* Bösenberg & Strand 1906	-	-	-	-	-	1
	Aprifrontalia mascula (Karsch 1879)	-	-	-	-	-	1
	Ceratinopsis setoensis (Oi 1960)	-	-	-	-	-	4
Linyphiidae	*Floronia exornata* (L. Koch 1878)	-	-	-	-	9	-
	Gonatium japonicum Simon 1984	-	-	-	-	-	1
	Neolinyphia fusca Oi 1960	-	-	-	-	4	-
	Neriene brongersmai (van Helsdingen 1969)	-	-	5	-	-	-
	Neriene spp.	-	-	-	-	-	3
	Nippononeta obliqua (Oi 1960)	-	-	1	-	-	35
	Porrhomma spp.	-	-	-	-	-	2
	Prolinyphia limbatinella (Bösenberg & Strand 1906)	4	11	-	-	-	-
	Pseudomicrargus latitegulatus (Oi 1960)	-	1	-	-	-	4
	Strandella yaginumai H. Saito 1982	-	1	-	-	-	-
	Tmrinyphia ymohamensis (Bösenberg & Strand 1906)	8	6	-	-	11	-
Theridiidae	*Anelosimus crassipes* (Bösenberg et Strand 1906)	1	-	-	-	-	-
	Chikunia albipes (S. Saito 1935)	-	-	-	-	3	-
	Chrysso foliata (L. Koch 1878)	-	-	-	-	3	-
	Coleosoma octomaculatum (Bösenberg & Strand 1906)	1	-	-	-	-	-
	Enoplognatha abrupta (Karsch 1879)	1	-	-	-	-	-
	Enoplognatha caricis (Fickert 1876)	-	-	-	-	1	-
	Episinus affinis Bösenberg et Strand 1906	2	3	-	2	4	-
	Euryopis flavomaculata (C. L. Koch 1836)	7	-	-	-	-	-
	Parasteatoda japonica (Bösenberg & Strand 1906)	1	1	-	-	-	-
	Phoroncidia altiventris Yoshida 1985	-	1	-	-	-	-
	Takayus chikunii (Yaginuma 1960)	5	1	-	-	-	-
	Takayus takayensis (S. Saito 1939)	37	11	-	277	273	-
	Yunohamella lyrica (Walckenaer 1842)	1	-	-	-	28	-
Orb-web weavers		**147**	**281**	**3**	**53**	**92**	**1**
Araneidae	*Alenatea fuscocoloratus* (Bösenberg & Strand 1906)	1	1	-	-	1	-
	Araneus acusisetus Zhu & Song 1994	21	23	-	2	13	-
	Araneus macacus Uyemura 1961	-	1	-	-	-	-
	Araneus rotundicornis Yaginuma 1972	-	-	-	-	1	-
	Araneus stella (Karsch 1879)	1	-	-	-	-	-
	Araneus uyemurai Yaginuma 1960	1	-	-	-	-	-
	Araneus viridiventris Yaginuma 1969	-	1	-	-	-	-
	Araneus spp.	6	12	-	-	-	-
	Araniella displicata (Hentz 1847)	6	-	-	-	-	-
	Araniella yaginumai Tanikawa 1995	-	-	-	-	6	-
	Cyclosa ginnaga Yaginuma 1959	-	-	-	-	2	-
	Eriophora sachalinensis (S. Saito 1934)	3	4	1	-	9	-

Appendix 1.—Continued.

Functional group		CJ			LK		
family	species	UC	LC	FF	UC	LC	FF
	Neoscona punctigera (Doleschall 1857)	1	-	-	-	-	-
	Neoscona scylla (Karsch 1879)	1	-	-	-	2	-
	Neoscona subpullata (Bösenberg & Strand 1906)	-	1	-	-	1	-
	Parazygiella disper (Kulczyński 1885)	-	2	-	-	9	-
	Yaginumia sia (Strand 1906)	3	2	-	-	-	-
	Araneidae juvenile *	1	-	-	-	-	-
Tetragnathidae	*Leucauge subblanda* Bösenberg & Strand 1906	-	-	1	-	-	-
	Leucauge sp.	-	-	-	-	1	-
	Tetragnatha shinanoensis Okuma & Chikuni 1978	11	21	-	-	2	-
	Tetragnatha yesoensis S. Saito 1934	79	99	-	51	45	1
Uloboridae	*Octonoba sybotides* (Bösenberg & Strand 1906)	13	114	1	-	-	-
Wandering spiders		**554**	**519**	**5**	**330**	**460**	**199**
Anyphaenidae	*Anyphaena pugil* Karsch 1879	51	46	-	8	3	-
Araneidae	*Chorizopes nipponicus* Yaginuma 1963	1	1	-	-	-	-
Clubionidae	*Clubiona jucunda* (Karsch 1879)	69	22	-	-	-	-
	Clubiona kurosawai Ono 1986	2	5	-	-	5	-
	Clubiona lena Bösenberg & Strand 1906	-	-	-	-	-	1
	Clubiona spp.	46	30	1	11	29	1
Corinnidae	*Otacilia komurai* (Yaginuma 1952)	-	-	-	-	-	1
Gnaphosidae	*Drassyllus shaanxicnsis* Platnick & Song 1986	-	-	-	-	-	9
	Drassyllus sasakawai Kamura 1987	-	-	-	-	-	2
	Drassyllus spp.	-	-	-	-	-	3
	Gnaphosa akagiensis Hayashi 1994	-	-	-	-	-	1
Lycosidae	*Pardosa laura* Karsch 1879	-	-	-	-	-	170
	Pirata clercki (Bösenberg et Strand 1906)	-	-	-	-	-	8
	Pirata yaginumai Tanaka 1974	-	-	4	-	-	-
Philodromidae	*Philodromus subaureolus* Bösenberg & Strand 1906	60	8	-	25	3	-
Salticidae	*Evarcha albaria* (L. Koch 1878)	-	-	-	-	3	-
	Evarcha sp.	-	-	-	-	1	-
	Plexippoides annulipedis (S. Saito 1939)	3	1	-	-	-	-
	Plexippoides doenitzi (Karsch 1879)	-	-	-	13	21	-
	Rhene atrata (Karsch 1881)	1	-	-	-	-	-
	Sibianor kochicnsis (Bohdanowicz & Prószyński 1987)	-	-	-	-	-	1
	Sibianor spp.	-	-	-	-	-	2
	Sitticus spp.	-	-	-	-	5	-
	Stertinius kumadai Logunov, Ikeda & Ono 1997	6	34	-	-	-	-
	Yaginumaella striatipes (Grube 1861)	15	20	-	-	-	-
	Salticidae juvenile *	-	1	-	-	-	-
Theridiidae	*Argyrodes cylindratus* Thorell 1898	-	5	-	-	-	-
	Ariamnes cylindrogaster Simon 1888	-	1	-	-	-	-
	Keijia sterninotata (Bösenberg et Strand 1906)	81	212	-	-	-	-
	Phycosoma anamiense (Yoshida 1985)	1	-	-	-	8	-
	Phycosoma mustelinum (Simon 1888)	45	11	-	-	-	-
	Rhomphaea sagana (Dönitz et Strand 1906)	-	1	-	-	-	-
Thomisidae	*Diaea subdola* O. Pickard-Cambridge 1885	128	13	-	74	71	-
	Lysiteles coronatus (Grube 1861)	32	15	-	160	241	-
	Synaema chikunii Ono 1983	12	94	-	39	61	-
	Tmarus rimosus Paik 1973	1	-	-	-	-	-
	Xysticus spp.	-	-	-	-	9	-
	Unidentified *	13	9	3	4	2	-
	Total	**848**	**907**	**50**	**666**	**890**	**299**

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Assessing spider diversity on the forest floor: expert knowledge beats systematic design

Elvira Sereda[1], Theo Blick[2], Wolfgang H. O. Dorow[2], Volkmar Wolters[1] and Klaus Birkhofer[1,3]: [1]Department of Animal Ecology, Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany. E-mail: Elvira.Sereda@allzool.bio.uni-giessen.de; [2]Senckenberg Gesellschaft für Naturforschung, Senckenberganlage 25, 60325 Frankfurt am Main, Germany; [3]Department of Biology, Biodiversity and Conservation Science, Lund University, Sölvegatan 37, 223 62 Lund, Sweden

Abstract. The design of sampling schemes affects the results of biodiversity inventories. As an approach for quantifying the implications of such effects, we compared data on spider communities sampled in a beech-dominated forest floor habitat by 1) a regular grid of pitfall traps (systematic design) and 2) an expert-based distribution of traps (stratified design). We tested whether the two designs would lead to similar conclusions about the diversity and composition of ground-dwelling spider communities. Estimates of species richness, rarefied species richness and activity density calculated per trap were significantly higher in the stratified than in the systematic design. The community composition based on the presence or absence of sampled species or based on log-transformed activity densities differed significantly. Most of the dissimilarity between the community estimates of the two designs was attributable to three species, with *Pardosa saltans* Töpfer-Hofmann 2000 being more common in traps of the stratified design and *Tenuiphantes zimmermanni* (Bertkau 1890) and *Walckenaeria cuspidata* Blackwall 1833 being more frequently observed in traps of the systematic design. Our study suggests that a stratified sampling design is better suited for inventory surveys of spider communities of forest-floor habitats, as trap locations of this design reflect specific habitat needs. It is important to note that inventories are a major field for the application of such designs and that greater care is needed for the application of inferential statistics. For example, the non-randomness that is caused by expert selection of sampling sites may violate fundamental assumptions of simple linear models.

Keywords: Araneae, biodiversity, inventory, expert-based sampling, regular sampling, sampling design

Biodiversity research provides crucial information for the development of conservation strategies (Brooks et al. 2004). Strict inventories that generate comprehensive taxonomic lists for a discrete spatiotemporal unit are thus prerequisites for protecting species richness (Longino & Colwell 1997). Moreover, reliable estimates of species composition are needed to enable researchers to monitor biodiversity changes successfully (Dorow et al. 1992; Colwell & Coddington 1994; Buckley & Roughgarden 2004). As a contribution to this issue, we compared estimates of diversity and species composition of ground-dwelling spiders in a forest-floor habitat of a beech forest with two different sampling designs (systematic vs. stratified; e.g., Southwood & Henderson 2000).

Systematic designs that are based on a regular distribution of sampling locations in a study area (Woodcock 2005) are a common approach in diversity surveys (e.g., sampling transects for flower-visiting insects: Rundlöf et al. 2008). However, such a design depends on a priori decisions on the distance between sampling points in relation to the scale of environmental heterogeneity and the mobility of the focal taxa. A regular placement of sampling locations further assumes that environmental gradients which affect the analysed taxa are constant over the study area and do not vary over different spatial scales (Quinn & Keough 2002). Systematic designs may therefore be most appropriate for homogeneous habitats with weak or very simple environmental gradients. Dorow et al. (2007) suggested that stratified sampling of pre-defined subpopulations provides an appropriate alternative for biodiversity inventories, since it may improve precision by taking account of specific habitat types (see also Hayek & Buzas 1997). In stratified designs, specific microhabitats can be selected based on expert knowledge, and this approach may thus provide a more precise estimate of diversity in heterogeneous study regions than random sampling (Southwood & Henderson 2000). In general, subjective selection of sampling locations biases analyses of ecological data by preconceptions of the investigator (Hirzel & Guisan 2002). However, subjectivity may be necessary and valid for certain research questions (McCune & Grace 2002). A strict inventory of species richness in heterogeneous habitats, for example, may only be reliable if the sampling design is biased by expert knowledge toward locations that support rare species and habitat specialists. An important assumption for using data from stratified designs is that information about the stratum is included as a predictor in statistical models (Quinn and Keough 2002). Comparative studies on the trade-offs between systematic and stratified designs are generally rare (Hirzel & Guisan 2002) and not available for invertebrate communities in temperate forests.

Our study focused on spiders, because this taxon forms a diverse group in temperate forests, and species are sensitive to environmental heterogeneity (Wunderlich & Blick 2006; Ziesche & Roth 2008; Birkhofer et al. 2010). Data were collected with pitfall traps in a 34.8 ha area for 16 months. The spatial arrangement of traps either followed a systematic design (regular grid) or a stratified design (expert-based selection of 14 pre-defined habitat structures). We hypothesize that the design based on expert knowledge would provide a more complete estimate of spider diversity than the systematic design based on a regular grid.

METHODS

Study site and sampling.—The study was conducted in the strict forest reserve "Locheiche" located in the National Park Kellerwald-Edersee in the northern part of Hesse, Germany

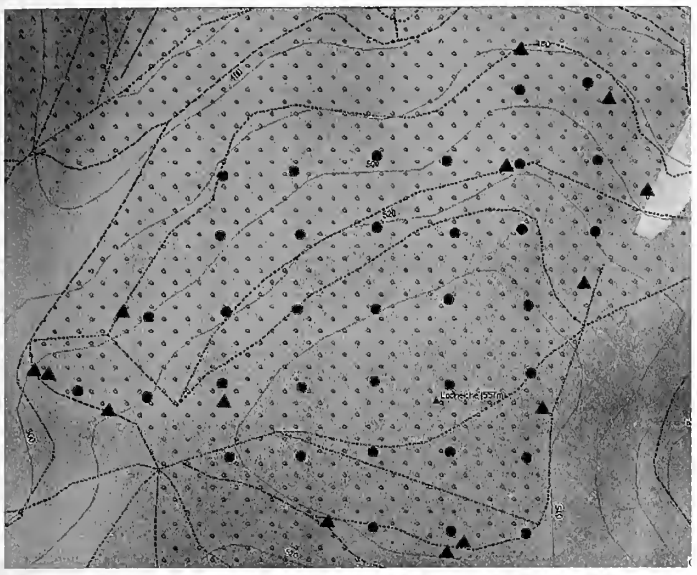

Figure 1.—Trap locations (points) in the 34.8 ha study area in the Kellerwald strict forest reserve "Locheiche" with the systematic (circles, one trap per point) and the stratified design (triangles, three traps per point).

(480–555 m a.s.l.; 51°08′30.45″N, 08°59′21.82″E) as a part of the long term studies in the forest reserves of Hesse (Dorow et al. 2010). The forest has not been managed since 1988, and beech trees (*Fagus sylvatica* L.) of an age of 81–120 years grow on the north and west exposed slopes of the study area. Additional tree species are *Quercus petraea* (Mattuschka) Liebl., *Larix decidua* Mill., *Acer pseudoplatanus* L. and *Picea abies* (L.) H. Karst. The annual mean temperature is 7.6°C, and the average annual precipitation is 765 mm (www.naturwaelder.de). The soil type is a cambisol with a pH of 5.1 in the uppermost horizon (Harmonized World Soil Database 2009).

In total, 77 funnel pitfall traps (diameter 10 cm, filled with approximately 200 ml of 70% ethanol and 99.5% glycerin at a ratio of 2:1) were placed on the forest floor (for details see Dorow et al. 1992). Thirty-five traps were arranged in a regular grid with an inter-trap distance of 100 m (systematic design, referred to as SYS below: Fig. 1), and 14 triplets of traps (42 traps in total) were placed at pre-defined locations with a distance of 5 m between traps in a triplet (stratified design, referred to as STR below: Fig. 1, Table 1). We account for these differences in inter-trap distances within and between designs in our analyses (see statistical analysis). Forest inventory points at 100×100 m grid intersections were established on the forest floor, and pitfall traps of the systematic design were placed next to these standardized locations. Locations of the traps in the stratified design were defined based on an inspection of the study area and structures outlined in Table 1. Traps were open for 16 months (29 October 2008 to 23 March 2010) and were emptied every 4 weeks. In winter, traps were not emptied before spring due to snow cover from 11 December 2008 to 25 March 2009 and from 25 November 2009 to 23 March 2010. Spiders were determined using standard keys (Roberts 1987, 1995; Nentwig et al. 2013), and the nomenclature followed Platnick (2013). Juveniles were only identified to the family level and were not included in the analysis.

Statistical analysis.—Before analyses commenced diversity metrics were corrected for differences in sampling effort between designs (systematic: 35 traps vs. stratified: 42 traps) by using the following approach. Traditional diversity metrics, such as species richness (including species richness that was rarefied to a minimum of 24 individuals observed in one trap), activity density or the inverse Simpson index were calculated as means per trap over the 16 month study period and are presented as average values per trap. As the capture probability of pitfall traps varies with both activity and density of the species, the term activity density should be used (Heydemann 1957). To make the results more intuitive we used the inverse of the Simpson index instead of its original formulation, as an increase in the inverse index reflects an increase in diversity (Magurran 1988).

Table 1.—Description of trap locations in the stratified (1–14) and systematic design (201–235). Note that each location of the stratified design was sampled with three pitfall traps.

Trap ID	Description
1	Beech-spruce-larch forest with needle and leaf litter
2	Border of forest-driveway with *Avenella flexuosa*
3	Border of forest-driveway with grasses and *Urtica dioica*
4	Woodrush beech forest, underlayer without herb layer
5	Woodrush beech forest, stony hilltop
6	Dense beech young stands with maple
7	Charcoal pile with *Cardamine bulbifera*
8	Edge of the forest with several shrub species
9	Charcoal pile with grass and young beech stands
10	Glade with grass and young beech stands
11	Young spruce plantation
12	Mixed beech-oak-larch forest
13	Border of forest-driveway, stony, poor herb layer
14	Border of forest-driveway, with young stands of beech and larch
201–206, 208–224, 226, 227, 230–232, 235	Forest floor covered with beech litter, without herb layer
225, 228, 229, 233, 234	Forest floor covered with beech and needle litter, without herb layer
207	Forest floor with grass and young stands of beech

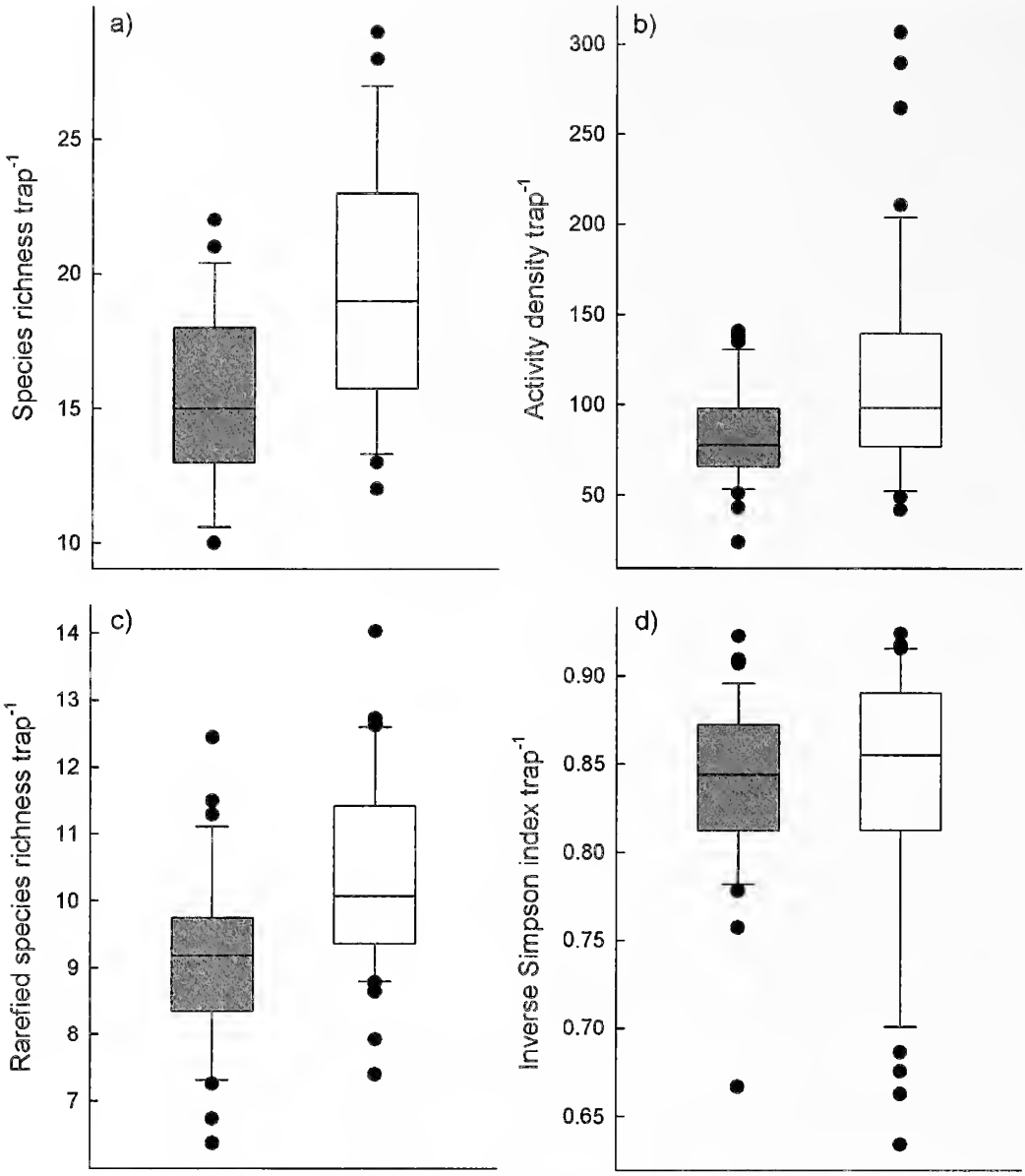

Figure 2.—Median, 75 and 95% quartiles and outliers for a) species richness, b) activity density, c) rarefied species richness (n = 24) and d) inverse Simpson index per pitfall trap for spider assemblages sampled in a systematic (SYS, gray) or stratified (STR, white) design.

Diversity and abundance metrics were compared between designs by one-way permutational analysis of variance with permutation of residuals under a reduced model and design (systematic vs. stratified) as fixed factor (PERMANOVA: Anderson 2001). We included X and Y coordinates of all trap locations as co-variables in our models to account for the fact that some traps within, but also between, designs were located more closely to each other. All univariate tests were based on Euclidean distances and 10,000 permutations. The univariate PERMANOVA based on Euclidean distances is analogous to a traditional one-way ANOVA, but P-values are obtained from permutations (Anderson and Millar 2004). We thus avoid the assumption of normality in our statistical models (e.g., Anderson et al. 2008) and show all results using box and whisker plots as recommended by Dytham (2003) for such data.

To assess the differences in community composition between sampling designs, we calculated resemblance matriees based on Sørensen (presence or absence of species) or Bray-Curtis (log x+1-transformed activity densities) distances between traps in both designs. We log-transformed activity density data to weigh down the contribution of abundant species to differences between the two designs and to emphasize the importance of rare species (Clarke et al. 2006). We used principal coordinate analysis (PCO) based on Bray-Curtis distances to visualize the dissimilarity of communities between traps from both designs (Clarke & Warwick 2001). To explore the individual contribution of species to dissimilarities between the two designs, we used similarity percentage analysis (SIMPER; Clarke & Warwick 2001). We further tested for homogeneity of multivariate dispersion by comparing the distanees of communities per traps to group centroids between both designs (PERMDISP routine). All analyses were performed using PRIMER version 6.1.13 with the PERMANOVA + add-on version 1.0.3 (PRIMER-E, Plymouth, UK: Anderson et al. 2008).

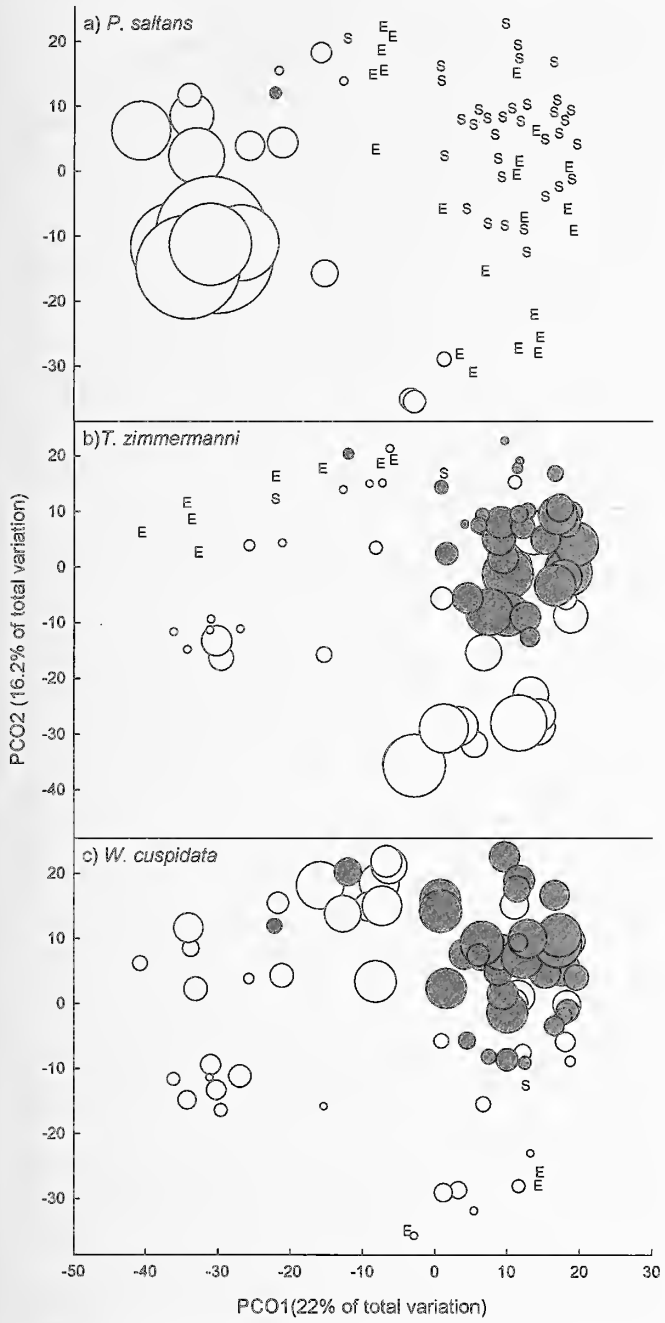

Figure 3.—Principal coordinates analysis based on Bray-Curtis similarities of log-transformed activity density data from all traps. The size of the bubbles corresponds to the number of individuals sampled in each pitfall trap in the systematic (SYS, gray) or stratified (STR, white) design for a) *Pardosa saltans* (bubble size range: 1–173 individuals), b) *Tenuiphantes zimmermanni* (1–62 individuals) and c) *Walckenaeria cuspidata* (1–39 individuals). Letters stand for traps that did not contain any individuals from the species in the stratified (expert-based, E) or systematic (S) sampling. Note that bubbles may overlap.

RESULTS

In total, 8012 adult spiders were sampled from 96 species in 14 families (see Appendix 1). Traps in the STR design contained 90 species, of which 42 were exclusively found in the STR design. Traps of the SYS design contained 54 species, of which 6 were exclusively found in the SYS design. Species richness (pseudo-$F_{1,74} = 31.59$, $P < 0.001$) and activity density (pseudo-$F_{1,74} = 13.99$, $P < 0.001$) per trap were significantly lower in the SYS than in the STR design (Figs. 2a,b). Rarefied species richness was also significantly lower in the SYS design (Fig. 2c; pseudo-$F_{1,74} = 17.18$, $P < 0.001$). The inverse Simpson index did not differ significantly between designs (Fig. 2d; pseudo-$F_{1,74} = 1.10$, $P = 0.301$).

Community composition based on the presence or absence of species in traps (Sørensen similarity, pseudo-$F_{1,74} = 7.04$, $P < 0.001$) or based on log-transformed activity densities (Fig. 3, Bray-Curtis similarity, pseudo-$F_{1,75} = 8.02$, $P < 0.001$) differed significantly between the two designs. Although both designs shared 47 out of 96 species, similarity percentage analyses indicated that three common species contributed most to the significant dissimilarity between communities (Fig. 3). *Pardosa saltans* Töpfer-Hofmann 2000 was more common in traps of the STR design (mean abundance of 51 individuals across all traps) and almost absent from the SYS design (only two individuals were collected in one trap of the systematic design). In contrast, *Tenuiphantes zimmermanni* (Bertkau 1890) and *Walckenaeria cuspidata* Blackwall 1833 were more frequently observed in traps of the SYS design. In general, the multivariate dispersion of community composition was significantly smaller in the SYS design, indicating that community composition varied less between traps than in the STR design (PERMDISP; $F_{1,75} = 49.18$, $P < 0.001$).

DISCUSSION

Our study suggests that the stratified design provides a more representative estimate of diversity and a more comprehensive summary of community composition in the study area than a systematic design. Species richness was higher in the stratified design, and the number of exclusive species only sampled with this design was almost an order of magnitude higher than for the systematic design. However, expert knowledge is needed to select sample locations in stratified designs in order to sample all relevant microhabitats. In contrast, systematic designs do not require such knowledge, but decisions about the extent of the sampling area, the number of sample points and the interpoint distances also require a priori assumptions.

It has been previously suggested that systematic designs may not adequately represent the composition of communities, since environmental gradients that acted on the mammal species studied were not covered (Read et al. 1988; Pearson & Ruggiero 2003). The effectiveness of stratified methods to sample rare species in heterogeneous habitats was also highlighted for plant communities in coastal wetlands (Croft & Chow-Fraser 2009). In our study, the number of unique spider species was seven times higher in the stratified design, even though the same sampling technique was used and the survey lasted over the same period (16 months). Differences between designs were attributed to some common spider species; for example, *P. saltans* was predominantly collected by traps in the stratified design. This pattern highlights preferences of *P. saltans* for particular forest habitats (e.g., Hendrickx et al. 2001) that were only sampled in the stratified design. This observation also demonstrates the danger of missing specific habitat types if trap locations are arranged in a regular grid that is related to the number, size and distribution of habitats in the study area.

Tenuiphantes zimmermanni and *W. cuspidata* were more frequently observed in traps of the systematic design, but both species were also present at particular locations of the stratified design. This pattern reflects the rather broad habitat preferences of these two sheet-web weavers.

Community composition of spiders in individual traps was significantly more homogeneous in the systematic design than in the stratified design, reflecting a more diverse range of microhabitats sampled in the stratified design. The vast majority of Central European beech forests consist of a relatively uniform stand of dense beech trees without a shrub and herb layer (Standovár & Kenderes 2003; Gálhidy et al. 2006). These areas are interspersed by small patches of different structure (e.g., wayside herbs, seepage springs, glades, rocks). To cover such elements in a systematic design requires an enormous effort and resources that may not be available for biodiversity inventories. Although the study presented here clearly illustrates that a stratified sampling design is more efficient than a systematic design, we acknowledge that the observed differences may be limited to the study location. Thus additional studies are needed to confirm our results for other habitats in general.

To conclude, our results suggest that forest surveys aiming at strict inventories of ground-active arthropods should not be based on systematic designs even in moderately heterogeneous study areas. That approach is more expensive and provides a less precise estimate of diversity and community composition. We propose, instead, that stratified designs should be used for strict inventories in European forests if expert knowledge is available and that the use of systematic designs should be reserved for spatial analyses (e.g., Birkhofer et al. 2011; Sereda et al. 2012) or surveys in more homogeneous habitats (e.g., Diekötter et al. 2010). It is important to note that inventories are a major field for the application of such designs and that greater care is needed for the application of inferential statistics. For example, the non-randomness that is caused by expert selection of sampling sites may violate fundamental assumptions of simple linear models.

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Appendix 1.—Species and number of individuals (juveniles excluded) sampled by the systematic (SYS) and the stratified (STR) approach.

Family/Species	SYS	STR
Agelenidae		
Coelotes terrestris (Wider 1834)	507	479
Histopona torpida (C. L. Koch 1837)	115	87
Inermocoelotes inermis (L. Koch 1855)	129	213
Malthonica silvestris (L. Koch 1872)	21	18
Amaurobiidae		
Amaurobius fenestralis (Ström 1768)	26	29
Clubionidae		
Clubiona comta C. L. Koch 1839	0	1
C. reclusa O. P.-Cambridge 1863	0	1
C. terrestris Westring 1851	1	6
Dictynidae		
Cicurina cicur (Fabricius 1793)	128	211
Dysderidae		
Dysdera erythrina (Walckenaer 1802)	0	1
Harpactea hombergi (Scopoli 1763)	0	8
Gnaphosidae		
Drassodex lesserti (Schenkel 1936)	0	2
Haplodrassus signifer (C. L. Koch 1839)	0	1
H. silvestris (Blackwall 1833)	1	16
H. umbratilis (L. Koch 1866)	0	1
Micaria pulicaria (Sundevall 1831)	0	2
Zelotes clivicola (L. Koch 1870)	0	17
Z. erebeus (Thorell 1871)	0	3
Z. subterraneus (C. L. Koch 1833)	0	128
Hahniidae		
Hahnia helveola Simon 1875	3	7
H. pusilla C. L. Koch 1841	0	17
Linyphiidae		
Agyneta conigera (O. P.-Cambridge 1863)	0	1
Asthenargus paganus (Simon 1884)	0	2
Bathyphantes gracilis (Blackwall 1841)	0	1
B. nigrinus (Westring 1851)	0	1
Bolyphantes alticeps (Sundevall 1833)	0	5
Centromerus brevivulvatus Dahl 1912	1	0
C. cavernarum (L. Koeh 1872)	2	66
C. dilutus (O. P.-Cambridge 1875)	54	167
C. pabulator (O. P.-Cambridge 1875)	1	48
C. sylvaticus (Blackwall 1841)	21	134

Appendix 1.—Continued.

Family/Species	SYS	STR
Ceratinella brevis (Wider 1834)	1	35
Linyphiidae		
Dicymbium tibiale (Blackwall 1836)	28	35
Diplocephalus cristatus (Blackwall 1833)	0	1
D. latifrons (O. P.-Cambridge 1863)	1	13
D. picinus (Blackwall 1841)	51	122
Diplostyla concolor (Wider 1834)	1	65
Drapetisca socialis (Sundevall 1833)	3	0
Entelecara erythropus (Westring 1851)	0	1
Erigone atra Blackwall 1833	0	1
Formiphantes lephthyphantiformis (Strand 1907)	0	1
Gonatium rubellum (Blackwall 1841)	26	40
Helophora insignis (Blackwall 1841)	0	42
Jacksonella falconeri (Jackson 1908)	8	0
Lepthyphantes minutus (Blackwall 1833)	0	1
L. nodifer Simon 1884	0	1
Linyphia hortensis Sundevall 1830	0	3
Macrargus rufus (Wider 1834)	36	34
Maso sundevalli (Westring 1851)	0	2
Micrargus herbigradus (Blackwall 1854)	26	130
Microneta viaria (Blackwall 1841)	36	26
Monocephalus fuscipes (Blackwall 1836)	0	1
Neriene clathrata (Sundevall 1830)	1	1
N. emphana (Walckenaer 1841)	1	0
Nusoncus nasutus (Schenkel 1925)	1	1
Obscuriphantes obscurus (Blackwall 1841)	0	1
Palliduphantes pallidus (O. P.-Cambridge 1871)	1	3
Pocadicnemis pumila (Blackwall 1841)	0	1
Porrhomma campbelli F. O. P.-Cambridge 1894	4	1
P. pallidum Jackson 1913	7	13
Pseudocarorita thaleri (Saaristo 1971)	4	3
Saloca diceros (O. P.-Cambridge 1871)	26	64
Tapinocyba insecta (L. Koch 1869)	327	256
T. pallens (O. P.-Cambridge 1872)	64	161
T. praecox (O. P.-Cambridge 1873)	0	1
Tenuiphantes alacris (Blackwall 1853)	4	2
T. cristatus (Menge 1866)	1	15
T. flavipes (Blackwall 1854)	10	73
T. mengei (Kulczyñski 1887)	4	12
T. tenebricola (Wider 1834)	1	34
T. tenuis (Blackwall 1852)	0	7
T. zimmermanni (Bertkau 1890)	443	487
Thyreosthenius parasiticus (Westring 1851)	1	0
Walckenaeria acuminata Blackwall 1833	0	11
W. corniculans (O. P.-Cambridge 1875)	24	45
Linyphiidae		
W. cucullata (C. L. Koch 1836)	47	111
W. cuspidata Blackwall 1833	566	407
W. dysderoides (Wider 1834)	8	7
W. mitrata (Menge 1868)	0	1
W. obtusa Blackwall 1836	4	14
Liocranidae		
Agroeca brunnea (Blackwall 1833)	0	4
Apostenus fuscus Westring 1851	0	2
Lycosidae		
Alopecosa pulverulenta (Clerck 1757)	0	12
Pardosa amentata (Clerck 1757)	0	1
P. pullata (Clerck 1757)	0	1
P. lugubris (Walckenaer 1802)	0	45
P. saltans Töpfer-Hofmann 2000	2	1018

Appendix 1.—Continued.

Family/Species	SYS	STR
Trochosa terricola Thorell 1856	2	75
Xerolycosa nemoralis (Westring 1861)	0	1
Salticidae		
Euophrys frontalis (Walckenaer 1802)	0	3
Neon reticulatus (Blackwall 1853)	9	9
Segestriidae		
Segestria senoculata (Linnaeus 1758)	0	3
Tetragnathidae		
Metellina segmentata (Clerck 1757)	1	0
Pachygnatha degeeri Sundevall 1830	3	1
Theridiidae		
Robertus lividus (Blackwall 1836)	33	41
R. scoticus Jackson 1914	7	4
Total:	2833	5179

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The conservation value of secondary forests in the southern Brazilian Mata Atlântica from a spider perspective

Florian Raub[1], **Hubert Höfer**[1,4], **Ludger Scheuermann**[2] and **Roland Brandl**[3]: [1]Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstr. 13, D-76133 Karlsruhe, Germany; [2]Lenzstraße 6, D-76137 Karlsruhe, Germany; [3]Department of Animal Ecology, Philipps-Universität Marburg, Karl-von-Frisch-Str. 3, D-35032 Marburg, Germany

Abstract. In many tropical areas of the world, pristine forests have become rare. Nevertheless, due to shifts in the human population the area covered by secondary forests is increasing. These forests may harbor a rich flora and fauna and are considered to be main refuges for species of primary forests. However, this issue is far from clear. To assess the conservation value of secondary forests in the Atlantic Forest of Brazil, we compared the diversity of spiders in differently aged secondary forests with old-growth forests. Within a larger project treating several invertebrate taxa, we sampled spiders using a standard protocol in 24 sites of three successional stages (5–8, 15–20, 30–50 years old) and old-growth forests (> 100 years untouched) in two nature reserves. We describe the diversity and structure of the assemblages using morphospecies and genera and analyze richness at the genus level. Generic richness and diversity showed no differences between successional stages; i.e., did not increase from the youngest to older forests, but guild diversity did increase. The youngest stage showed the highest variability in generic composition, and the turnover of genera and species was strong between the younger forests (5–20 years old) and forests older than 30 years. High alpha diversity, high turnover among sites and the lack of differences in richness between stages support the value of secondary forests for species conservation in the region studied.

Keywords: Araneae, diversity, guild structure, Atlantic Forests, Brazil

The Brazilian Atlantic forest (Mata Atlântica) is one of the "hottest hotspots" of biodiversity (Laurance 2009), due to its exceptional species richness and high number of endemic taxa in the various forest types (Forzza et al. 2012). However, the coastal region of Brazil has experienced an exceptionally high degree of forest conversion and deforestation (Myers et al. 2000; Ribeiro et al. 2009) for more than 500 years. In contrast to the more strongly deforested areas of the Atlantic coast, in the state of Paraná in southern Brazil large remnants of Atlantic forests still exist, forming a mosaic of patches of old-growth forests (sensu Clark 1996; also see Wirth et al. 2009) and secondary forests in various stages of succession. These secondary forests originate mainly from abandoned buffalo pastures. Recently the issue of the importance of these secondary forests for the conservation of biodiversity initiated a controversial discussion (see Bihn et al. 2008b).

Conservation strategies and management in the tropics are often based on large, exotic and beautiful or rare, endangered vertebrate species. However, the overwhelming part of biodiversity consists of invertebrates. Furthermore, invertebrates are involved in numerous important ecosystem functions (e.g., nutrient cycling or pollination). The analyses of invertebrate diversity for conservation are usually restricted to species numbers or lists of species of selected taxa. Although the number of species is not a quality measure per se, richness and diversity measures, which include the relative abundance of species, are valuable approximations to biodiversity and the conservation value of a habitat (Gaston 1996; Gotelli & Colwell 2001; Brose et al. 2003; Magurran 2004). This is especially true when autecological data are lacking; i.e., when knowledge of the distribution, natural history traits and habitat preferences for most of the species is sparse. However, to evaluate the richness of an assemblage a reference is needed. Comparing species numbers of assemblages in secondary vegetation with the original (primary) vegetation seems to be a meaningful approach to estimate degradation, to recognize the loss of functional diversity (Bihn et al. 2008b, 2010) and to classify areas with regard to their conservation value (Dunn 2004), although there is some evidence of functional redundancy (Lawton et al. 1998; Loreau et al. 2001).

The Brazilian-German cooperative project SOLOBIOMA (Höfer et al. 2007, 2011) studied the biogeochemistry and, in a multi-taxon approach, the diversity of earthworms (Römbke et al. 2009), enchytraeids (Schmelz et al. 2009, 2011), ants (Bihn et al. 2008a,b), beetles (Hopp et al. 2010, 2011; Ottermanns et al. 2011) and spiders in order to evaluate the conservation value of secondary forests in the Mata Atlântica. The overall aim of this projeet was to cheek the possibility of classifying secondary forest stages by their soil fauna and comparing that with the "traditional" classification by age and vegetation. In the absence of true primary vegetation in this region, we had to rely on "old-growth" forests as a reference.

Spiders are a species-rich taxon in the tropics. In Brazil the taxonomy is comparatively well studied (Brescovit et al. 2011), and meaningful faunistic inventories are available (Höfer 1990, 1997; Silva 1996; Silva & Coddington 1996; Höfer & Brescovit 2001; Rego et al. 2007; Venticinque et al. 2008; Bonaldo et al. 2009). However, in these studies there is a strong bias toward the Amazonian region. During the most recent years and based on taxonomic advances and faunistic knowledge, several studies in the Mata Atlântica, foeusing on spiders, have approached ecological questions (effects of disturbanee, fragmentation and vegetation type: Benati et al. 2005; Candiani et al. 2005; Oliveira-Alves et al. 2005; Podgaiski et al. 2007). However, studies with well-replicated designs are still rare (Dias et al. 2005; Bonaldo et al. 2007; Lo-Man-Hung et al. 2008; Pinto-Leite et al. 2008; Ricetti & Bonaldo 2008). To assess the eonservation value of secondary forests, we sampled spiders on

[4]Corresponding author. E-mail: hubert.hoefer@smnk.de

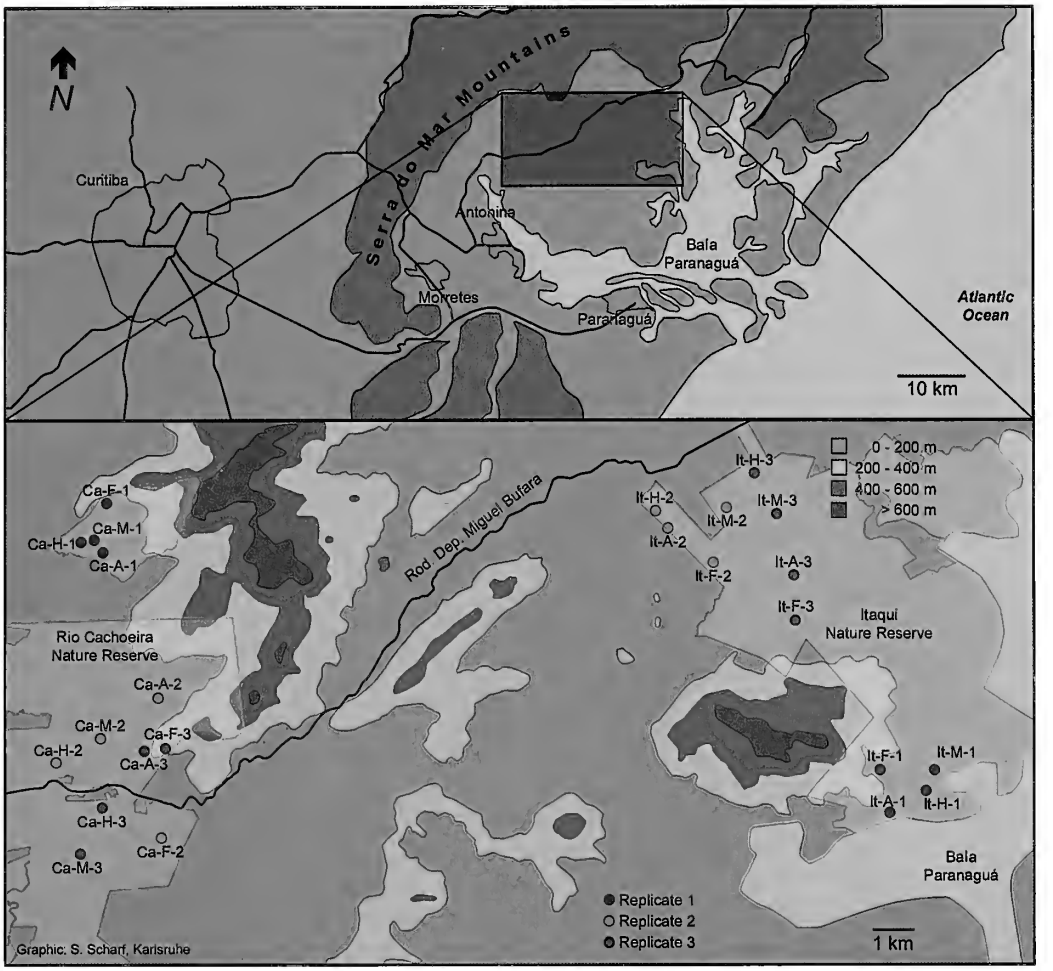

Figure 1.—Location of the study region in Paraná state, Brazil and the sampling sites in the two Nature Reserves Rio Cachoeira (Ca) and Itaqui (It); successional stages: H – herbaceous, A – arboreal, M – medium, F – old-growth forest.

the ground and in the lower vegetation in three different stages of secondary and old-growth forests, appraising the changes in richness and composition of genera across the successional gradient.

METHODS

Study area.—This study was conducted in the coastal region of Paraná State in southeastern Brazil. Originally the region was covered by dense ombrophilous lowland and submontane forests (IBGE 1992), but these ecosystems suffered massive exploitation and were largely converted to buffalo pastures (IPARDES 1995). Today, the landscape is characterized by a mosaic of open land, secondary forests and few relatively large patches of old-growth forests. The regional climate is humid subtropical (Köppen's Cfa: Strahler & Strahler 2005), with mean temperatures between 16.2 °C in July and 24.5 °C in February (IPARDES 2001). Average precipitation ranges between 2000 and 3000 mm year^{-1} (Roderjan & Kunyoshi 1988). Rainfall is more or less evenly distributed throughout the year, although with some seasonality (low rainfall from April to August).

The areas studied are part of an ecological restoration program (Feretti & Britez 2006). Sampled sites were located in two private nature reserves (RPPN) "Reserva Natural do Rio Cachoeira" and "Reserva Natural Serra do Itaqui" (Fig. 1). Both are owned and managed by the Brazilian NGO "Society for wildlife research and environmental education" (SPVS) and are part of the Environmental Protection Area (EPA) of Guaraqueçaba and also the Mata Atlântica Biosphere Reserve. Within their areas of 12,000 and 6,700 ha, respectively, ranging from sea level to elevations of 700 m a.s.l., different successional stages from pasture to forest were categorized a priori by the SPVS using age and vegetational structure, based on orthophotos from 1952, 1980 and 2002 and knowledge of the residents on historical use.

Study design.—In both reserves (subsequently called localities), which are located approximately 25 km apart (Cachoeira: 25.3142°S, 48.6958°W; Itaqui: 25.2733°S, 48.4872°W), we sampled spiders along a chronosequence of four forest stages: 5–8 years old (H – herbaceous stage), 10–15 years old (A – arboreal stage), 35–50 years old (M – medium stage) and > 100 years old (F – old-growth); the latter was used as a reference stage. In each stage we sampled three spatially separated replicate sites of 30 × 50 m^2 each. In total, 12 sites (3 replicates × 4 stages) were studied in both localities (Fig. 1) during several days of sampling (see below) in springtime (October/November) of 2005 (Cachoeira) and 2007 (Itaqui). The springtime period provides a high degree of sampling completeness without the necessity of resampling throughout the year (Baldissera et al. 2003; Rodrigues 2005; Podgaiski et al. 2007).

Table 1.—Absolute and relative abundance and richness of the spider families captured on lower vegetation (by beating and looking up). N = number of individuals, G = number of genera, S = number of morphospecies.

Family	N	% N	G	% G	S	% S
Theridiidae	1010	37.4	29	19.6	96	30.2
Linyphiidae	374	13.8	9	6.1	23	7.2
Salticidae	370	13.7	29	19.6	52	16.4
Araneidae	234	8.7	21	14.2	53	16.7
Anyphaenidae	148	5.5	8	5.4	10	3.1
Thomisidae	119	4.4	7	4.7	10	3.1
Pholcidae	98	3.6	3	2.0	12	3.8
Uloboridae	69	2.6	3	2.0	5	1.6
Tetragnathidae	60	2.2	5	3.4	9	2.8
Dictynidae	58	2.2	1	0.7	1	0.3
Mimetidae	33	1.2	3	2.0	4	1.3
Scytodidae	32	1.2	1	0.7	2	0.6
Oonopidae	24	0.9	5	3.4	5	1.6
Theridiosomatidae	24	0.9	5	3.4	12	3.8
Corinnidae	10	0.4	4	2.7	7	2.2
Oxyopidae	9	0.3	3	2.0	4	1.3
Hahniidae	8	0.3	1	0.7	1	0.3
Zoridae	5	0.2	1	0.7	2	0.6
Miturgidae	4	0.2	2	1.4	2	0.6
Lycosidae	3	0.1	1	0.7	1	0.3
Deinopidae	2	0.1	1	0.7	1	0.3
Hersiliidae	2	0.1	1	0.7	1	0.3
Sparassidae	2	0.1	1	0.7	1	0.3
Amaurobiidae	1	0.0	1	0.7	1	0.3
Ctenidae	1	0.0	1	0.7	1	0.3
Philodromidae	1	0.0	1	0.7	1	0.3
Synotaxidae	1	0.0	1	0.7	1	0.3
Sum: 27	2702		148		318	

Sampling methods and identification.—A structured sampling, following a widely accepted standard protocol (Coddington et al. 1991), was applied to sample spider diversity in these forests:

a) Ground hand sampling ("looking down" of Coddington et al. 1991): two experienced persons sampled for one hour at night with headlights, exploring all structures below knee level, resulting in one sample per person, two samples per site.
b) Aerial hand sampling ("looking up" of Coddington et al. 1991): one person sampled for one hour at night, exploring all structures from knee height upwards to overhead arm's reach; i.e., lower vegetation, resulting in 1 sample per site.
c) Beating: Three persons striking vegetation at any reachable level (i.e., lower vegetation) with a stick, collecting the spiders falling on a 50 × 50-cm tray held below, for one hour. Twenty beating points made one sample. Depending on the person sampling, a different number of samples per site (3–9) resulted.
d) Pitfall trapping: Ten traps per site were installed to capture active ground spiders for one week, usually resulting in 10 samples per site, with a few failures. Traps were 330 ml PE cups with an opening diameter of 7.5 cm, filled with 100 ml of 4% formaldehyde solution and protected against rain by transparent plastic plates.

The spiders sampled were stored in 75% ethanol. All adult spiders were determined to morphospecies or to morphogenera if possible, using a conservative approach to delimit morphospecies and morphogenera. All analyses are based on adult spiders. Notwithstanding the progress in spider taxonomy in the Neotropics, severe shortcomings in the analyses of the diversity of tropical faunas remains a prime difficulty in identifying specimens to the species level or to sort all adult specimens to the level of morphospecies. This is due to the high number of inadequately described species and the lack of identification keys (Uehara-Prado et al. 2009). We therefore used genera as a surrogate for the comparison of species richness and diversity, which has been shown to be a successful strategy even at local scales (Andersen & Hauge 1995; Balmford et al. 1996; Baldissera et al. 2008; Bihn et al. 2008b).

Identifications were made by the first and third authors, with help from Brazilian experts at Butantan Institute, São Paulo (IBSP) and Museu de Ciências Naturais da Fundação Zoobotânica, Porto Alegre (MCN). Morphospecies numbers (in the appendix) were assigned according to IBSP and MCN numeration to assure future comparability. Voucher material is deposited at the entomological department of Universidade Federal do Paraná in Curitiba (UFPR), at IBSP and MCN.

Data analysis.—We pooled the complementary captures from the different methods and strata for all analyses. Richness and diversity of the spider assemblages per site (alpha diversity) were described by the numbers of genera (G) observed, the ratio of genera/individuals (G/N), the Shannon index (H), the Shannon evenness measure (E) and log series α (Magurran 2004). We used rarefaction (Hurlbert 1971; Coleman 1982; Gotelli & Entsminger 2004; Magurran 2004) for the

Table 2.—Absolute and relative abundance and richness of the spider families captured on the ground (by pitfall traps and looking down). N = number of individuals, G = number of genera, S = number of morphospecies.

Family	N	% N	G	% G	S	% S
Zoridae	855	47.7	1	0.9	8	3.6
Theridiidae	210	11.7	25	21.6	48	21.6
Linyphiidae	126	7.0	12	10.3	29	13.1
Ctenidae	121	6.8	2	1.7	6	2.7
Pholcidae	69	3.9	5	4.3	13	5.9
Lycosidae	62	3.5	5	4.3	9	4.1
Pisauridae	54	3.0	1	0.9	2	0.9
Araneidae	49	2.7	10	8.6	22	9.9
Mysmenidae	40	2.2	3	2.6	4	1.8
Hahniidae	26	1.5	1	0.9	7	3.2
Salticidae	25	1.4	8	6.9	14	6.3
Corinnidae	18	1.0	3	2.6	6	2.7
Amaurobiidae	17	1.0	1	0.9	3	1.4
Oonopidae	16	0.9	4	3.5	5	2.3
Ochyroceratidae	14	0.8	1	0.9	3	1.4
Theridiosomatidae	12	0.7	2	1.7	4	1.8
Tetragnathidae	11	0.6	5	4.3	7	3.2
Thomisidae	11	0.6	3	2.6	6	2.7
Anyphaenidae	10	0.6	3	2.6	3	1.4
Scytodidae	10	0.6	1	0.9	2	0.9
Nemesiidae	8	0.5	2	1.7	2	0.9
Titanoecidae	7	0.4	1	0.9	1	0.5
Mimetidae	4	0.2	1	0.9	2	0.9
Gnaphosidae	2	0.1	1	0.9	1	0.5
Palpimanidae	2	0.1	2	1.7	2	0.9
Prodidomidae	2	0.1	1	0.9	1	0.5
Anapidae	1	0.1	1	0.9	1	0.5
Caponiidae	1	0.1	1	0.9	1	0.5
Deinopidae	1	0.1	1	0.9	1	0.5
Dipluridae	1	0.1	1	0.9	1	0.5
Liocranidae	1	0.1	1	0.9	1	0.5
Miturgidae	1	0.1	1	0.9	1	0.5
Nesticidae	1	0.1	1	0.9	1	0.5
Symphytognathidae	1	0.1	1	0.9	1	0.5
Synotaxidae	1	0.1	1	0.9	1	0.5
Trechaleidae	1	0.1	1	0.9	1	0.5
Uloboridae	1	0.1	1	0.9	1	0.5
Zodariidae	1	0.1	1	0.9	1	0.5
Sum: 38	1793		116		222	

direct comparison of generic richness between the single sites. It was calculated with R version 2.10.2 (R Development Core Team 2009), using the rarefy function of the package VEGAN 1.17-2 (Oksanen et al. 2009). To evaluate the proportion of rare genera at the single site, we calculated the relative abundance of singletons (proportion of genera with one individual from the total genera number per site: Magurran 2004). We calculated the nonparametric sample-based estimators Chao 2 and ICE (Magurran 2004) with EstimateS 8.0 (Colwell 2005). A coverage measure was calculated for each site using the number of observed genera as a percent of the estimated richness.

Similarity across stages (beta diversity) was analyzed with qualitative presence/absence (Sørensen index) and quantitative (abundance) data for assemblage structure (NESS = Normalized Expected Species Shared: Grassle & Smith 1976). In contrast to the Sørensen Index, NESS is a quantitative similarity measure, which accounts for the individual numbers of shared species in the sites compared or assemblages (as in the Renkonen or Bray-Curtis qualitative index), but weights the rare species with ascending values for the sample size. Therefore it seems to be a good measure for tropical communities, where rare species account for a considerable part of the recorded species (Chazdon et al. 1998; Novotny & Basset 2000). We calculated NESS with the program BIODIV 97 for Excel.

To visualize differences in spider assemblages of the forest stages and localities, we used a three-dimensional ordination based on a non-metric multidimensional scaling (nMDS) analysis, calculated on Bray-Curtis similarities of square-root transformed abundances of genera using Winkyst 1.0 (100 random perturbations) and Canoco for Windows 4.53 (Ter Braak 2002). The similarity matrix was tested for spatial autocorrelation using the mantel function of the R package ECODIST. The spatial distribution of the study sites had no effect on the patterns of beta diversity ($P = 0.98$).

To complete the comparison of the forest stages, we used available guild classifications for the Neotropical spider fauna

Table 3.—Alpha diversity of spiders in the Cachoeira sites (genus based, samples from all methods pooled). Site codes: Ca H1–3 = Cachoeira sites of herbaceous stage, Ca A1–3 of arboreal stage, Ca M1–3 of medium stage, Ca F1–3 of old-growth. N = number of individuals, G = number of genera, H = Shannon Index, E = evenness, α = Fishers's alpha index, Ra = rarefied genera number, Sg = portion of singletons, Chao 2 = estimated generic richness, ICE = sample-based richness estimate, Coverage = number of observed genera as a percentage of Chao 2-estimated richness, SD = standard deviation, CV = coefficient of variation.

Site	N	G	G/N	H	E	A	Ra (SD)	Sg	Chao 2 (SD)	ICE	Coverage
Ca H1	165	51	0.31	3.5	0.66	25.3	44.0 (2.1)	0.39	61.6 (6.2)	73.5	82.8
Ca H2	139	40	0.29	3.1	0.57	18.8	36.4 (1.6)	0.55	69.0 (16.1)	86.8	58
Ca H3	194	51	0.26	3.3	0.52	22.5	40.5 (2.4)	0.45	79.0 (14.8)	83.0	64.6
Ca A1	169	37	0.22	2.6	0.36	14.6	30.7 (2.0)	0.49	53.3 (10.2)	62.1	69.4
Ca A2	134	50	0.37	3.4	0.59	28.9	46.4 (1.6)	0.54	94.4 (22.0)	119.7	53
Ca A3	185	52	0.28	3.4	0.58	24.0	41.5 (2.4)	0.46	100.6 (25.4)	103.7	51.7
Ca M1	137	49	0.36	3.2	0.51	27.3	44.5 (1.7)	0.61	86.9 (18.8)	115.0	56.4
Ca M2	155	46	0.30	3.4	0.56	22.1	39.8 (2.0)	0.52	94.1 (26.2)	93.1	48.9
Ca M3	169	44	0.26	3.0	0.45	19.3	36.4 (2.1)	0.50	70.3 (14.5)	78.1	62.6
Ca F1	216	54	0.25	3.3	0.50	23.1	40.6 (2.5)	0.46	95.7 (22.5)	88.4	56.4
Ca F2	212	49	0.23	3.2	0.51	20.0	36.7 (2.5)	0.47	83.7 (18.6)	89.5	58.5
Ca F3	241	57	0.24	3.3	0.48	23.6	39.9 (2.7)	0.46	79.6 (11.3)	92.8	71.6
Total	2116	157							220.4 (25.2)	200.0	71.3
Mean	176.3	49.3	0.28	3.2	0.52	22.5	39.8	0.49	80.7	90.5	61.2
CV	19%	12%	17%	8%	15%	17%	11%	12%	18%	18%	16%

(Höfer & Brescovit 2001; Dias et al. 2010). We assigned the specimens to 16 distinct guilds. The assignment of a species to a guild is usually based on the family, in some cases on the genus, which was possible for almost all specimens in our samples. In a few cases we had to apply personal knowledge of the biology of a taxon based on our own observations in the field, the sampling method and information in the literature (Silva & Coddington 1996; Álvares et al. 2004). Only the Amaurobiidae (18 individuals) were not assigned to a guild due to the unclear taxonomic status and lack of ecological information for Neotropical species. For the comparison of guild structure in the different stages, data from the two localities were pooled.

The rarefied genera numbers, the estimated richness and the alpha diversity values were tested for significant effects of the stage (four levels) and the locality (two levels) with two-way ANOVAs using Statistica 8.0 (StatSoft 2007). Permutational multivariate analysis of variance (Permanova, Version 1.6: Anderson 2001, 2005) was used to analyze the generic turnover in the spider assemblage of different forest stages and to underpin the ordination with a statistical analysis. We tested the main factors of the residuals and their interaction terms with 9999 permutations using Bray-Curtis dissimilarities between the study sites.

Indicator analysis was done with R, version 2.10.1 (R Development Core Team 2009) and the packages MASS (Venables & Ripley 2002) and labdsv (Roberts 2007). Because indicators of single stages were weak, we pooled the beating tray data of the two younger and the two older stages to one group each [stages H and A = young (Y), stages M and F = old (O)] in order to achieve a distinctive separation with indicator genera of high indicator values for younger and older forests, respectively.

Table 4.—Alpha diversity of spiders in the Itaqui sites (genera based, samples from all methods pooled). Site codes: It H1–3 = Itaqui sites of herbaceous stage, It A1–3 of arboreal stage, It M1–3 of medium stage, It F1–3 of old growth. N = number of individuals, G = number of genera, H = Shannon index, E = evenness, α = Fishers's alpha index, Ra = rarefied genera number, Sg = portion of singletons, Chao 2 = estimated generic richness, ICE = sample-based richness estimate, Coverage = number of observed genera as a percentage of Chao 2-estimated richness, SD = standard deviation, CV = coefficient of variation.

Site	N	G	G/N	H	E	α	Ra (SD)	Sg	Chao2 (SD)	ICE	Coverage
It H1	121	38	0.31	3.2	0.64	19.0	37.5 (0.7)	0.42	51.6 (8.3)	64.7	73.6
It H2	253	54	0.21	3.2	0.45	21.0	37.1 (2.7)	0.48	97.2 (22.2)	103.3	55.6
It H3	125	34	0.28	2.4	0.31	15.4	32.5 (1.1)	0.68	71.7 (21.4)	128.6	47.4
It A1	150	44	0.29	3.2	0.53	21.0	38.6 (1.9)	0.52	75.7 (17.3)	90.6	58.1
It A2	230	50	0.22	2.5	0.26	18.5	32.7 (2.6)	0.54	117.3 (37.4)	101.8	42.6
It A3	160	42	0.26	3.1	0.54	17.8	35.4 (1.9)	0.46	61.1 (12.0)	71.1	68.7
It M1	205	43	0.21	3.0	0.49	16.6	33.4 (2.3)	0.44	70.7 (16.1)	79.7	60.8
It M2	277	54	0.20	3.2	0.46	20.0	37.3 (2.7)	0.37	78.2 (13.6)	77.3	69.1
It M3	281	57	0.20	3.3	0.46	21.6	38.7 (2.8)	0.37	73.5 (8.9)	85.2	77.6
It F1	123	33	0.27	3.1	0.69	14.8	32.4 (0.7)	0.36	47.3 (9.9)	51.3	69.8
It F2	274	60	0.22	3.4	0.47	23.7	39.4 (2.9)	0.45	109.2 (24.7)	105.5	54.9
It F3	180	44	0.24	3.2	0.58	18.6	37.2 (2.0)	0.36	60.4 (9.7)	67.0	72.8
Total	2379	154							196.8 (17.3)	191.5	78.3
Mean	198.3	46.1	0.24	3.1	0.49	19.0	36.0	0.45	76.2	85.5	62.6
CV	32%	19%	16%	10%	25%	14%	7%	21%	29%	25%	18%

Table 5.—Sampling effort, generic richness (observed and estimated) and diversity per stage (means and standard deviations from three replicates, all samples pooled). N = number of individuals, G = number of genera, α = Fishers's alpha index, Ra = rarefied genera number, Sg = portion of singletons (pooled data for the three replicates), Chao 2 = estimated generic richness, ICE = sample-based richness estimate, Coverage = number of observed genera as a percentage of Chao 2-estimated richness. SD = standard deviation. Abbreviations for stage as in Tables 3 and 4.

Stage	Samples	Total N	Mean N (SD)	Total G	Ra (SD)	Sg	α (SD)	Chao 2 (SD)	ICE	Coverage
Ca H	60	498	166 (28)	89	47.6 (3.4)	0.34	31.6 (2.3)	116.6 (12.5)	125.2	76.3
Ca A	69	488	163 (26)	85	43.7 (3.4)	0.34	29.8 (2.2)	111.3 (11.9)	126.2	76.3
Ca M	70	461	153 (16)	83	42.9 (3.3)	0.41	29.5 (2.3)	115.8 (14.3)	135.5	71.7
Ca F	72	669	223 (16)	84	40.9 (3.2)	0.36	25.4 (1.7)	119.3 (17.0)	113.6	70.4
It H	69	499	166 (75)	86	44.9 (3.4)	0.34	30.0 (2.2)	102.5 (8.0)	115.8	83.9
It A	65	540	180 (44)	82	39.3 (3.3)	0.43	26.9 (2.0)	116.5 (15.2)	132.2	70.4
It M	72	763	254 (43)	89	40.6 (3.3)	0.33	26.1 (1.7)	117.6 (13.9)	118.6	75.7
It F	62	577	192 (76)	81	41.4 (3.2)	0.40	25.7 (1.8)	123.5 (19.7)	127.4	65.6
Total	539	4495	187 (51)	192	49.8 (3.8)	0.23	40.7 (1.4)	248.8 (22.3)	229.3	77.2

RESULTS

A total of 11,293 individuals were collected from 539 samples, of which only the 4,495 (39.8%) adults were identified and sorted to 43 families, 192 genera and 440 morphospecies (Appendix 1). We were able to identify and name 155 species according to the available literature. Although the two localities were sampled in different years, similar numbers of spiders were collected: 2,116 individuals of 33 families and 157 genera in Cachoeira (2005) and 2,379 individuals of 37 families and 154 genera in Itaqui (2007). The ratios of females/males (0.941, 0.948) and adults/juveniles (0.673, 0.669) were also similar.

Overall, Theridiidae ranked first in abundance, accounting for 27% of all adults, and also in species richness with 117 morphospecies in 34 genera. The theridiid genera *Dipoena* (19 morphospecies), *Theridion* (16), *Cryptachaea* (13) and *Thymoites* (10) showed the highest species richness. Only the araneid genus *Mangora* was represented by a comparably high number of morphospecies (10). Zoridae ranked second with 19% of the individuals, but only eight morphospecies. The spider assemblages in Cachoeira and Itaqui showed a similar ranking (Spearman $r = 0.36$) of family abundance values, but Theridiidae and Linyphiidae were nearly twice as abundant in Itaqui as in Cachoeira. The Araneidae (58 morphospecies/21 genera), Salticidae (55/29) and Linyphiidae (43/15) accounted together for more than 35% of all speeies and 34% of all genera collected.

As expected, sampling in different strata (ground/vegetation) yielded strongly eomplementary sets of lineages. In the vegetation 74% of all spiders captured were web-builders. Theridiidae and Linyphiidae alone accounted for more than 50% (Table 1), with more than 100 species. The only abundant hunting spiders in the vegetation were Salticidae (55 morphospecies) and Anyphaenidae (10 species). There was no dominant (10% criterion) species or genus in the vegetation, and the 316 morphospecies (148 genera) collected showed that this stratum houses a large part of the total diversity. In strong contrast, half of all spiders captured on the ground belong to one genus of small hunting zorids, and 70% of all were hunting spiders (Table 2). All abundant hunting-spider families (Zoridae, Ctenidae, Lycosidae, Pisauridae) were represented by few genera and species and thus overall richness (216 morphospeeies, 116 genera) was lower than in the vegetation. Very few mygalomorphs (i.e., Nemesiidae, Dipluridae) were collected.

Alpha diversity.—The number of individuals ranged from 134 to 241, representing 37 to 57 genera, in Cachoeira and from 121 to 277, representing 33 to 60 genera, in Itaqui. Means of all generic richness values were very close, and the coefficient of variation rarely exceeded 20% (Tables 3, 4). The same applied to the diversity indices. Typical for nonrecurring sampling of tropical habitats, nearly half of the morphospecies or genera were represented by only one adult specimen per site of a stage (singletons: Tables 3, 4, Appendix 1). Both estimators produced very similar values (mean of 81 genera in Cachoeira, 76 in Itaqui), corresponding to a coverage of over 60%. The richness of genera (total, mean rarefied, estimated) was very similar aeross the stages of forest succession (see means in Table 5).

After correcting for the sampling effort (number of samples, individuals), no differences between the stages were found. None of the statistical tests (two-way ANOVAs with stage and locality as factors and rarefied and estimated generic richness and the two diversity indices as dependent variables) showed a significant effect of stage or locality. The spider assemblages in younger stages were as rich in genera and as diverse as in the old-growth forests. At the stage level the portion of singletons was 33% or higher, the estimated number of genera, based on the Chao 2 and ICE estimators, was mostly less than twice the number of observed genera and, consequently, coverage was higher than 66% (Table 5). At the morphospecies level the portion of singletons in the stages was even higher.

Beta diversity.—Qualitative similarity (Sørensen index) of the different stages at each locality ranged from 0.53 (youngest stage with older) to around 0.7 (between older stages) (Tables 6, 7), reflecting a turnover of genera (and species)

Table 6.—Qualitative (Sørensen index, upper right) and quantitative (NESS index, lower left, m = 228) similarities between the forest stages in Cachoeira reserve, based on genera data, all methods pooled. Abbreviations for stage as in Table 3.

	Ca H	Ca A	Ca M	Ca F
Ca H		0.58	0.56	0.53
Ca A	0.76		0.70	0.67
Ca M	0.72	0.93		0.71
Ca F	0.70	0.92	0.92	

Table 7.—Qualitative (Sørensen index, upper right) and quantitative (NESS index, lower left, m = 248) similarities between the forest stages in Itaqui reserve, based on genera data, all methods pooled. Abbreviations for stage as in Table 4.

	It H	It A	It M	It F
It H		0.59	0.54	0.53
It A	0.82		0.65	0.58
It M	0.72	0.85		0.66
It F	0.67	0.76	0.91	

along the successional gradient. Furthermore, within-stage similarities were not higher, ranging from 0.4 (stage H) to 0.7 (stage F) in Cachoeira and from 0.3 (H) to 0.6 (M) in Itaqui. Similarities of the same stages from the two reserves were usually higher (Tables 8, 9) than of the different stages within the same locality (Tables 6, 7). Similarities between stage H and other stages were always lowest; the spider assemblage of the herbaceous stage differed strongly from the older stages.

Quantitative similarity (NESS) is generally higher than qualitative similarity (Tables 6–9), indicating that the dominant genera (respectively, species) were abundant in all stages. This is also obvious in the list of the ten most abundant genera (respectively, species) of the two localities, representing 49% and 50%, respectively, of all adults (Tables 10, 11). One zorid genus clearly dominated in all stages, and the positions of many abundant genera in the list are also very similar. Abundant spider species reflecting the turnover between younger (H, A) and older forests (M, F) are the linyphiids of the genus *Anodoration* and several theriidid genera (*Spintharus*, *Theridion*, *Thwaitesia*) in Itaqui and the dictynid *Thallumetus* and pholcids of the genus *Mesabolivar* in Cachoeira. In Itaqui the latter was also found exclusively in the two older stages, but was not among the ten most abundant genera (see also indicator analysis).

Multivariate analysis.—The ordination (Stress = 0.12: Fig. 2) shows the stages of both localities arranged along the first axis. The younger stages (H, A) are especially well separated from each other and from the older stages, with the exception of one herbaceous site in Cachoeira. A much higher variability of the youngest (H) stage is obvious. Sites of the two older stages (M, F) ordinate close to each other. Sites at Itaqui and Cachoeira separate along the second and third axes. Although the nMDS is based on Bray-Curtis distances, which are more biased to dominant species than the NESS measures, the ordination visualizes the same differences between sites as the NESS values (Tables 6, 7, 9). Several genera (mainly orb- and sheet-weavers, some anyphaenids) characterized the youngest herbaceous stage, whereas the older stages grouped apart from the younger by pholcids (*Mesabolivar* spp.), the anyphaenid genus *Patrera*, the uloborid genus *Miagrammopes* and the theridiid genus *Spintharus*. The nMDS ordination was confirmed by a Permanova analysis. The four stages showed highly significant differences concerning their composition of spider assemblages ($F = 2.34$; $P = 0.0007$).

Table 8.—Qualitative similarity (Sørensen) between the forest stages in both reserves, based on genera data, all methods pooled. Abbreviations for stage as in Tables 3 and 4.

	It H	It A	It M	It F
Ca H	0.65	0.56	0.49	0.51
Ca A	0.58	0.62	0.65	0.66
Ca M	0.56	0.66	0.69	0.69
Ca F	0.50	0.64	0.68	0.68

Table 9.—Quantitative similarity (NESS, m = 228) between the forest stages in both reserves, based on genera data, all methods pooled. Abbreviations for stage as in Tables 3 and 4.

	It H	It A	It M	It F
Ca H	0.82	0.75	0.65	0.65
Ca A	0.76	0.85	0.91	0.85
Ca M	0.68	0.80	0.89	0.86
Ca F	0.57	0.80	0.91	0.92

Functional diversity.—Weavers were more abundant than hunting spiders in all stages (62/38%–56/44%), with the exception of the young arboreal stage (49/51%). Most spiders (40%) belonged to the diurnal space-web weavers, and these were more abundant in the herbaceous stage than in the older ones. Twenty-one percent were ground runners, most abundant in the young arboreal stage and less in the herbaceous. Spiders known to be diurnal dominated the collections with 44% of all individuals, while nocturnal spiders accounted for 20%. The portion of diurnal spiders decreased with the age of the stages from 53% to 41%. In older forests distinctly more orb weavers (e.g., near the ground), sedentary sheet-web weavers and nocturnal ground ambushers (i.e., ctenids) were caught than in the younger stages. Ground runners were most abundant in the more open young arboreal stage (due to a higher proportion of lycosids). The number of guilds in the stages was nearly equal, but the diversity of guilds appeared to increase from the young herbaceous to the old forest stages (Table 12).

Indicator analysis.—Indicators of single stages were weak, so the two younger (H + A) and the two older (M + F) stages were pooled to show a clear separation by genera (Table 13). *Spintharus* and *Miagrammopes* showed high indicator values for the older forest stages, whereas *Anodoration* and *Titidius* were indicatory taxa for the younger forests. The same genera fitted best to the nMDS ordination space, but species arrows are not shown in Fig. 2 to maintain legibility.

DISCUSSION

Given the project's approach, we put time and effort into the use of replicates to allow for a statistical analysis of biodiversity patterns of spiders in secondary forests, rather than to attempt to inventory the entire spider assemblage. We therefore did not undertake a special effort to sample cryptic, specialized or rare species, but rather used an accepted and widely used protocol to sample the spider assemblage on the ground and lower vegetation. By doing so we also made our samples per site comparable within our study and to other studies in the Neotropics. Due to difficulties in identifying the species and to avoid a biased result by wrong morphospecification of the partly undescribed tropical species, we based our richness measures and estimates on genera. According to other studies, genera serve as a reliable base for evaluating species richness (Baldissera et al. 2008; Bihn et al. 2010).

Table 10.—Assemblage structure (relative abundance of the ten most abundant genera) and total number of individuals (Ind.) in the four forest stages in Cachoeira reserve, pooled from all methods in all sites Abbreviations for stage as in Table 3.

Family	Genus	Ca H %	Ca A %	Ca M %	Ca F %	Ca total %	Ca Ind.
Zoridae	gen. 1	15.0	16.1	22.3	29.7	20.3	429
Salticidae	*Tariona*	5.1	6.7	3.8	4.1	5.1	108
Theridiidae	*Dipoena*	5.3	3.9	5.6	5.4	5.0	105
Linyphiidae	*Sphecozone*	6.4	3.3	0.4	7.6	4.3	90
Theridiidae	*Theridion*	3.1	2.4	0.8	6.7	3.1	66
Pholcidae	*Mesabolivar*	0.2	1.8	1.2	9.1	2.9	61
Araneidae	*Mangora*	2.0	0.9	2.8	6.5	2.8	60
Anyphaenidae	*Patrera*	0.0	1.5	3.8	3.9	2.2	47
Dictynidae	*Thallumetus*	0.0	0.0	4.0	5.4	2.1	45
Theridiidae	*Spintharus*	0.4	1.5	1.6	5.4	2.1	45

The temporal distant sampling of the two localities had no effect on any of the analyzed variables (total number of individuals, genera, families; ratios of female/male and adult/juvenile). The absence of autocorrelation in the dataset indicates that neither the temporal distance of the two sampling campaigns nor the spatial distance between the two localities had significant effects on the sampled spider assemblages.

Shortcomings in the methods, sampling protocol and identification could have masked differences in richness between the stages. Probably old-growth forests offer more speeific microhabitats (e.g., in bromeliads or dead wood) for specialized, cryptically living or rare (less abundant, not widely distributed, not active during the whole year) species. These species cannot be assessed by either strongly vision-based sampling or by beating the easily accessible lower vegetation (Dias et al. 2000; Rinaldi et al. 2002). Thus, to assess and evaluate the diversity of complex habitats such as an old-growth forest in an unbiased way, more effort might be necessary, using special sampling techniques for specialized species. It is even questionable whether the old-growth sites studied, although not strongly altered by humans, were suitable as a reference in place of primary forests. They could have obscured differences between stages or a directed succession, being "old-growth successional states" in themselves. The lack of any native earthworm speeies in the investigated sites and the high dominance of the invasive species *Pontoscolex corethrurus* in all, even the oldest, forest sites (Römbke et al. 2009) shed some light on the long history of anthropogenic influence in the region.

Notwithstanding these possible constraints, our survey of spiders revealed a high richness at the genus and speeies level when compared to other studies in the realm of the Atlantic Forests. Some of them, however, sampled in urban parks, plantations or small forest fragments (Rinaldi & Ruiz 2002; Benati et al. 2005; Candiani et al. 2005; Oliveira-Alves et al. 2005). Comparably high richness values were recorded by Brescovit et al. (2004), Podgaiski et al. (2007) and Baldissera et al. (2008) for Atlantic forests and Ricetti & Bonaldo (2008) for Amazonian forests. The differences in both sampled and estimated alpha-diversity values between sites (of all types) and also between the two sampled reserves of our study were low and not significant. Even the youngest successional stages in the study area house a considerable diversity of spiders. This is not unusual, because such habitats often show high structural heterogeneity, prey availability and ecotone characteristics, which increase species numbers (Kotze & Samways 1999; Baldissera et al. 2003; Platen 2006; Pétillon & Garbutt 2008).

The high turnover of species between all sites, independent of the stage, was interesting. Stages differ in their species composition, not in richness. Variability within the a priori defined stages originates from the heterogeneity of structural and microclimatic conditions (openness, plant density), which in all stages is based on physical and pedological heterogeneity (exposition, inclination, soil type, groundwater level). The higher variability within the youngest stage (visible in the ordination) is probably caused by differences in historical (largely unknown) land use (e.g., the use of machines, fertilizers or pesticides), which mainly influences early

Table 11.—Assemblage structure (relative abundance of the ten most abundant genera) and total number of individuals (Ind.) in the four forest stages in Itaqui reserve, pooled from all methods in all sites. Abbreviations for stage as in Table 4.

Family	Genus	It H %	It A %	It M %	It F %	It total %	It Ind.
Zoridae	gen. 1	7.4	30.9	18.3	15.1	18.1	431
Theridiidae	*Dipoena*	2.4	6.1	7.9	3.6	5.3	126
Linyphiidae	*Sphecozone*	5.0	2.8	6.0	5.4	4.9	117
Theridiidae	*Spintharus*	0.2	1.9	9.7	5.2	4.8	115
Linyphiidae	*Anodoration*	16.2	2.8	0.0	0.0	4.0	96
Salticidae	*Tariona*	0.4	1.5	4.3	4.9	3.0	71
Theridiidae	*Thwaitesia*	0.4	1.3	1.6	7.1	2.6	62
Ctenidae	*Isoctenus*	0.8	2.4	2.9	3.8	2.6	61
Theridiidae	*Episinus*	6.4	1.3	2.2	0.9	2.6	61
Theridiidae	*Theridion*	9.2	0.2	0.1	1.9	2.5	59

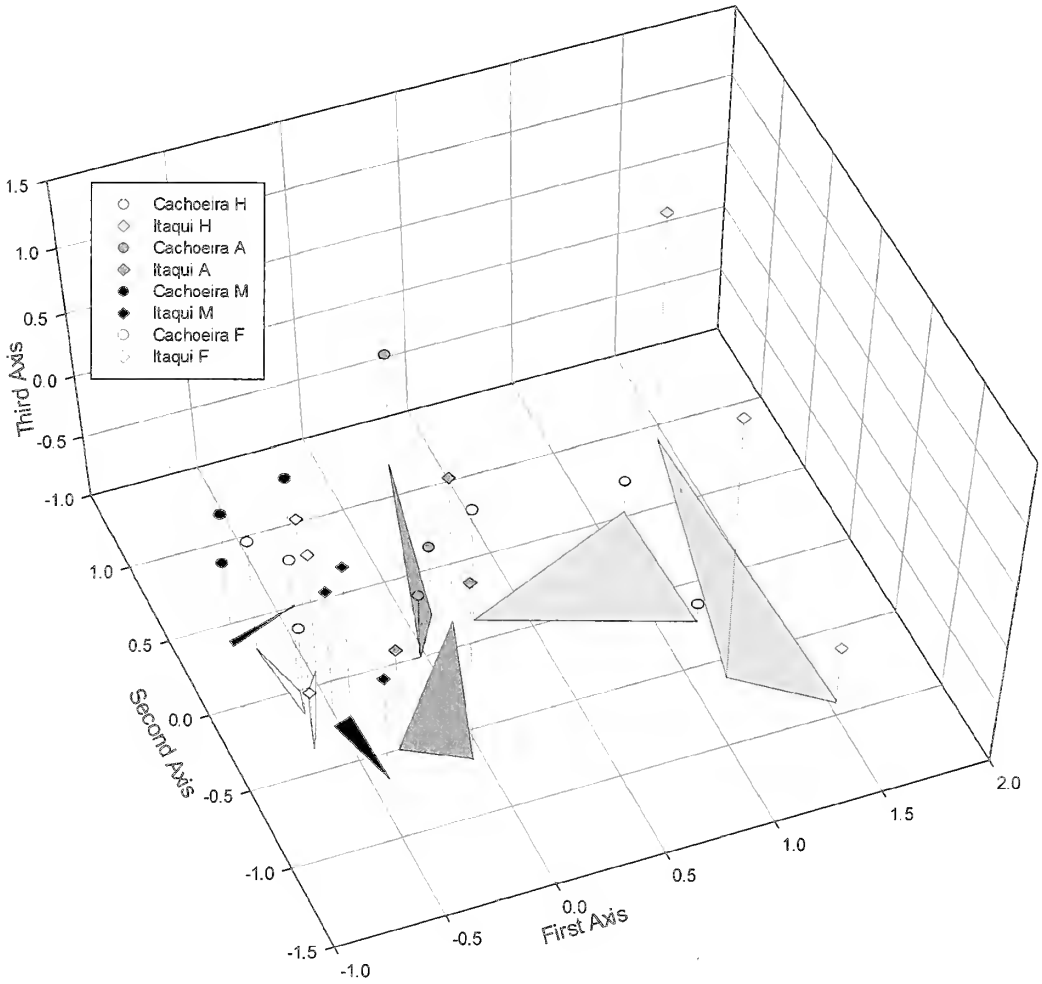

Figure 2.—Three-dimensional representation of a non-metric multidimensional scaling analysis (nMDS), based on Bray-Curtis distances; generic data pooled from all methods and sites in Cachoeira and Itaqui and square-root transformed (stress = 0.12).

Table 12.—Guild structure of the spider assemblage in the four stages, data of both localities and all methods pooled. Taxa assigned to guilds following Dias (2010) or Höfer and Brescovit (2001)[1]. H – herbaceous, A – arboreal, M – medium, F – old-growth forest.

Guild	Stages				Assigned families (genera)
	H	A	M	F	
Diurnal aerial ambushers	32	46	34	19	Thomisidae, Philodromidae
Diurnal aerial hunters	5	4	2	1	Miturgidae 2 (*Radulphius*), Oxyopidae
Diurnal ground runners	1	0	0	0	Liocranidae
Nocturnal aerial ambushers	2	2	0	1	Hersiliidae, Sparassidae,Trechaleidae
Nocturnal aerial hunters	77	37	68	46	Anyphaenidae, Scytodidae, Corinnidae
Aerial runners	91	101	121	110	Salticidae, Mimetidae
Nocturnal ground ambushers	13	29	39	49	Ctenidae, Nemesiidae
Nocturnal ground hunters	10	15	20	12	Salticidae 2 (*Asaphobelis*), Oonopidae, Palpimanidae, Caponiidae, Zodariidae, Prodidomidae
Ground runners/Nocturnal ground hunters	34	13	0	20	Lycosidae 1, Gnaphosidae
Ground runners	111	276	251	224	Miturgidae 1 (*Teminius, Strotarchis*), Zoridae
Diurnal ground orb weavers[1]	3	8	5	25	Mysmenidae, Symphytognathidae
Diurnal space-web weavers	487	374	460	459	Dictynidae, Linyphiidae, Synotaxidae, Theridiidae, Nesticidae
Nocturnal ground weavers[1]	12	2	20	12	Deinopidae, Diplluridae, Titanoecidae Anapidae, Hahniidae
Nocturnal space web weavers	1	2	4	7	Ochyroceratidae
Sedentary sheet weavers[1]	16	54	58	97	Pholcidae and Pisauridae 2 (*Architis*)
Orb weavers	103	65	138	154	Araneidae, Tetragnathidae, Theridiosomatidae, Uloboridae
Shannon Index H	1.75	1.83	1.87	1.94	
Evenness E	0.64	0.68	0.69	0.72	

Table 13.—Indicator analysis of the vegetation-bound spiders (beating tray data): O = older stages (M & F); Y = younger stages (H & A).

	Cluster	Indicator value	Probability
Spintharus	O	0.82	0.003
Miagrammopes	O	0.82	0.003
Patrera	O	0.76	0.002
Mangora	O	0.69	0.007
Thallumetus	O	0.67	0.003
Mesabolivar	O	0.63	0.041
Faiditus	O	0.50	0.013
Chrosiothes	O	0.42	0.043
Onoculus	O	0.42	0.043
Anodoration	Y	0.92	0.001
Titidius	Y	0.77	0.003
Hetschkia	Y	0.59	0.027

succession. During further succession, differences in biotic (prey availability, structure) and abiotic (climate) habitat parameters within and between the stages appear to decrease. An experimental manipulation of food and structure in one arboreal stage and the old-growth forest suggested food limitation of the decomposer fauna, but also revealed no effect of food or structure or any influence of stage on the spiders (Raub et al. 2014). Spiders are mostly generalist predators and seem to adapt easily to different food conditions and prey types (Uetz 1992), as long as suitable habitat structures and climate are provided. Baldissera et al. (2008) also found no differences in family, generic and species composition of the spider assemblages of natural *Araucaria* forest fragments and *Eucalyptus* monocultures, when appropriate habitat structures where provided.

The richness of our sites is comparable to other studies in Atlantic forests (see above), but some studies showed a different (family level) composition of assemblages (Rinaldi et al. 2002; Rinaldi & Ruiz 2002) and also significant differences in richness between young secondary and old-growth forest sites (Pinto-Leite et al. 2008; Uehara-Prado et al. 2009). We assume sueh differences to be caused by different uses of the sampled areas; for example, the use of pesticides or heavy machinery, and by the influence of the matrix of a forest fragment (see above).

Studies from tropical forest regions in the Brazilian Amazon revealed distinctly lower species richness of spiders in anthropogenic altered landscapes with forest patches than in a continuous forest cover (Lo-Man-Hung et al. 2011). However, as shown by Rego et al. (2005), taxa-specific responses can also lead to opposite responses in Neotropical forest fragments. High spider richness in the younger secondary sites should be regarded carefully in the context of conservation issues and not be taken as an absolute measure of habitat quality. Other invertebrate groups investigated in the same area showed an increase in richness along the successional gradient (Bihn et al. 2008b; Hopp et al. 2010).

The use of indicator taxa is becoming more and more important in the context of the growing anthropogenic pressure on highly diverse and threatened tropieal ecosystems. For the evaluation of the conservation potential and state of secondary and old-growth tropical forests, precise but quick and cheap tools such as indicators are needed (Uehara-Prado et al. 2009). However, the use of indicator taxa in the evaluation of ecosystems is a controversial topic, especially because of the indirect effects in food webs (Abrams et al. 1996), together with the lack of knowledge of the interrelations between the taxa. Therefore a multi-taxon approach with a carefully selected set of organisms (Kotze & Samways 1999; Cabra-García et al. 2012) should be used. Nonetheless, the results of our indicator analysis can be used for evaluations of secondary forest areas in the southern Mata Atlântica region. The identified genera can serve as indicator taxa for the evaluation of priority areas for forest conservation. For future evaluations they should be combined with the outcomes of other arthropod studies (Bihn et al. 2008b; Hopp et al. 2011; Ottermanns et al. 2011), and ecological traits should also be included to establish a reliable multi-taxon approach for the implementation of conservation strategies (Kotze & Samways 1999; Uehara-Prado et al. 2009).

Recovery of (species) richness can be relatively fast. Dunn (2004) reported a time span of 20–40 years for ant and bird richness recovery, which is comparable to the age of our medium-aged secondary forests. However, the regeneration of the original forest community often needs much more time (Dunn 2004; Bihn et al. 2008b). Among spiders, some forest-dwelling Lycosidae still do not seem to find adequate habitat in the oldest secondary stage. We therefore assume that mature secondary forests can host a highly diverse spider community, but do not serve as surrogate habitats for all old/primary forest dwelling genera or species. A classification of forests by the diversity and structure of spider assemblages would separate young (< 15 years) from median to old forests (> 30 years), in good accordance with results on beetles (Hopp et al. 2010), but not on ants (Bihn et al. 2008a).

Our study did not show a succession of spider diversity from species-poor young secondary vegetation toward a species-rich old-growth fauna, but rather a turnover of spider genera along the successional gradient, strongest between the two young and the two older stages; i.e., between ages of 20 to 30 years. We interpret the high alpha diversity and turnover between sites of the same stage as an expression of a rich regional spider fauna, maintained by the mosaic landscape of forests of different ages and mainly stochastic processes in the establishment of spider assemblages in early successional stages. Our study region presents a highly diverse mosaic texture, with large patches of old-growth forest acting as refuges for spiders (Rodrigues et al. 2009), never far away even from the youngest secondary stages. This variation in vegetation complexity, and the large set of microhabitats provided, is able to host highly diverse spider assemblages (Ricetti & Bonaldo 2008). We assume that ideal preconditions for colonization and repopulation of secondary habitats have been met in the region. Spiders survived the deforestation and fragmentation of the coastal forests in Paraná due to the constant availability of retreat habitats for later resettlement.

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Appendix 1.—List of morphospecies of adult spiders recorded in the forest stages (H – herbaceous, A – arboreal, M – medium, F – old-growth forest) of the two nature reserves Cachoeira (Ca) and Itaqui (It) (specimens from all methods and replicate sites pooled).

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Amaurobiidae								
Amaurobiidae sp. 1	0	0	0	1	0	0	0	6
Amaurobiidae sp. 2	0	0	0	0	0	1	0	0
Amaurobiidae sp. 3	0	0	0	1	0	0	0	0
Amaurobiidae sp. 4	0	0	3	2	0	0	3	1
Anapidae								
Anapidae sp.	0	0	0	0	0	0	1	0
Anyphaenidae								
Amaurobioidinae sp. 1	1	0	0	0	0	0	0	0
Aysha sp. 1	11	2	0	1	7	1	1	1
Aysha sp. 2	1	0	0	0	0	0	0	0
Aysha sp. 4	0	0	0	1	0	0	0	0
Buckupiella imperatriz Brescovit 1997	4	1	0	0	4	0	0	0
gen. 1 sp. 1	16	0	0	0	0	0	0	0
Patrera cita (Keyserling 1891)	0	10	19	18	0	2	15	9
Temnida sp. 1	0	0	0	0	1	0	0	0
Wulfila sp. 1	0	0	0	0	0	1	0	0
Wulfilopsis sp. 1	2	1	1	3	8	2	10	4
Araneidae								
Araneidae sp. 8	0	1	0	0	0	0	0	0
Acacesia tenella (L. Koch 1871)	1	0	0	0	7	0	0	0
Acacesia yacuiensis Glueck 1994	0	0	0	0	0	2	1	0
Alpaida biasii Levi 1988	0	0	0	0	3	0	0	0
Alpaida canoa Levi 1988	0	0	2	0	0	0	1	0
Alpaida rubellula (Keyserling 1892)	0	0	0	1	0	0	0	0
Alpaida septemmammata (O.P.-Cambridge 1889)	0	0	0	0	0	0	0	1
Alpaida sp. 3	0	0	0	1	0	0	0	0
Alpaida sp. 5	2	0	0	0	0	0	0	0
Alpaida tijuca Levi 1988	1	0	1	0	0	1	3	0
Alpaida truncata (Keyserling 1865)	0	0	1	0	0	0	0	0
Araneus iguacu Levi 1991	0	1	0	2	0	2	3	3
Araneus tijuca Levi 1991	0	0	2	1	2	1	1	0
Araneus unanimus (Keyserling 1879)	0	0	0	1	0	0	0	0
Araneus uniformis (Keyserling 1879)	0	0	0	0	1	0	0	0
Bertrana rufostriata Simon 1893	1	0	0	0	7	0	0	0
Bertrana sp. 1	0	0	0	0	2	0	0	0
Cyclosa fililineata Hingston 1932	1	0	4	8	1	2	1	2
Cyclosa morretes Levi 1999	0	0	0	3	0	0	0	2
Enacrosoma anomalum (Taczanowski 1873)	0	0	0	0	0	1	0	0
Eustala sp. 1	1	0	0	1	0	0	0	0
Eustala sp. 2	1	0	0	0	1	0	0	0
Eustala sp. 3	0	0	0	0	3	0	0	0
Eustala sp. 4	0	0	0	0	2	0	0	0
Eustala sp. 6	1	0	0	0	1	0	0	0
Eustala sp. 8	0	0	0	0	3	0	0	0
Hypognatha sp. 1	0	0	1	0	0	0	0	0
Kaira echinus (Simon 1897)	0	1	0	0	0	0	0	0
Kapogea sellata (Simon 1895)	0	0	1	0	1	0	0	0
Mangora blumenau Levi 2007	4	0	2	14	0	4	6	2
Mangora bocaina Levi 2007	0	1	0	0	1	4	1	0
Mangora botelho Levi 2007	0	0	1	0	0	0	0	0
Mangora caparu Levi 2007	1	0	1	3	0	0	3	1
Mangora chacobo Levi 2007	0	0	0	0	0	1	0	0
Mangora manicore Levi 2007	4	3	3	3	1	0	1	5
Mangora melanocephala (Taczanowski 1874)	1	0	0	0	2	0	0	0
Mangora missa Levi 2007	0	1	0	0	0	0	1	1
Mangora sp. 1	0	1	7	10	0	2	2	0
Mangora sp. 2	0	0	0	0	0	0	1	2

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Metazygia manu Levi 1995	0	0	0	0	1	0	1	0
Micrathena crassispina (C.L. Koch 1836)	0	0	0	0	0	0	0	1
Micrathena excavata (C.L. Koch 1836)	0	0	1	1	0	1	0	1
Micrathena sanctispiritus Brignoli 1983	0	0	0	1	0	0	0	0
Micrathena triangularis (C.L. Koch 1836)	1	2	0	0	0	0	1	0
Micrepeira albomaculata Schenkel 1953	0	0	0	1	0	0	0	0
Parawixia audax (Blackwall 1863)	3	0	0	0	2	0	1	2
Parawixia kochi (Taczanowski 1873)	0	0	0	0	1	0	0	0
Parawixia monticola (Keyserling 1892)	0	0	0	0	0	0	3	1
Scoloderus cordatus (Taczanowski 1879)	0	0	1	0	2	0	0	4
Scoloderus gibber (O.P.-Cambridge 1898)	1	1	0	0	0	0	0	0
Testudinaria gravatai Levi 2005	0	0	0	1	0	2	0	0
Verrucosa sp. 1	0	1	1	2	0	1	1	3
Wagneriana eupalaestra (Mello-Leitão 1943)	0	0	2	1	0	0	0	0
Wagneriana heteracantha (Mello-Leitão 1943)	0	0	2	0	0	0	0	0
Wagneriana iguape Levi 1991	0	1	1	1	0	1	2	1
Wagneriana janeiro Levi 1991	0	1	1	6	0	1	3	1
Wagneriana taim Levi 1991	3	0	0	0	2	0	0	1
Wixia sp. 1	0	1	0	0	0	0	0	0
Caponiidae								
Caponiidae sp.	0	0	0	0	0	0	1	0
Corinnidae								
Castianeira sp. 1	0	0	0	0	1	0	0	0
Castianeira sp. 2	0	1	0	0	0	0	0	0
Corinna sp. 1	0	1	0	1	0	0	1	0
Corinna sp. 2	1	1	0	0	0	0	0	0
Corinna sp. 3	0	1	0	0	0	0	0	0
Corinna sp. 4	0	0	0	0	0	0	1	0
Corinna sp. 5	1	0	0	0	2	0	1	0
Corinna sp. 6	0	0	0	0	0	1	0	0
Corinna sp. 7	0	0	0	0	0	0	1	0
Ianduba varia (Keyserling 1891)	3	2	0	1	1	0	0	0
Myrmecium sp. 1	0	0	1	0	0	0	0	1
Trachelas sp. 1	0	1	0	1	0	0	1	0
Trachelas sp. 2	0	0	0	0	0	0	1	0
Ctenidae								
Ctenus medius Keyserling 1891	1	3	4	4	0	1	3	2
Ctenus ornatus (Keyserling 1877)	1	0	0	0	0	0	0	0
Ctenus sp. 1	0	0	0	1	0	0	0	0
Isoctenus janeirus (Walckenaer 1837)	0	0	0	1	0	0	4	0
Isoctenus ordinario Polotow & Brescovit 2009	0	0	0	1	0	2	4	1
Isoctenus strandi Mello-Leitão 1936	7	11	4	16	4	11	15	21
Deinopidae								
Deinopis sp. 1	0	0	1	2	0	0	0	0
Dictynidae								
Thallumetus sp. 1	0	0	20	25	0	0	6	7
Dipluridae								
Trechona rufa Vellard 1924	0	0	0	0	0	0	1	0
Gnaphosidae								
Gnaphosidae sp.	0	0	0	0	2	0	0	0
Hahniidae								
Hahniidae sp. 1	0	0	5	10	0	0	0	0
Hahniidae sp. 2	0	0	0	0	0	0	1	0
Hahniidae sp. 3	0	0	1	0	0	0	0	0
Hahniidae sp. 4	0	0	2	0	0	0	0	0
Hahniidae sp. 5	1	0	1	0	0	0	0	0

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Hahniidae sp. 6	0	0	0	0	0	0	1	0
Hahniidae sp. 7	0	0	0	0	4	0	8	0
Hersiliidae								
Ypypuera crucifera (Vellard 1924)	0	2	0	0	0	0	0	0
Linyphiidae								
Linyphiidae indet. 1	0	0	0	0	1	0	0	0
Linyphiidae indet. 2	0	0	0	0	3	0	0	0
Linyphiidae sp. 1	0	3	0	0	0	21	1	0
Linyphiidae sp. 2	2	0	0	0	0	1	0	0
Linyphiidae sp. 3	0	0	2	0	0	5	7	2
Anodoration claviferum Millidge 1991	22	5	0	0	0	0	0	0
Anodoration sp. 1	2	0	0	0	81	15	0	0
Asemostera tacuapi Rodrigues 2007	0	0	1	0	3	0	0	1
Dubiaranea sp.	0	0	1	0	0	0	0	0
Dubiaranea sp. 1	2	0	0	0	0	0	0	0
Dubiaranea sp. 2	0	0	0	0	0	1	0	0
Exechopsis conspicua Millidge 1991	0	0	0	0	0	0	0	2
Exechopsis sp.	1	6	2	0	0	16	5	0
Exechopsis sp. 1	0	1	0	0	0	0	1	2
Exechopsis sp. 2	1	0	0	0	0	0	0	0
Exocora sp.	0	0	0	2	0	3	0	0
Exocora sp. 1	0	0	2	0	0	0	0	1
Labicymbium sp.	3	0	0	0	0	0	0	0
Labicymbium sp. 1	0	3	0	0	0	1	0	0
Lepthyphantes sp.	0	0	1	0	0	0	0	0
Lepthyphantes sp. 2	1	0	0	0	0	0	0	0
Linyphiinae indet. 1	0	0	0	1	0	0	0	0
Linyphiinae indet. 2	2	0	0	0	0	0	0	0
Meioneta sp.	0	0	0	0	0	0	0	1
Meioneta sp. 1	0	2	0	0	1	1	8	8
Meioneta sp. 2	0	0	0	0	0	0	8	0
Meioneta sp. 3	0	0	0	0	3	0	0	0
Meioneta sp. B	0	3	0	0	0	0	0	0
Moyosi prativaga (Keyserling 1886)	0	1	0	0	0	0	0	0
Moyosi sp.	0	1	0	0	0	0	0	0
Moyosi sp. 1	1	0	0	0	0	1	0	0
Psilocymbium sp.	5	0	0	0	0	0	0	0
Psilocymbium sp. 1	6	0	1	0	0	0	0	0
Psilocymbium sp. 2	1	0	0	0	0	0	0	0
Scolecura sp.	1	0	0	0	0	0	0	0
Sphecozone diversicolor (Keyserling 1886)	17	0	0	0	19	0	0	0
Sphecozone labiata (Keyserling 1886)	0	3	0	0	0	0	0	5
Sphecozone personata (Simon 1894)	10	13	2	1	0	5	7	7
Sphecozone sp.	1	0	0	0	0	0	0	0
Sphecozone sp. 1	3	5	0	0	6	0	0	0
Sphecozone tumidosa (Keyserling 1886)	0	1	0	0	0	2	0	0
Sphecozone venialis (Keyserling 1886)	0	0	0	34	0	8	39	19
Vesicapalpus simplex Millidge 1991	0	0	0	0	0	0	1	0
Liocranidae								
Gen. 1 sp. 1	1	0	0	0	0	0	0	0
Lycosidae								
Agalenocosa sp. 1	7	0	0	0	0	0	0	0
Hogna sp. 1	0	2	0	0	2	0	0	0
Hogna sp. 2	4	2	0	0	0	0	0	0
Hogna sternalis (Bertkau 1880)	0	5	0	0	3	0	0	0
Lobizon sp. 1	0	0	0	9	0	0	0	3
Lobizon sp. 2	0	4	0	3	11	0	0	0
Lycosa erythrognatha Lucas 1836	0	0	0	0	1	0	0	0

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Lycosa inornata Blackwall 1862	3	0	0	0	1	0	0	0
Lycosinae sp. 1	0	0	0	5	0	0	0	0
Mimetidae								
Ero sp. 1	2	2	8	7	2	2	3	5
Ero sp. 2	2	0	0	0	0	1	0	0
Gelanor sp. 1	0	0	0	1	0	0	0	1
Mimetinae sp. 1	0	0	1	0	0	0	0	0
Miturgidae								
Radulphius sp. 1	1	1	0	0	0	0	0	1
Strotarchus sp. 1	0	1	0	0	0	0	0	0
Teminius insularis (Lucas 1857)	0	0	0	0	1	0	0	0
Mysmenidae								
Mysmenidae sp. 1	0	0	0	1	0	0	0	0
Itapua sp.	1	2	1	0	0	0	0	0
Itapua sp. 1	0	0	1	0	0	0	0	0
Microdipoena sp. 1	0	0	0	0	2	6	2	24
Nemesiidae								
Acanthogonatus sp. 1	0	0	1	1	0	1	2	0
Pycnothele sp. 1	0	0	2	1	0	0	0	0
Nesticidae								
Eidmanella sp.	0	0	0	1	0	0	0	0
Ochyroceratidae								
Ochyrocera sp. 1	0	0	0	0	1	1	3	1
Ochyrocera sp. 2	0	0	0	3	0	0	1	3
Ochyrocera sp. 3	0	0	0	0	0	1	0	0
Oonopidae								
Gamasomorpha sp.	0	0	1	0	0	0	0	0
gen. 2 sp. 1	0	0	0	0	0	1	0	0
Neoxyphinus sp. 1	0	0	0	3	0	1	0	0
Oonops sp. 1	2	2	1	1	3	3	3	1
Oonops sp. 2	0	0	0	0	0	4	2	0
Orchestina sp. 1	0	0	0	0	0	0	4	0
Predatoroonops sp. 1	0	1	0	3	0	0	2	1
Triaeris stenaspis Simon 1891	0	0	0	0	1	0	0	0
Oxyopidae								
Hamataliwa sp. 1	0	2	1	0	0	0	0	0
Hamataliwa sp. 2	0	1	0	0	2	0	1	0
Oxyopes salticus Hentz 1845	1	0	0	0	0	0	0	0
Peucetia flava Keyserling 1877	0	0	0	0	1	0	0	0
Palpimanidae								
Palpimanidae sp.	0	0	1	0	0	0	0	0
Notiothops birabeni (Zapfe 1961)	0	0	0	0	0	0	0	1
Philodromidae								
Cleocnemis sp. 1	0	0	0	0	0	1	0	0
Pholcidae								
Pholcidae sp. 1	11	5	3	3	0	0	0	0
Mesabolivar aff. *brasiliensis* (Moenkhaus 1898)	0	0	0	1	0	0	0	0
Mesabolivar aff. *cyaneotaeniatus* (Keyserling 1891)	0	0	0	3	0	0	0	0
Mesabolivar aff. *guapiara* Huber 2000	0	0	0	1	0	4	1	6
Mesabolivar brasiliensis (Moenkhaus 1898)	0	1	3	11	0	0	0	0
Mesabolivar cyaneotaeniatus (Keyserling 1891)	0	0	0	2	0	0	0	0
Mesabolivar luteus (Keyserling 1891)	0	9	1	24	0	4	7	4
Mesabolivar rudilapsi Machado, Brescovit & Francisco 2007	1	0	2	0	0	6	1	2
Mesabolivar sp. 1	0	0	0	0	1	0	3	0

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Mesabolivar sp. 2	0	2	0	0	0	3	0	2
Metagonia aff. *bonaldoi* Huber 2000	0	1	0	1	0	0	0	0
Metagonia furcata Huber 2000	0	0	1	1	0	1	0	0
Metagonia sp. 1	0	3	2	5	0	5	4	0
Metagonia sp. 2	0	0	0	0	0	1	0	0
Ninetines sp. 1	0	0	0	0	0	0	0	2
Ninetines sp. 2	0	1	1	0	0	0	1	1
Tupigea nadleri Huber 2000	0	2	0	1	0	0	6	0
Tupigea sp. 1	0	1	3	0	0	0	0	0
Pisauridae								
Architis brasiliensis (Mello-Leitão 1940)	2	2	18	20	0	3	1	7
Architis capricorna Carico 1981	1	0	0	0	0	0	0	0
Prodidomidae								
gen. sp.	0	0	0	0	0	0	2	0
Salticidae								
Salticidae sp. 26	1	0	0	0	0	0	0	0
Salticidae sp. 38	0	0	0	0	1	0	0	0
Salticidae sp. 45	0	0	0	0	0	1	0	0
Salticidae sp. 46	0	0	0	0	0	1	0	0
Salticidae sp. 54	0	0	0	0	0	0	3	0
Salticidae sp.	0	0	0	0	0	0	2	0
Amphidraus sp. 1	0	1	1	0	0	0	0	0
Amycinae sp.1	0	0	2	0	0	0	1	1
Amycinae sp.2	0	1	0	0	0	1	0	0
Arnoliseus graciosa Braul & Lise 2002	0	4	1	4	0	0	2	3
Asaphobelis physonychus Simon 1902	3	2	2	1	1	1	0	1
Atelurius sp. 1	1	0	0	0	0	0	1	0
Chira spinosa Mello-Leitão 1945	1	0	0	0	1	0	0	0
Chira thysbe Simon 1902	2	0	0	0	0	0	0	0
Coryphasia sp. 1	0	0	0	1	0	0	0	0
Coryphasia sp. 2	0	2	3	0	0	1	1	0
Cotinusa sp. 1	2	1	2	0	0	0	0	1
Cotinusa sp. 2	0	0	0	0	0	1	1	0
Cylistella sp. 1	0	0	1	1	0	3	2	0
Cyllodania sp. 1	0	0	0	0	1	0	0	0
Dendryphantinae sp.1	1	0	0	0	0	0	0	0
Dendryphantinae sp.2	0	0	0	0	1	0	0	0
Euophryinae sp. 2	0	0	0	2	0	0	1	0
Euophryinae sp. 3	2	0	0	0	0	0	0	0
Euophryinae sp. 4	0	0	0	2	0	0	0	0
Euophryinae sp. 5	1	0	0	0	0	0	0	0
Euophryinae sp. 6	0	3	0	0	1	1	0	0
Euophryinae sp. 7	0	0	1	0	0	1	1	2
Euophryinae sp. 8	1	0	0	0	1	0	0	0
Euophryinae sp. 9	1	0	0	1	0	0	0	0
Euophryinae sp. 10	0	0	1	0	0	0	1	2
Euophryinae sp. 11	0	0	0	0	0	0	0	1
Euophryinae sp. 12	0	0	0	0	0	0	1	0
Euophryinae sp. 13	0	0	0	0	0	1	0	0
Euophryinae sp. 14	0	0	0	0	0	1	0	0
gen. n. sp. 1	0	0	1	0	0	0	0	0
Hyetussinae sp. 1	0	0	1	3	0	0	0	2
Ilargus sp. 1	0	0	0	0	1	0	0	0
Itata sp. 1	0	0	0	1	0	0	0	0
Lyssomanes sp. 1	0	0	0	0	0	0	0	1
Lyssomanes sp. 2	0	0	0	0	0	0	1	0
Maeota dichrura Simon 1901	0	1	0	0	5	1	0	1
Noegus sp. 1	8	9	11	7	5	4	7	10
Ramboia sp. 1	3	0	0	0	2	0	4	0

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Romitia sp. 1	2	0	0	0	1	1	0	0
Semnolius sp. 1	0	1	0	0	1	0	0	1
Semora napaea Peckham & Peckham 1892	0	0	0	0	4	0	0	0
Synemosyna lauretta Peckham & Peckham 1892	1	0	0	0	3	0	0	0
Synemosyninae sp. 1	0	0	0	1	0	2	3	0
Tanybelus aeneiceps Simon 1902	0	0	0	0	0	0	0	1
Tariona sp. 1	18	38	19	19	1	7	33	28
Tariona sp. 3	0	3	0	0	0	0	0	0
Tariona sp. 4	5	4	0	0	0	0	0	0
Tariona sp. 5	2	0	0	0	0	0	0	0
Tariona sp. 6	0	0	0	0	1	1	0	0
Vinnius uncatus Simon 1902	3	0	0	0	0	0	0	0
Scytodidae								
Scytodes antonina Rheims & Brescovit 2009	6	2	9	0	7	6	3	3
Scytodes globula Nicolet 1849	0	0	0	1	0	0	0	0
Scytodes sp. 1	0	0	2	1	0	1	1	0
Sparassidae								
Olios sp. 1	1	0	0	1	0	0	0	0
Symphytognathidae								
Anapistula sp. 1	0	0	1	0	0	0	0	0
Synotaxidae								
Synotaxus sp. 1	0	0	0	0	0	1	0	1
Tetragnathidae								
Azilia histrio Simon 1895	1	0	0	0	0	0	0	1
Chrysometa boraceia Levi 1986	0	0	0	0	2	0	2	0
Chrysometa ludibunda (Keyserling 1893)	0	2	1	2	1	1	13	2
Chrysometa sp.	0	0	0	1	0	0	3	0
Cyrtognatha sp. 1	9	3	1	0	4	0	2	0
Glenognatha sp. 1	1	0	0	0	0	0	0	0
Leucauge sp.	0	0	0	0	0	1	0	1
Leucauge sp. 1	0	1	1	0	0	2	0	5
Leucauge sp. 2	0	0	1	0	0	0	5	0
Tetragnatha sp.	0	2	0	0	0	0	0	0
Theridiidae								
Theridiidae sp. 1	0	0	0	0	0	0	0	1
Achaearanea sp. 1	0	0	0	0	0	1	0	0
Achaearanea sp. 18	0	0	0	1	0	0	0	0
Achaearanea tingo Levi 1963	0	1	1	0	0	1	0	0
Ameridion unanimum (Keyserling 1891)	0	0	0	0	4	0	0	0
Anelosimus ethicus (Keyserling 1884)	0	0	0	0	1	0	0	0
Anelosimus nigrescens (Keyserling 1884)	1	0	0	0	0	0	0	0
Argyrodes elevatus Taczanowski 1873	0	0	0	1	0	0	0	0
Argyrodes sp. 1	0	0	0	0	0	1	0	1
Ariamnes attenuatus (O.P.-Cambridge 1881)	0	0	2	1	0	0	1	2
Ariamnes longissimus Keyserling 1891	1	1	0	0	1	1	0	0
Ariamnes sp. 2	0	0	0	0	0	0	1	0
Chrosiothes niteroi Levi 1964	0	0	0	3	13	4	7	9
Chrosiothes perfidus Marques & Buckup 1997	0	1	1	3	2	3	0	5
Chrosiothes sp. 1	0	0	0	2	0	0	0	0
Chrysso gounellei Levi 1962	1	13	0	0	0	1	0	0
Chrysso nigrosterna Keyserling 1891	0	0	0	0	0	3	3	0
Chrysso rubrovittata (Keyserling 1884)	0	0	0	0	1	0	0	0
Chrysso sp. 3	1	2	0	1	0	0	0	6
Chrysso sp. 32	0	0	0	0	0	0	0	1
Coleosoma sp. 1	0	0	0	0	2	0	0	0
Cryptachaea altiventer (Keyserling 1884)	2	1	0	0	1	0	0	0
Cryptachaea digitus (Buckup & Marques 2006)	0	0	0	0	0	0	0	3

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Cryptachaea cinnabarina (Levi 1963)	0	0	0	0	0	0	1	2
Cryptachaea hirta (Taczanowski 1873)	0	0	0	0	8	0	0	0
Cryptachaea isana (Levi 1963)	3	0	0	0	0	0	0	0
Cryptachaea jequirituba (Levi 1963)	0	0	0	0	0	2	0	0
Cryptachaea migrans (Keyserling 1884)	0	0	0	3	0	0	0	0
Cryptachaea passiva (Keyserling 1891)	2	2	3	6	2	5	2	7
Cryptachaea rioensis (Levi 1963)	0	0	0	0	0	0	1	0
Cryptachaea sicki (Levi 1963)	0	0	0	0	0	0	0	1
Cryptachaea sp. 1	0	0	0	0	0	0	1	0
Cryptachaea taim (Buckup & Marques 2006)	1	0	1	1	0	2	4	3
Cryptachaea triguttata (Keyserling 1891)	0	4	3	2	1	3	3	0
Dipoena atlantica Chickering 1943	0	0	0	0	1	1	0	1
Dipoena bryantae Chickering 1943	0	0	0	0	0	1	0	0
Dipoena cordiformis Keyserling 1886	0	0	1	0	0	0	0	0
Dipoena duodecimpunctata Chickering 1943	0	0	0	1	0	0	0	0
Dipoena ira Levi 1963	4	0	0	1	1	0	1	1
Dipoena keyserlingi Levi 1963	1	0	0	0	0	0	2	0
Dipoena pumicata (Keyserling 1886)	1	4	3	2	3	0	1	1
Dipoena pusilla (Keyserling 1886)	0	0	4	5	0	2	0	1
Dipoena santacatarinae Levi 1963	5	1	4	0	0	0	0	0
Dipoena sp.	1	0	0	0	0	0	1	2
Dipoena sp. 2	8	10	11	3	1	20	42	4
Dipoena sp. 3	0	0	0	0	0	0	1	0
Dipoena sp. 6	0	0	0	0	0	3	0	0
Dipoena sp. 8	1	0	0	0	3	0	0	0
Dipoena sp. 12	5	9	5	12	3	4	11	9
Dipoena sp. 21	0	1	0	0	0	0	0	0
Dipoena sp. 22	0	0	0	0	0	0	1	1
Dipoena sp. 58	0	0	0	0	0	2	0	0
Dipoena variabilis (Keyserling 1886)	0	1	0	1	0	0	0	1
Emertonella taczanowskii (Keyserling 1886)	0	0	0	1	1	1	0	0
Episinus sp.	0	0	0	0	0	1	0	0
Episinus sp. 1	3	2	6	4	9	6	17	5
Episinus sp. 2	0	0	0	0	21	0	0	0
Episinus teresopolis Levi 1964	1	0	0	0	2	0	0	0
Euryopis sp.	1	0	0	0	0	0	0	0
Euryopis sp. 1	2	0	0	0	2	1	0	0
Exalbidion sp. 1	6	1	0	3	1	1	0	0
Exalbidion sp. 2	0	1	0	0	0	0	0	0
Faiditus acuminatus (Keyserling 1891)	0	0	0	0	0	2	0	0
Faiditus sp. 1	0	0	1	1	0	0	0	0
Faiditus sp. 2	0	1	0	1	0	0	1	1
Faiditus sp. 3	0	0	0	3	0	0	1	0
gen. 2 sp. 2	0	0	0	0	0	0	1	0
Guaraniella mahnerti Baert 1984	0	0	0	0	4	15	31	5
Guaraniella sp.	0	0	0	0	0	0	1	0
Guaraniella sp. 1	3	0	0	5	0	0	0	0
Hadrotarsinae sp. 1	0	0	0	0	0	0	1	0
Hadrotarsinae sp. 2	0	0	0	0	3	0	0	0
Hadrotarsinae sp. 3	0	0	0	3	0	0	0	0
Helvibis sp. 1	2	3	0	0	0	0	1	0
Hetschkia gracilis Keyserling 1886	14	5	2	0	4	0	1	0
Janula bicorniger (Simon 1894)	0	1	13	6	0	2	21	32
Neopisinus cognatus (O.P.-Cambridge 1893)	0	1	0	0	0	0	0	0
Phycosoma altum (Keyserling 1886)	4	6	2	0	13	2	0	0
Rhomphaea sp. 1	1	0	1	1	0	0	0	0
Rhomphaea sp. 2	0	0	1	0	0	0	0	1
Spintharus gracilis Keyserling 1886	2	10	8	25	1	10	74	30
Stemmops sp. 2	1	0	0	0	0	0	0	0
Styposis selis Levi 1964	0	0	0	0	1	1	0	1
Tekellina crica Marques & Buckup 1993	1	0	0	0	0	0	2	0

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Tekellina sp. 3	0	0	0	1	0	0	0	0
Tekellina sp. 4	0	0	0	0	0	0	0	1
Theridion biezankoi Levi 1963	1	0	0	0	0	0	1	0
Theridion minutissimum Keyserling 1884	0	1	0	0	0	0	0	0
Theridion opolon Levi 1963	0	0	1	0	0	0	0	0
Theridion plaumanni Levi 1963	11	2	0	0	43	0	0	1
Theridion quadripartitum Keyserling 1891	0	0	1	3	0	0	0	0
Theridion sp. 1	1	0	0	0	1	0	0	0
Theridion sp. 2	0	0	0	1	0	0	0	0
Theridion sp. 11	0	0	0	0	0	1	0	0
Theridion sp. 28	0	0	0	0	0	0	0	1
Theridion sp. 32	1	12	1	23	0	0	0	8
Theridion sp. 36	0	0	0	1	0	0	0	0
Theridion sp. 40	0	1	0	0	0	0	0	0
Theridion sp. 47	0	0	1	1	0	0	0	0
Theridion sp. 63	0	0	0	1	0	0	0	0
Theridion sp. 68	0	0	0	1	1	0	0	0
Theridion teresae Levi 1963	1	0	0	0	1	0	0	1
Theridula gonygaster (Simon 1873)	0	0	0	0	1	0	0	0
Thwaitesia affinis O.P.-Cambridge 1882	7	9	7	1	2	7	12	41
Thwaitesia sp. 1	0	0	1	1	0	0	0	0
Thymoites anicus Levi 1964	8	2	0	0	4	0	0	0
Thymoites melloleitaoni (Bristowe 1938)	0	0	0	1	0	15	1	1
Thymoites sp.	1	0	0	0	0	1	0	0
Thymoites sp. ?	0	0	0	0	0	2	0	0
Thymoites sp. 1	4	1	2	4	1	0	1	0
Thymoites sp. 2	5	0	1	3	0	0	0	0
Thymoites sp. 4	1	0	0	0	0	0	1	2
Thymoites sp. 5	0	0	1	0	0	0	0	0
Thymoites sp. 7	0	0	2	3	0	7	1	0
Thymoites sp. 12	1	0	0	0	0	0	0	0
Wamba crispulus (Simon 1895)	4	1	0	0	0	0	0	0
Wirada sp. 1	0	0	0	0	0	0	1	0
Theridiosomatidae								
gen. indet. 1	0	1	0	0	1	0	0	0
gen. indet. 3	1	0	1	5	0	0	0	0
gen. indet. 4	0	0	0	1	0	0	0	1
gen. sp. 1	0	0	1	0	0	0	0	1
Chthonos sp.	1	0	0	0	0	0	0	0
Chthonos sp.1	0	0	0	0	0	1	3	0
Chthonos sp.2	0	0	0	0	0	0	1	0
Naatlo sp.	0	0	2	0	0	2	0	1
Naatlo sp. 1	0	0	0	0	0	0	1	0
Plato sp. 1	0	0	0	0	0	0	0	1
Theridiosoma sp. 1	5	0	0	1	0	0	0	0
Theridiosoma sp. 3	0	0	0	2	0	0	0	0
Theridiosoma sp. 4	0	0	0	1	0	0	0	1
Thomisidae								
Acentroscelus sp. 1	0	2	1	0	0	0	1	1
Aphantochilus taurifrons (O.P.-Cambridge 1881)	0	0	1	1	0	1	0	0
Deltoclita sp. 1	1	0	1	1	2	1	2	0
Epicadinus sp. 1	0	1	0	2	0	0	2	1
Misumenops sp. 1	1	0	0	0	0	0	0	0
Onocolus sp. 1	0	0	7	1	0	0	0	1
Titidius sp. 1	8	10	1	0	2	5	0	0
Tmarus sp. 1	3	12	4	3	9	3	9	5
Tmarus sp. 2	4	7	2	0	2	2	2	1
Tmarus sp. 3	0	0	1	1	0	0	0	0
Tmarus sp. 4	0	0	0	1	0	0	0	0
Tmarus sp. 5	0	1	0	0	0	0	0	0

Appendix 1.—Continued.

Taxon	Locality and Stage							
	Ca-H	Ca-A	Ca-M	Ca-F	It-H	It-A	It-M	It-F
Titanoecidae								
Goeldia sp. 1	0	0	0	0	7	0	0	0
Trechaleidae								
Neoctenus comosus Simon 1897	0	0	0	0	1	0	0	0
Uloboridae								
Conifaber sp. 1	0	1	0	0	0	0	0	0
Miagrammopes sp. 1	0	1	8	6	0	2	2	3
Miagrammopes sp. 2	4	0	8	8	0	3	9	9
Uloborus sp.	0	0	0	0	0	0	0	1
Uloborus sp. 1	0	0	0	1	0	0	1	3
Zodariidae								
Zodariidae sp.	0	0	0	0	0	0	1	0
Zoridae								
gen. 1 sp. 14	8	0	0	0	0	0	0	0
gen. 1 sp. A	30	74	88	103	21	110	107	64
gen. 1 sp. B	0	2	1	0	0	5	1	1
gen. 1 sp. C	20	28	2	21	16	50	31	20
gen. 1 sp. D	14	0	20	12	0	0	0	0
gen. 1 sp. E	1	0	0	0	0	0	0	0
gen. 1 sp. F	0	1	0	0	0	0	0	0
gen. 1 sp. G	0	3	0	1	0	2	1	2

* Nomenclature (order) of the morphospecies originates from the system of the experts/hosting institutions: IBSP - Instituto Butantan, São Paulo; MCN: Museu de Ciências Naturais da Fundação Zoobotânica, Porto Alegre.

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Trophic niche and predatory behavior of the goblin spider *Triaeris stenaspis* (Oonopidae): a springtail specialist?

Stanislav Korenko[1], **Kateřina Hamouzová**[1] and **Stano Pekár**[2]: [1]Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Suchdol, Czech Republic. E-mail: korenko.stanislav@yahoo.com; [2]Department of Botany and Zoology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Abstract. *Triaeris stenaspis* Simon 1891 is a parthenogenetic goblin spider that has been introduced into greenhouses all over Europe. Here we investigated its trophic niche and predatory behavior. Potential prey in the greenhouses included predominantly springtails, aphids, and other spiders. Out of ten potential prey types offered in the laboratory, *T. stenapsis* captured only three types, the primary one being springtails. The spider rarely caught the other two types, termites and crickets, and completely rejected beetles, ants, aphids, thrips, flies, spiders and mites. *Triaeris stenaspis* did not build webs for prey capture, but instead used the grasp-and-hold tactic. Prey-capture efficiency decreased with springtail body size, the spider using more than three bites to capture large springtails. Large springtails defended themselves by saltation with the spiders still attached to their backs. Our study supports the hypothesis that *T. stenaspis* is a specialized predator of springtails, being effective in the capture of this type of prey.

Keywords: Stenophagy, prey acceptance, capture behavior, prey

Spiders are mostly known as euryphagous predators, consuming a wide variety of prey (Nentwig 1987), but several spider species are prey specialists. Known examples of trophic specialists include myrmecophagous spiders of the genus *Zodarion* (Zodariidae) (e.g., Pekár 2004), oniscophagous spiders of the genus *Dysdera* (Dysderidae) (Řezáč et al. 2008), araneophagous spiders of the genus *Portia* (Salticidae) and *Palpimanus* (Palpimanidae) (Harland & Jackson 2001; Pekár et al. 2011), lepidopterophagous spiders of the genus *Mastophora* (Araneidae) (Yeargan 1988) and termitophagous spiders of the genus *Ammoxenus* (Ammoxenidae) (Dippenaar-Schoeman et al. 1996).

Trophic specialists possess various kinds of specific adaptations that are effective for their preferred prey. Pekár et al. (2011) documented specific behavioral and morphological adaptations in araneophagous *Palpimanus* spiders. Harland & Jackson (2001) identified a specific behavioral adaptation, "cryptic stalking", in araneophagous *Portia fimbriata* Doleschall 1859, which prevents prey dangerous to spiders from identifying *Portia* as a predator. Researchers observed unique physiological adaptations in ant-eating spiders of genus *Zodarion* (Pekár et al. 2008; Pekár & Toft 2009), one being that they cannot metabolize alternative prey at all, or at most to a much more limited degree.

Goblin spiders (Oonopidae) occur throughout the temperate and tropical regions of the world, in habitats as diverse as deserts, savannahs, mangroves and rainforest (e.g., Jocqué & Dippenaar-Schoeman 2006). To date, arachnologists have described more than one thousand species, making goblin spiders one of the largest families among the Haplogynae (Platnick 2012). Yet very little is known about the trophic niche of oonopid spiders. Scientists have found oonopids in a variety of underground microhabitats such as ant nests (Jacobson 1933, Weber 1957), termite mounds (Benoit 1964) and caves (Harvey & Edward 2007), but also on the soil surface (Ubick 2005) and on tree bark and in the canopy (e.g., Fannes et al. 2008). All oonopid species appear to be active cursorial hunters, as they do not build prey-capture webs. They have been observed to feed on springtails, mites, firebrats, psocids, and other spiders (Bristowe 1948; Knoflaeh et al. 2009; Korenko et al. 2009; Hansen 1992). Researchers have even detected some oonopids scavenging insect remains on webs of larger spiders (Bristowe 1948; Knoflach et al. 2009). To date, rigorous analysis of the trophic niche of oonopids is lacking.

Triaeris stenaspis Simon 1891 seems to be indigenous to West Africa (Platnick et al. 2012) and, according to Platniek (2012), its range stretches from Central to South America, including Antilles and Europe. It has become a resident in Europe and is successfully surviving in heated greenhouses (Korenko et al. 2007). It is known to be parthenogenic in Europe (Korenko et al. 2009).

Since greenhouses are rather poor in arthropod species diversity (Mahr et al. 2001), we expect *T. stenaspis* to be trophically specialized on an abundant prey. Based on knowledge from our previous study when *T. stenaspis* was successfully reared on a monotypic diet including only springtails (Korenko et al. 2009), we hypothesized that *T. stenaspis* prefers, or at least is adapted to, springtail prey. Such trophic specialization is either fixed across populations or is a plastic response of a population to locally abundant prey, as was recently found for *Oecobius* spiders (Líznarová et al. 2013). In the former case, specialized traits used for capture and utilization of prey occur in all populations and are leading to the evolution of species-specific adaptations for particular prey types. In the latter case, a predator does not possess specialized traits, but it can be locally specialized on profitable prey to enhance its versatility at a particular time and place. Our aim in this study was to investigate the trophic niche of *T. stenaspis*, namely the prey spectrum and the predatory behavior of this species in order to test our hypothesis on prey-specialization and to investigate whether it is effective in the capture of prey.

METHODS

On eight sampling dates during the autumn and winter 2006–2008, we collected all ground-dwelling arthropods occurring in the microhabitat of *T. stenaspis*' occurrence in the botanical garden of the Masaryk University in Brno (Czech Republic) to estimate the potential prey spectrum. Our team members hand-collected arthropods by lifting stones and rotten pieces of wood, inspecting the ground and plant roots underneath using a pooter, and then put the arthropods in tubes with ethanol and identified them to order in the laboratory. We only eonsidered specimens of a total body size less than six mm as potential prey.

Altogether, we collected 60 adult females of *T. stenaspis* in the greenhouse in order to perform observations in the laboratory. The body length of spiders was very similar, ranging between 1.6 and 1.7 mm. We placed spiders singly in cylindrical containers (diameter 35 mm, height 40 mm) with a layer of plaster of Paris at the bottom, and kept them at room temperature, 22 ± 3.5°C, to replicate conditions in the greenhouse. The plaster was moistened with a few drops of water to retain sufficient humidity. Spiders were fed springtails *Sinella curviseta* (Brook 1882) at 3–4 day intervals.

We tested the following 11 prey types of ground-living arthropods for acceptance: beetle imagoes (Curculionidae) (average body length = 1.93 mm, SD = 0.20), first instars of crickets *Acheta domestica* (Linnaeus 1758) (Gryllidae) (2.81 mm, SD = 0.46), aphids (Aphididae) (1.81 mm, SD = 0.25), thrips (Thysanoptera) (1.41 mm, SD = 0.15), mites (Trombidiidae) (0.87 mm, SD = 0.10), early instars of crab spiders of genus *Xysticus* (Thomisidae) (1.4 mm, SD = 0.38), ants *Tetramorium caespitum* (Linnaeus 1758) (Formicidae) (3.14 mm, SD = 0.20) and a mixture of springtail species (Collembola). Our team collected all of these prey from the soil in the greenhouse (1.06 mm, SD = 0.41). Termites *Reticulitermes* sp. (Isoptera), (3.54 mm, SD = 0.30), larvae and imagoes of *Drosophila melanogaster* Meigen 1830 (Diptera) (3.8 mm, SD = 0.54 for larvae and 2.4 mm, SD = 0.50 for imagoes) and springtails *Sinella curviseta* (Brook 1882) (1.45 mm, SD = 0.12) (Entomobryidae) came from laboratory cultures. The body length of prey was measured using an ocular ruler in the stereomicroscope before each trial.

Altogether we performed 150 trials over a period of 60 days to test prey acceptance. For each prey type, 12 individuals of adult female *T. stenaspis* were used. The order of tested prey was based on the availability of particular prey. Each tested spider was kept singly in a dish (diameter 35 mm, height 45 mm), which was marked by number, 1–60, in the order in which the individuals were collected. Twelve individuals from the set of 60 specimens were randomly (without replacement) selected for each prey type. When all individuals were used in the first round of tests, another 12 individuals were randomly (without replacement) selected from those that had already been used. Each individual was tested a maximum of two or three times. Each individual was used in the trial five days after being satiated with springtails. Individuals that did not feed during the day of satiation were not used in the next trial. At least six days elapsed between the repeated uses of the same individual.

Before each trial, spiders were placed singly in an experimental dish (diameter 35 mm, height 15 mm) with a thin (2–3 mm) layer of plaster of Paris at the bottom. After five minutes of acclimatization, the potential prey was released into the experimental dish, and the occurrence of capture (incidence) was recorded. If the spider accepted the prey, or did not catch the prey within 120 minutes following encounter with prey, we terminated the trial.

Table 1.—Relative incidence of potential prey types found on the soil in the greenhouse (n = 148).

Potential prey	Relative incidence
Isopoda	0.12
Myriapoda	0.02
Araneae (other than *Triaeris*)	0.09
Schizomida	0.07
Collembola	0.61
Ensifera	0.02
Formicidae	0.03
Coleoptera larvae	0.02
Other larvae	0.02

The capture efficiency was studied in detail using *S. curviseta* springtails as prey. Thirty adult female spiders, selected randomly (without replacement) from the set of 60, were starved for five days and placed in arenas as described above. After five minutes of spider acclimatization in the dish, a springtail *S. curviseta* of body length between 0.5 mm–2 mm (prey-predator body length ratio = 0.3–1.2) was released. Spiders were selected randomly from the same experimental group as in the previous experiment. We recorded the attack latency, the capture behavior and the number of attempts required to capture springtails. The attack latency was the time between when the spider oriented itself toward the springtail and successfully completed the attack, (i.e., when prey was held in the chelicerae). We measured the body length of springtails before each experimental trial.

Statistical analyses were conducted within the R environment (R Development Core Team 2011). The relationship between prey size and attack latency, springtail size and number of attacks were analyzed using Generalized Linear Models with Gamma (GLM-g) or Poisson (GLM-p) error structure, respectively (Pekár & Brabec 2009). We used Generalized Estimating Equations with binomial error structure (GEE-b) to compare the incidence of captured prey assessed in a binary form. GEE is an extension of GLM used for repeated measurements by specifying correlation among measurements (Pekár & Brabec 2012). GEE was used since measurements were not independent due to repeated use of the same individual spiders.

RESULTS

The most abundant potential prey arthropods in the greenhouse (n = 148) were springtails (Entomobryidae) followed by aphids, other spiders, schizomids, ants, myriapods, coleopteran larvae, other larvae and crickets (Table 1). From 11 potential prey types tested in the laboratory, *T. stenaspis* accepted only springtails at high incidence rates (83%, n = 42, Fig. 1). Crickets and termites were rarely accepted (8%, n = 12, both prey types). The prey-predator body-length ratio of accepted prey was on average 0.6 (min–max = 0.3–1.1) in springtails, 1.2 in crickets, and 2.0 in termites. Mites, spiders, thrips, aphids, beetles, ants and flies

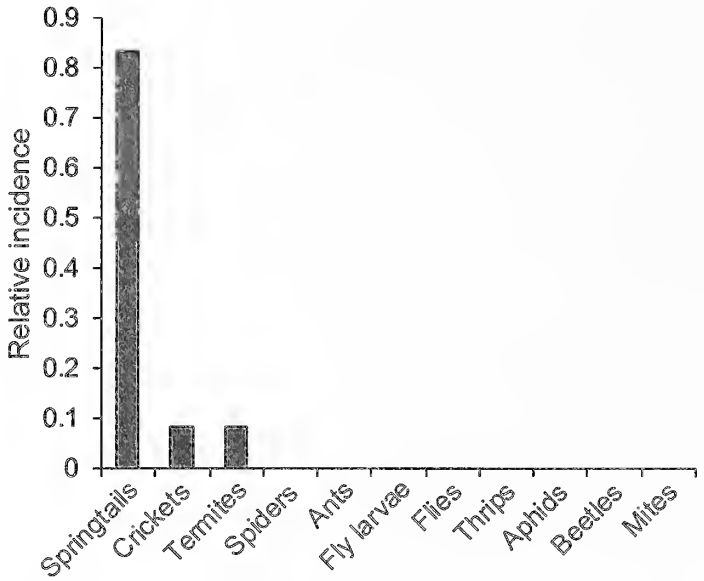

Figure 1.—Comparison of the relative capture incidence of 11 prey types.

(larvae and imagoes) were never accepted. The prey capture incidence thus differed among groups (GEE-b, $X^2_{10} = 45.7$, $P < 0.0001$).

Triaeris stenaspis did not build a web for prey capture; instead, it caught springtails and other prey using the grasp-and-hold tactic. After an attack, springtails attempted to escape by means of saltation. Ten percent of springtails jumped up with the spider attached to the springtail's back and hit the top of the experimental arena. All jumping springtails were large adults with a prey-predator ratio > 1.2.

The attack latency increased significantly with prey size (GLM-g, $F_{1,23} = 6.4$, $P = 0.02$, Fig. 2). The number of attacks required for prey immobilization increased significantly with

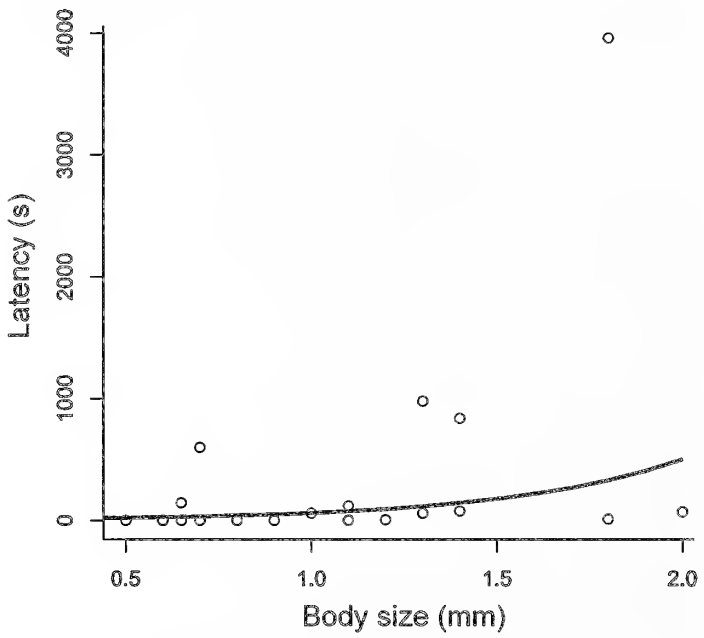

Figure 2.—Relationship between the attack latency and the springtail body size with estimated model.

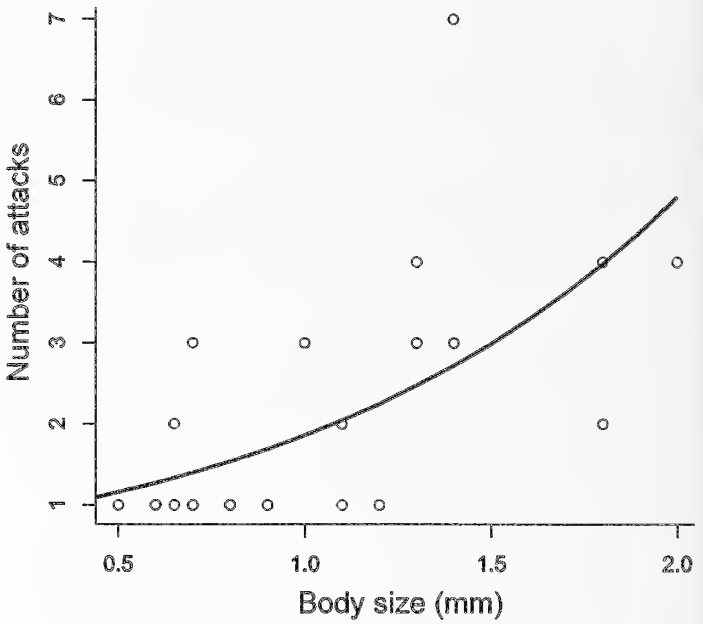

Figure 3.—Relationship between the number of attacks and prey size with estimated model.

prey size (GLM-p, $X^2_1 = 9.5$, $P = 0.002$, Fig. 3). *Triaeris stenaspis* required significantly fewer attacks to catch small springtails than to catch large springtails. The spiders made on average 1.4 attacks (SD = 0.71, $n = 17$) to catch small springtails (prey-predator ratio < 0.7), compared to 3.8 attacks (SD = 1.48, $n = 8$) to catch large springtails (prey-predator ratio > 0.7).

DISCUSSION

We found a moderate diversity of potential prey for *T. stenaspis* on the surface of the greenhouse soil. All of these arthropods were expected to be potential prey for *T. stenaspis*, at least at the life stage when of suitable body size. *Triaeris stenaspis* is a ground dweller that inhabits various structures of the soil and encounters all of these taxa. Although we did not record prey capture by *T. stenaspis* in the field, acceptance trials clearly indicate that *T. stenaspis* likely utilizes only a very small portion of available prey.

Little is known about the prey consumption of goblin spiders. There is only one report of the natural prey of *T. stenaspis*: Weber (1957) stated that it consumed *Cyphomyrmex costatus* (Mann 1922) ants and springtails. Our laboratory study confirmed the spiders feeding on springtails but not on ants. Since Weber (1957) observed only ant remnants in the spider surrounding, not the actual feeding on ants, we propose that *T. stenaspis* was not feeding on ants. Ants are dangerous prey and feeding on them requires morphological and behavioral specialization (e.g., Pekár et al. 2008, 2011). We have not observed any adaptation useful for capture of ants in this spider species. *Triaeris stenaspis* is thus likely myrmecophilous but not myrmecophagous.

In the laboratory, *T. stenaspis* consumed springtails almost exclusively and haphazardly preyed on other prey. Spiders uniformly used the grasp-and-hold predatory tactic to capture springtails, termites, and crickets. Several other spiders use this tactic (Nentwig 1987). The grasp-and-hold tactic seems efficient for the capture of mobile prey, because after a bite such prey could escape the spider. This is particularly

important for species capable of fast escape, such as springtails with furca.

The venom of *T. stenaspis* seems to be extremely effective in immobilizing springtail prey. Springtails with bodies larger than the spiders were not able to jump more than onee. The spider injected the venom behind the head, near the central neural system, and the paralysis latency was only a few seconds. Thus *T. stenaspis* was able to immobilize the large springtail within a few seeonds.

Most cursorial spider species prey on much smaller prey than themselves (Nentwig 1987). *Triaeris stenaspis* was able to eatch springtails larger than itself, even capturing one termite double its size. The ability to capture much larger prey is typical for many stenophagous predators (e.g., Pekár et al. 2008) that possess various adaptations to capture their specific prey.

Springtails are consumed by many spider species either of cursorial or web-building habit, such as corinnids (Pekár & Jarab 2011), linyphiids (Sunderland et al. 1986; Nyffeler & Benz 1988; Alderweireldt 1994), lycosids (Hallander 1970; Gettmann 1978; Punzo 2006), salticids (Guseinov et al. 2004; Huseynov et al. 2005), theridiids (Ibarra- Núñez et al. 2001) and thomisids (Guseinov 2006). The spiders differ in the way they capture springtails. Although web-builders such as *Lepthyphantes* (Linyphiidae) rely on the use of webs, cursorial species such as *Erigone*, *Oedothorax* (both Linyphiidae) or *Mexcala* (Salticidae) grasp springtails with their forelegs and chelicerae (Alderweireldt 1994; Pekár & Haddad 2011). The linyphiid *Bathyphantes simillimus* (L. Koch 1879) captures springtails by means of two different strategies (Rybak 2007): juvenile individuals rely on the web (a springtail gets entangled in a web by its hairs), while adult spiders grasp springtails with their chelicerae.

For spiders, many springtails are palatable prey, except for some species that are toxic (Toft & Wise 1999). Springtails, however, possess efficient defenses: species having furca can escape by jumping either from a web or from a spider's forelegs. For a predator that is of similar or smaller size, the springtails can jump after being grasped. Such "rodeo-riding" on the springtail may carry a high risk of injury to the spider. Springtails with a spider attached to their back jumped high enough to hit the lid of the experimental arena. This strong knoek could cause serious injury to the spider's soft abdomen. However, three scuta located on both sides of the soft abdomen in *T. stenaspis* seem to prevent such injury. Whether the scuta can be considered morphological adaptations used for defense against prey remains to be investigated.

Our study found that springtails are readily accepted as prey by *T. stenaspis*, even when the prey is longer than the spider. It is yet to be discovered whether *T. stenaspis* is a collembolan specialist and whether such prey-specificity is a fundamental property of all populations of this species or only a case of local specialization. Although data gathered so far do not provide clear evidence for specific adaptations, we found a high efficiency of capture of collembolan prey. This efficiency is attributed to their powerful grasp-and-hold tactic and the fast-ensuing paralysis from the spider's bite. Riding the "rodeo" on the springtail back is a behavioral trait serving to avoid the loss of the escaping prey. Strong scuta on the spider abdomen could be a morphological trait that protects the spider's soft parts against injury during "rodeo." The short paralysis latency suggests that the venom is used for fast immobilization and minimizing the duration of the dangerous "rodeo." Finally, in our previous study, we reared this spider species on a monotypic diet of springtails (Korenko et al. 2009); the spiders suffered low mortality, were able to develop completely and produce viable offspring. They also seem to possess physiological adaptations to utilize a monotypic springtail flesh. Whether these traits have evolved as an adaptation to collembolan prey remains to be proven.

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Use of locomotor performance capacities reflects the risk level associated with specific cue types in two cursorial spider species

Joseph A. Dillon[1] and **Jonathan N. Pruitt**[2,3]: [1]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996 USA; [2]Department of Biological Sciences, University of Pittsburgh, Pittsburgh Pennsylvania 15260 USA

Abstract. Understanding when and how animals use their performance capacities can yield insights into the selection pressures driving high performance. Using two species of cursorial spiders, *Schizocosa ocreata* (Hentz 1844) and *Rabidosa rabida* (Walckenaer 1837) (Araneae: Lycosidae), we investigated the escape speeds exhibited by individuals of various body sizes in response to three aversive stimuli (jets of air, seismic cue, prodding) to determine how individuals use their performance capacity in response to different stimuli. We found that large individuals of both species exhibited their highest observed escape speeds in response to jets of air, whereas smaller individuals exhibited their fastest observed escape performances in response to prodding. We hypothesized that differences in escape behavior may reflect differences in risk associated with each cue type: fast moving jets of air may announce the arrival of an avian predator, and large individuals may be at greater risk of avian predation owing to their more conspicuous body size; whereas smaller individuals may be more susceptible to arthropod predators, which attack from the level of the spider, similar to a prod. We then performed an unreplicated mark-recapture, avian-exclosure experiment for both species, where we tracked individuals' persistence for 30 d. Consistent with our predictions, we found that larger individuals enjoyed greater persistence in our avian exclosure treatment, but their advantage was lost when avian predators were allowed to enter. Our results suggest that these spiders express their highest performances in only their most dire situations.

Keywords: Behavioral compensation, foraging mode, locomotion, Lycosidae

Variation in performance capacities has been linked with fitness components in a variety of species. For instance, higher running speed may be associated with greater survivorship (Calsbeek & Irschick 2007; Pruitt 2010), stronger bite forces may help individuals establish dominance (Lailvaux et al. 2004; Perry et al. 2004) and greater endurance may help animals obtain prospective mates (Stoltz & Andrade 2010; Stoltz et al. 2009). Although everyday tasks require that animals use their performance capacity somewhat regularly, identifying the specific selection pressures driving maximum performance can be difficult. This difficulty stems, in part, from the fact that the same actions are used across many tasks: capturing prey, avoiding predators, maintaining territories and displaying courtship (Irschick & Garland 2001). Thus, any number of context-specific selection pressures could drive the evolution of animals' maximum performance. One way to gain insights into the selective pressures driving maximum performance is to understand when and how animals use their performance capacity. The hypothesis underlying this notion is that individuals are expected to approximate maximum performance capacity in situations most important for their survival or reproductive success and express sub-maximal performance in other, less dire situations (Irschick 2000a,b; Irschick & Garland 2001; Husak & Fox 2006; Pruitt & Husak 2010). Here we define individuals' "maximum" or "highest" performance as the peak performance individuals exhibited across all observed contexts (after Husak & Fox 2006), although we acknowledge that even these values may fall short of absolute potential.

A small number of studies have shown that individuals tend to express their highest performance in the ecological contexts most pertinent to their success. For example, within a population of collared lizards (*Crotaphytus collaris*), females exhibit their highest observed running speeds when escaping predators. Males, however, reserve their highest speeds for territorial encounters. Neither sex uses high speeds when attempting to capture prey (Husak & Fox 2006). Males and females experience differing selective pressures in this system, as evidenced by differences in how the sexes use their performance capacities. Males are territorial and risk suffering a potentially high cost to their fitness if they do not rapidly respond to intruding rivals (Husak et al. 2006; Husak et al. 2008), whereas females are non-territorial and have little selective pressure to respond intensely to rival females. Thus, strength of selection on locomotor importance may differ considerably across demographic groups within a single population.

Similarly, in the funnel-web spider *Agelenopsis aperta* Gertsch 1934, individuals show population-level divergence in how they use their maximum running speeds (Pruitt & Husak 2010). This species occupies a habitat mosaic of arid and riparian zones; arid zones are characterized by few foraging territories, low prey availability, and few predators, whereas riparian habitats are lush environments with many suitable foraging territories, high prey availability and many predators (Riechert & Hedrick 1990; Riechert 1993; Riechert et al. 2001). Consistent with our understanding of this species' ecology, offspring from parents collected in arid habitats exhibited their highest observed running speeds during territorial encounters, but responded slowly to simulated predator threats. In contrast, offspring from parents collected from riparian sites expressed their highest observed speeds in response to simulated predator threats, but were slower to attack rivals (Pruitt & Husak 2010). Here again, data suggest animals scale the use of their performance capacities to the significance of the task or challenge at hand.

[3]Corresponding author. E-mail: pruittj@pitt.edu

A primary insight gleaned from the studies detailed above is that different populations or demographic groups within them experience divergent selection pressures, and these pressures are echoed in how animals use their performance capacities. However, the possibility that individuals may differ in their responsiveness to different cue types within a context (e.g., predator avoidance) has been largely overlooked. Here we explore this topic using two species of temperate wolf spider, *Schizocosa ocreata* (Hentz 1844) and *Rabidosa rabida* (Walckenaer 1837 (Araneae, Lycosidae). Specifically, we test whether individual variation in body size is associated with how individuals respond to different cue types. For example, large individuals might exhibit slow running speeds in response to cues resembling arthropod predators, but high-speed responses towards cues resembling avian/vertebrate predators. We then test whether performance matches risk of predation in the field using an unreplicated mark-recapture, predator exclusion experiment. Documenting associations between performance use and body size has important general implications for understanding how animals of different body states (e.g., age, viability, reproductive status) use their performance capacities.

METHODS

Collection and laboratory maintenance.—Wolf spiders (family Lycosidae) are cursorial, non-web building spiders that traverse leaf litter and low-lying vegetation in search of prey. We collected spiders for use in our aversive stimuli trials in March–April (*Schizocosa*) and July–August (*Rabidosa*) of 2010. *Schizocosa ocreata* (n = 46) were collected amid fallen leaf litter from a deciduous forest habitat (35°47′56″N, 84°14′01″W), and *Rabidosa rabida* (n = 28) were collected among mixed herbs and grasses from an early successional meadow habitat (36°00′49″N, 84°02′32″W) in East Tennessee. We selected our two test species because they occur commonly at our test sites and because of their divergent ecologies: they occupy different habitats, exhibit divergent phenologies, and differ distinctly in body size (see Results section). Spiders were collected at night using a spotlight. They were spotted, chased into pill vials, and transported to a laboratory at the University of Tennessee, Knoxville. They were housed individually in 490-ml opaque deli cups, provided a maintenance diet of two three-week old crickets once weekly, and maintained under ambient lighting conditions. A moist paper towel was provided as a water source. Upon reaching maturity (≈1–3 weeks), individuals were selected for use in our aversive stimuli trials, and all aversive stimuli trials were completed within two weeks of individuals' final molts. Only mature females were used in our aversive stimuli trials and mark-recapture experiments.

Individuals' body measurements were collected one day after their first routine feeding as mature individuals. Each individual was weighed to the nearest 0. 1 mg, (Mettler-Toledo XP205) and its cephalothorax length and abdomen length and width were measured to the nearest millimeter using Leica digital imaging software and a stereomicroscope (Leica M80).

Aversive stimuli trials occurred three days after a routine feeding, and 24 h elapsed between trials. We used a standardized feeding-level for our trials because previous data indicate recent meal size (via the resulting increase in body mass) can negatively impact spiders' escape performance (Pruitt 2010), and we limit our investigation to recently matured females. Sex and reproductive status have significant impacts on spiders' escape performance (Pruitt and Troupe 2010). To avoid potential confounding of trial order, the sequence of the trials was alternated among individuals. Thus, each individual was tested three times, once with each stimulus.

Aversive stimuli.—Aversive stimuli trials were run at 2000–2200 at low light conditions (30–50 lux) to mimic the nocturnal/crepuscular nature of most wolf spiders (*Schizocosa*: Cady 1983; general wolf spider ecology: Foelix 1996; but see Uetz et al. 1999). Spiders were placed on a 30-cm track in a clear plastic collection vial and allowed 30 sec to acclimate before the vial was lifted. Spiders were then given another 30 sec of acclimation before an aversive stimulus was applied. Tracks were lined with graphing paper with 0.5 cm demarcations. Tracks were 6 cm wide and the walls extended 8 em up on all sides. We video-recorded spiders' eseape responses using an infrared Bullet Security Camera (Sony CSP-LR560IR) and noted individuals' flight speed (cm/s) over 15 cm of track. Data from trials where spiders paused or turned were removed from our analyses, as is standard in the animal performance literature. In such instances (n = 6), spiders were given 15 min to recover before the trial was repeated.

Our three aversive stimuli were a prod, a puff of air, and a seismic cue. For our prod test, we touched the rear end of the spider with a thin (3 mm wide) paintbrush. This prod was designed to mimic the tactic cue of an approaching predatory spider or insect. For our 'puff' test, we used a camera lens cleaning bulb to apply rapid jets of air on the dorsal, posterior portion of the spiders from 5 cm distance: two fast, consecutive puffs were applied. Puffs of air have been used to simulate the approach of an avian predator in a number of investigations on spiders (Riechert & Hedrick 1990; Riechert 1993; Riechert et al. 2001; Pruitt et al. 2008; Jones et al. 2011a,b). This cue is relevant because most spiders lack acute vision and instead detect the approach of avian predators using minute vibratory-sensing setae, the trichobothria (Foelix 1996). We applied a seismic cue by dropping a 1.2 kg biology textbook from 1m off the ground, 30 cm away from the spider. This seismic cue was modeled to resemble the distant approach of a large vertebrate (e.g., a deer, coyote or a human eollecting spiders). After we reeorded their reaction, spiders were returned to their individual cups.

Selection on body size.—To assess whether individuals of different body sizes were more or less susceptible to avian predation, we estimated selection on body size in field exclosures from March–July 2008 (March for *S. ocreata* and July for *R. rabida*). We established four 3 m × 3 m exclosures: two to assess selection on body size for *R. rabida* at a meadow site (Riechert Farm: 36°00′49″N, 84°02′32″W) and two to assess selection on *S. ocreata* at a deciduous forest site (House Mountain: 35°47′56″N, 84°14′01″W). Thus, our predator present/absent treatment was unreplicated within each species, but two parallel studies were conducted with two different species.

Exclosures were lined with aluminum flashing around the perimeter; flashing extended 40 cm above the ground and 15 cm below. To prevent immigration and emigration across the aluminum border, herbaceous plants were trimmed back

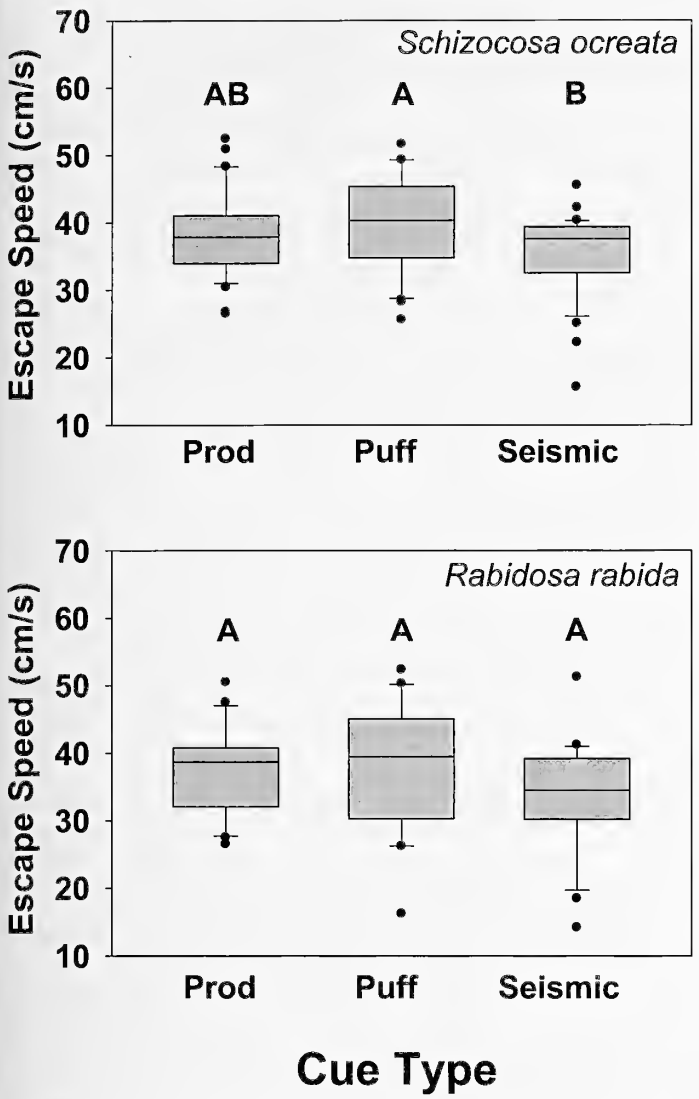

Figure 1.—Box plot depicting the raw (cm/s) escape speeds exhibited by both test species (*Schizocosa ocreata*, *Rabidosa rabida*) in response to our three aversive stimuli. Vertical shaded bars represent interquartile range and vertical lines represent the 90th and 10th percentiles. Within BT compositions, bars not sharing letter flagging are significantly different at $\alpha = 0.05$ using post-hoc Tukey tests.

on either side of the flashing using pruning shears, and both sides of the flashing were topped with a 7-cm-wide strip of Tanglefoot tree pest barrier. For each pair of exclosures, one was left with an open top (avian predators present) and the second was topped with a grid of two perpendicular series of monofilament fishing lines placed every 4 cm in either direction. Fishing lines were anchored into a wooden frame, which we fastened atop the aluminum flashing, thus generating a monofilament 'table top' that effectively excluded birds (Hubbs & Boonstra 1997; Krebs et al. 1995). Although we did not directly observe avian predation in this study, birds are known to be important predators for at least one of our test species (*Schizocosa ocreata*: Lohrey 2007; Lohrey et al. 2009) and are thought to be major predators of spiders in general (Foelix 1996; reviewed in Riechert & Hedrick 1990; Gunnarsson 2007). Although our exclosure design likely excluded other vertebrate predators (e.g., bats, raccoons) and not just birds, we will refer to our exclosures as 'avian exclosures' throughout this paper.

Table 1.—Summary of our general linear models predicting relative speed in response to three aversive stimuli. Relative speed was obtained by dividing the flight speed (cm/s) obtained by an individual in response to each stimulus by the mean flight speed exhibited across all three stimuli; *df* indicates degrees of freedom.

Schizocosa ocreata			
Source	*df*	*F*	*P*
Combined Model, $R^2 = 0.36$	5	3.866	0.03
Intercept	1	58.791	<0.001
Body Size	1	0.45	0.87
Stimulus type	2	10.227	<0.001
Body Size*Stimulus Type	2	7.2	<0.001
Error *df*	91		
Rabidosa rabida			
Source	*df*	*F*	*P*
Combined Model, $R^2 = 0.34$	5	7.289	<0.001
Intercept	1	40.826	<0.001
Body Size	1	0.03	0.99
Stimulus type	2	13.006	<0.001
Body Size*Stimulus Type	2	13.455	<0.001
Error *df*	57		

Before initiating an experiment, we systematically sampled each exclosure by sifting through leaf litter and removing all *S. ocreata* and *R. rabida* therein. Non-focal arthropods (spiders or otherwise) were replaced within our exclosures before the start of our marked-recapture experiment. We removed 41 and 26 *S. ocreata* from our exclosures at House Mountain, and 12 and 5 *R. rabida* at the Riechert Farm. A pool of mature female conspecifics was collected from adjacent habitats, measured using digital calipers, individually marked, and placed within our exclosures. Individuals' assignment to treatment (avian predators present/absent) was determined randomly using statistical software. We placed 35 *S. ocreata* in each plot at House Mountain and 20 *R. rabida* in each plot at the Riechert Farm. Spider densities were a compromise between 1) our desire to replicate natural densities of both test species at our test sites, and 2), our desire to maximize the statistical power of our experiment. Each test spider was assigned a unique letter/number combination, and a corresponding tag was adhered to its prosoma using non-toxic liquid adhesive (Testors). Care was taken to use a minimal amount of adhesive, since excessive adhesive may reduce spider mobility. Exclosures were left undisturbed for 30 d.

After the 30-d selection period, we resampled each plot and collected all individuals therein. Plots were sampled for four days by sifting through leaf litter and turning over each individual leaf. Individuals that were not recovered during our four-day search period were assumed to be dead. Importantly, all of the spiders recovered from within our plots were marked, thus indicating a low probability of emigration or immigration across our exclosure barriers.

Statistical analyses.—To test for differences in escape speed (cm/s) in response to our three aversive stimuli, we used one-way ANOVAs with post-hoc Tukey tests. To assess the relationship between body size and performance, we used

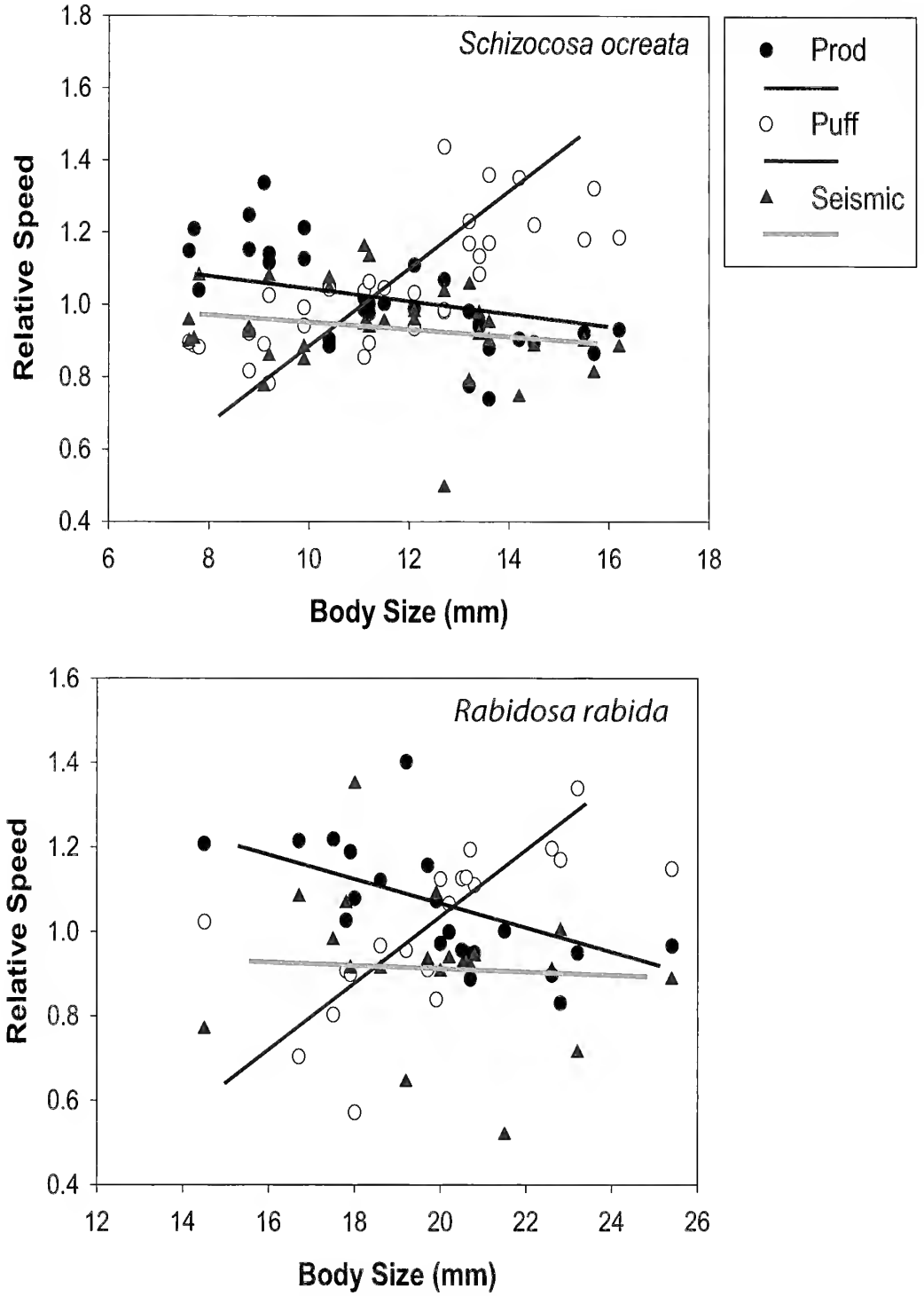

Figure 2.—Relationship between individuals' body size (abdomen + cephalothorax length) and relative speed exhibited (speed displayed with a given stimulus/average speed displayed across stimuli) in response to various stimuli for *Schizocosa ocreata* (top) and *Rabidosa rabida* (bottom).

repeated measure ANOVAs and analyzed each species independently. We included stimulus type, body size (cephalothorax length + abdomen length) and their interaction term in our models, and used 'relative speed' as our response variable. Relative speed was calculated by dividing the flight speed (cm/s) obtained by an individual in response to each stimulus by the mean flight speed exhibited across all three stimuli.

To assess the effects of body size on survival in *S. ocreata* and *R. rabida*, we calculated selection gradients for eaeh species independently by transforming trait values to mean zero and unit variance, and survival scores (1,0) were transformed into relative fitness (individuals' fitness/average fitness of their cohort). Selection gradients (i.e., the change in expected fitness per standard deviation of trait value) were ealculated for each exclosure, independently using multiple

Table 2.—Summary of our combined models predicting spiders' persistence in our avian exclosure, mark-recapture experiments. The effects of body size on persistence differed significantly among treatments (predators present vs. absent).

Schizocosa ocreata			
Source	*df*	*F*	*P*
Combined Model	3	10.35	0.002
Intercept	1	0.11	0.74
Treatment	1	5.09	0.02
Body Size	1	0.19	0.66
Treatment*Body Size	1	6.55	0.01
Rabidosa rabida			
Source	*df*	*F*	*P*
Combined Model	3	11.51	<0.001
Intercept	1	0.51	0.64
Treatment	1	5.85	0.03
Body Size	1	0.43	0.45
Treatment*Body Size	1	6.36	0.01

linear regression (Lande & Arnold 1983; Calsbeek & Irschick 2007). To test whether selection gradients differed among exclosures (avian predators present vs. absent treatment) we used a combined multiple logistic regression model with treatment, body size, and the interaction term treatment × body size as predictor variables. For all analyses we used logistic regression for our significance tests (after Janzen & Stern 1998) and multiple linear regression to estimate selection gradients (after Calsbeek & Irschick 2007). We did not include non-linear selection terms in our models owing to limited degrees of freedom, but visual inspection of the data indicated no non-linearity.

RESULTS

We detected significant differences in the escape speeds exhibited in response to our three aversive stimuli for *S. ocreata* ($F_{2,93}$ = 4.21, P = 0.02), but not *R. rabida* ($F_{2,62}$ = 1.48, P = 0.16). In *S. ocreata*, individuals exhibited higher eseape speeds in response to rapid jets of air (mean speed = 39.95 cm/s, SE = 1.31) than to seismic cues (mean speed = 35.23 cm/s, SE = 1.11), and their responses to prodding (mean speed = 38.36 cm/s, SE = 1.12) were intermediate and indistinguishable from responses to the other stimuli (Fig. 1).

Our combined models predicting individuals' relative escape speeds were significant for both *S. ocreata* ($F_{5,93}$ = 3.86, P = 0.03) and *R. rabida* ($F_{5,62}$ = 7.29, P <0.001). We detected significant effects of stimulus type and the interaction term stimulus type × body size for both species (Table 1). From examination of the interaction plot between body size and stimulus type (Fig. 2), we found a strong positive relationship between body size and individuals' relative escape speed in response to puffs of air. In contrast, we observed negative trends in the relationship between relative escape performance and body size with both the prod and seismic cues (Fig. 2). That is, larger individuals responded more slowly to prod and seismic cues, but responded faster after experiencing a puff of air.

Our combined models predicting persistence in our mark-recapture avian exclosure experiment were significant for both species: *S. ocreata* ($F_{3,67}$ = 10.35, P = 0.002) and *R. rabida* ($F_{3,37}$ = 11.51, P <0.001). We detected a significant effect of treatment (avian predators present vs. absent) and a significant interaction between individuals' body size and treatment for both species (summarized in Table 2). From examination of body size distribution of the "surviving" versus "dead" spiders in each treatment (Fig. 3), we found that surviving individuals tended to be larger in our predator exclusion treatments. However, this trend disappeared (*S. ocreata*) or was reversed (*R. rabida*) in our predator inclusion treatment.

DISCUSSION

Documenting how animals employ their performance capacities can help to elueidate the selective pressures driving performance. Specifically, data from a wide range of taxa suggest that animals express their highest speeds in the contexts/situations most vital in determining their direct fitness and survival (Domenici & Blake 1997; Husak & Fox 2006; Husak et al. 2008; Irschick & Garland 2001; Pruitt & Husak 2010). Our data here demonstrate that even within a single context (anti-predator behavior) animals may differ in their responsiveness. Specifically, we demonstrate that, in *R. rabida* and *S. ocreata*, larger individuals tend to exhibit higher running speeds in response to puffs of air, whereas smaller individuals of both species tended to exhibit greater burst speeds in response to mechanical stimuli (i.e., prodding). Concordantly, we found that in the absence of avian predators, larger individuals enjoy higher survivorship in the field. In contrast, when avian predators were not excluded, the advantage of large body size was lost or reversed. Our data add an additional point of note to standard investigations on animal performance and indicate experiments that use only a single cue type may overlook important condition- or trait-dependent variation in performance utilization.

Individuals of different body sizes tended to express their highest observed performances in response to different aversive stimuli, and we infer that this finding reflects differences in the selection pressures they experience. In both species, larger spiders were more likely to express their highest observed speeds in response to puffs of air. Given that birds are primarily visual predators, it is plausible that larger spiders are more likely to be spotted and attacked owing to greater conspicuousness, and thus large individuals express higher escape speeds in response to sudden jets of air. Certainly, behavioral data in at least one of our test species (*S. ocreata*) have demonstrated that individuals exhibit anti-predator behavior in response to bird songs (Lohrey et al. 2009), and a number of bird speeies have been observed feeding directly on *S. ocreata* (Lohrey 2007); thus, we argue that birds are likely some of the more important vertebrate predators of these spiders. In contrast, smaller spiders are perhaps less conspicuous to avian predators, but are no doubt more susceptible to attacks by other spiders and predaceous insects, which attack cursorially (i.e., similar to a prod). Hence, we argue that the greater responsiveness of smaller individuals to the prod stimulus may reflect a greater threat of arthropod predation.

Given that larger individuals tended to exhibit higher speeds in response to puffs of air, we predicted that larger individuals would suffer greater mortality as a result of avian predation.

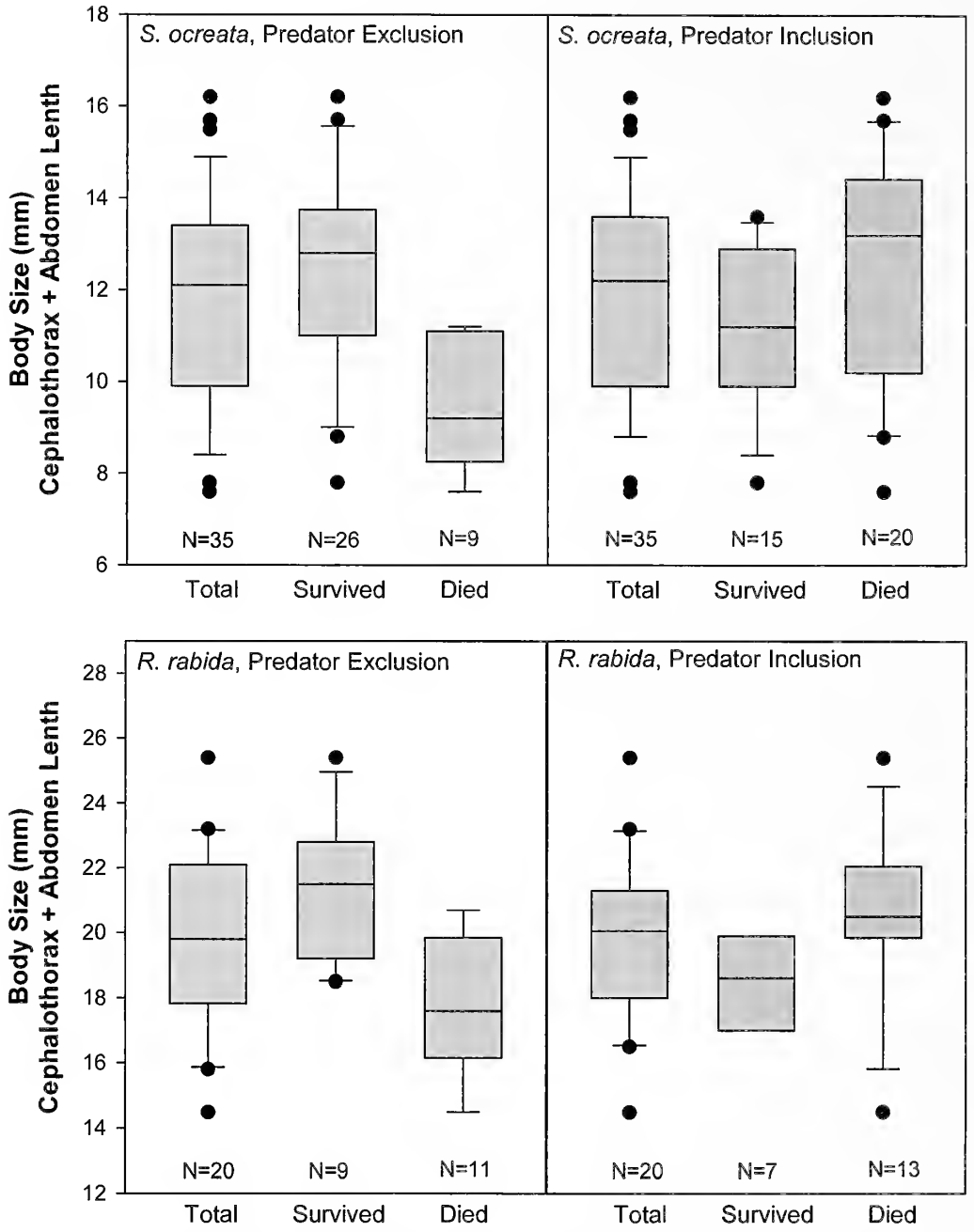

Figure 3.—Relationship between individuals' body length and survivorship in an avian predator exclusion experiment for the wolf spiders *Schizocosa ocreata* (top) and *Rabidosa rabida* (bottom). Larger body sizes were associated with higher survivorship in the absence of large vertebrate predators, and this advantage was lost with large vertebrate predators were present. Vertical shaded bars represent interquartile range and vertical lines represent the 90^{th} and 10^{th} percentiles.

Concordantly, we found that larger individuals of both species exhibited greater persistence in our avian exclusion treatments. However, this advantage was diminished or reversed in our avian inclusion treatment (Fig. 3). Thus, our results are consistent with the interpretation that avian predation is a major selective force on large spiders, and is one plausible driver of escape speed in these animals. These findings are at odds with the results of Wise and Chen (1999), whose data suggested that avian predators were not influential in *Schizocosa* population dynamics. In contrast, the data herein and those of Lohrey and collaborators (Lohrey 2007; Lohrey et al. 2009) do document significant effects/responses of *Schizicosa* to the threat of avian predation. Our parallel prediction that smaller individuals are more likely to fall victim to predation by other arthropods remains untested. However, it seems plausible that heterospecific arthropods and/or cannibalism by larger conspecifics could impose selection on small individuals. Consistent with this hypothesis, smaller individuals in our predator exclusion treatment were generally less likely to survive our 30-day selection period, perhaps as a result of size-dependent cannibalism or predation by other arthropods. Data from congeners of *S. ocreata* provide some evidence that invertebrate predators impose significant effects on individual survival (e.g., Punzo 1997).

However, we caution that all of these results must be interpreted as tentative, since our predator exclosure treatments were not replicated within each species.

Finally, our results bring to light an important methodological concern for the animal performance literature. Specifically, the vast majority of studies on animal performance assess individuals' behavior in only a single ecological context (typically an escape response) and use only a single aversive stimulus such as chasing the animal with a broom/brush (e.g., Prenter et al. 2012; Prenter et al. 2010; Pruitt 2010). A potential criticism of these studies is that they could limit our understanding of animal performance by assessing performance under a narrow range of conditions and thus lead to erroneous conclusions.

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A review and redescription of the cosmopolitan pseudoscorpion *Chelifer cancroides* (Pseudoscorpiones: Cheliferidae)

Mark S. Harvey: Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia; Research Associate: Division of Invertebrate Zoology, American Museum of Natural History, 79th Street at Central Park West, New York, New York 10024–5192, USA; Research Associate: Department of Entomology, California Academy of Sciences, Golden Gate Park, San Francisco, California 94103–3009 USA; Adjunct Professor: School of Animal Biology, University of Western Australia, Crawley, Western Australia 6009, Australia; Adjunct Professor: School of Natural Sciences, Edith Cowan University, Joondalup, Western Australia 6027, Australia. E-mail: mark.harvey@museum.wa.gov.au

Abstract. The taxonomy of the cheliferid pseudoscorpion genus *Chelifer* Geoffroy 1762 is reviewed with a single cosmopolitan species, *Chelifer cancroides* (Linnaeus 1758), with the subspecies *C. cancroides orientalis* Morikawa 1954 from Japan newly synonymised with *C. cancroides*. Adults and the final two nymphal stages (tritonymph and deutonymph) are redescribed based on numerous specimens from Europe, North America, Asia and Australasia. The large size variation evident in the samples is documented. The latero-ventral process of the tarsal claws characteristically found in adults (except leg I of the male) is lacking in nymphs, a pattern that is also confirmed in the genera *Lissochelifer* Chamberlin 1932, *Lophochernes* Simon 1878 and *Parachelifer* Chamberlin 1932.

Keywords: Taxonomy, morphology, variation, new synonymy

The two pseudoscorpions described and named by Linnaeus (1758) in the 10th edition of *Systema Naturae* were both included in the mite genus *Acarus* Linnaeus 1758. *Acarus cancroides* Linnaeus 1758 was recorded from Europe and *A. scorpioides* Linnaeus 1758 from America. Both species were transferred to *Phalangium* Linnaeus 1758 by Linnaeus (1767) and then to *Scorpio* Linnaeus 1758 by Fabricius (1775), but these genera are now used exclusively in the arachnid orders Opiliones and Scorpiones, respectively. In the meantime the genus *Chelifer* was established in an anonymous work by Geoffroy (1762) to accommodate *A. cancroides* and the mite *A. longicornis* Linnaeus 1758, and has been used as a valid genus ever since despite being proposed in a publication that was eventually ruled as an unavailable work due to the inconsistent use of binominal system (International Commission on Zoological Nomenclature 1954). The genus name was conserved in a ruling by the International Commission on Zoological Nomenclature (1989) with the type species confirmed as *A. cancroides*. Since Geoffroy's publication, 346 additional species-group names have been described within the genus *Chelifer* and all except *C. cancroides orientalis* Morikawa 1954 have been either removed to other genera or synonymized with *C. cancroides* (e.g., Chamberlin 1931b, 1932; Beier 1932a, 1932b). The genus is currently monotypic (Harvey 2013), although 14 other species originally described in *Chelifer* are regarded as *nomina dubia* (Harvey 2013).

Chelifer cancroides (Linnaeus 1758) is the most widely distributed pseudoscorpion species in the world and has been recorded from 58 countries in all major biogeographic regions (summarized by Harvey 2013). Published records indicate that it occurs in a variety of habitats, but is most frequently found in human dwellings, associated buildings and bird's nests (e.g., Artault de Vevey 1901; Ewing 1911; Levi 1948; Beier 1963; Turienzo et al. 2010).

Chelifer cancroides is also one of the best known pseudoscorpions and has been used for a variety of studies including growth, feeding (Vachon 1932, 1933, 1934a), respiration (Sláma 1995) and oogenesis and genital morphology (Vachon 1934b, 1936; Badian & Ogorzalek 1982; Badian 1987; Jędrzejowska et al. 2013).

Despite its widespread distribution and the abundance of preserved specimens in some museum collections, there are relatively few published illustrations of *C. cancroides* and there is no comprehensive modern description. To facilitate the recognition of this species, *C. cancroides* is redescribed based on a variety of specimens collected from four continents.

METHODS

This study is based upon the examination of specimens that are lodged in the American Museum of Natural History, New York (AMNH); Australian National Insect Collection, Canberra (ANIC), California Academy of Sciences, San Francisco (CAS); Florida State Collection of Arthropods, Gainesville (FSCA); Naturhistoriska Riksmuseet, Stockholm (NHRS); Queensland Museum, Brisbane (QM); Queen Victoria Museum and Art Gallery, Launceston (QVMAG); Tasmanian Museum and Art Gallery, Hobart (TMAG) and Western Australian Museum, Perth (WAM). In addition to the specimens of *C. cancroides* examined for this study (Appendix 1), specimens of other cheliferid genera were also examined to determine differences in tarsal claw morphology between adults and nymphs (see Appendix 1).

Many specimens used in this study had been previously prepared as permanent slide mounts in Canada Balsam by other researchers including J.C. Chamberlin, C.C. Hoff and W.B. Muchmore. The methods they used to prepare the slides include the removal of soft body tissue through the immersion of the specimen in potassium hydroxide (KOH) and dismemberment of the specimen to facilitate examination of important morphological features as documented by Chamberlin (1931a) and Hoff (1949). Additional specimens examined were also studied using temporary slide mounts prepared

by immersion of the specimen in lactic acid at room temperature for several hours to days and mounting them on microscope slides with 10 or 12 mm coverslips supported by small sections of 0.25, 0.35 or 0.5 mm diameter nylon fishing line. After study the specimens were returned to 75% ethanol, with the dissected portions placed in 12 × 3 mm glass genitalia microvials (BioQuip Products, Inc.). Specimens were examined with a Leica MZ–16A dissecting microscope and an Olympus BH–2 or a Leica DM2500 compound microscope, the latter fitted with interference contrast, and illustrated with the aid of a drawing tube attached to the compound microscopes. Measurements were taken at the highest possible magnification using an ocular graticule. Terminology and mensuration mostly follow Chamberlin (1931a), with the exception of the nomenclature of the pedipalps, legs and with some minor modifications to the terminology of the trichobothria (Harvey 1992), chelicera (Judson 2007) and faces of the appendages (Harvey et al. 2012). Terminology for the male genitalia are taken from Vachon (1938b) and Legg (1975).

The length and width of the pedipalpal femur and chela were measured for every specimen, even though occasionally one or more measurements were not possible due to missing, damaged or poorly aligned appendages. Some specimens, including all of the Asian specimens (which included the smallest specimens; see "Variation and the identity of *Chelifer cancroides orientalis*"), and the six largest and the six smallest specimens of each sex (based on chela length) of the larger group were measured in detail to prepare the species description and capture the greatest range of variation. Additional features such as the number of setae on the tergites and the posterior margin of the carapace were also recorded for these specimens. Means and standard deviations for the pedipalpal femur and chela measurements listed above were calculated using the AVERAGE and STDEVA functions in Excel (Microsoft Office Professional 2010). The same program was used to prepare Figs. 39–44.

SYSTEMATICS

Family Cheliferidae Risso 1827
Chelifer Geoffroy 1762

Chelifer Geoffroy 1762:617–618.

Obisium Illiger 1798:501 (synonymised by Westwood 1836:10) (see Judson 2012 for the nomenclatural history of this genus).

Type species.—*Chelifer: Acarus cancroides* Linnaeus 1758, by subsequent designation of Latreille, 1810:484.

Obisium: Acarus cancroides Linnaeus 1758, by subsequent designation of Westwood, 1836:10.

Diagnosis and description.—See below under *C. cancroides*.

Remarks.—The genus-group name *Chelifer* was first proposed by Geoffroy (1762) in an anonymous publication that was not strictly binominal. The publication was placed on the International Commission of Zoological Nomenclature's Official Index of Rejected and Invalid Works in Zoology in 1954 (Opinion 228). After receiving a submission to validate the name *Chelifer* (Harvey 1987), the name was conserved by the Commission in Opinion 1542 (International Commission on Zoological Nomenclature 1989). Geoffroy's (1762) publication was later placed on the Official List of Works Approved as Available for Zoological Nomenclature in Opinion 1754 (International Commission on Zoological Nomenclature 1994). Geoffroy included two species in *Chelifer*, *Acarus cancroides* Linnaeus 1758 and *A. longicornis* Linnaeus 1758, and the Commission recognised *Acarus cancroides* as the type species by subsequent designation of Latreille (1810).

Chelifer is the type genus of Cheliferidae and all coordinate family-group names (Cheliferoidea, Cheliferinae and Cheliferini). The tribe Cheliferini is characterized by the presence in the male of a lateral rod in which the anterior margin is deeply invaginated and usually contains a sclerotic rod-like process (Fig. 32); coxal sacs of the male, when present, lack a clearly differentiated medial atrium (Fig. 12) and the median cribriform plates of the female are distinctly paired (Fig. 34) (e.g., Beier 1932a; Chamberlin 1932; Hoff 1956). In contrast, the other cheliferine tribe, Dactylocheliferini, has uninvaginated lateral rods and lacks a sclerotic rod; the coxal sacs, when present, usually have a differentiated atrium; and the median cribriform plates are unpaired (e.g., Beier 1932a; Chamberlin 1932; Hoff 1956). This tribal classification represents one of the few within the Pseudoscorpiones characterised solely by genitalic features. There are, however, some cheliferid genera that have characteristics of both tribes, including *Mexichelifer* Muchmore 1973 that has the invaginated lateral rods and paired median cribriform plates characteristic of the Cheliferini and the distinct atrium of the coxal sac found in Dactylocheliferini (Muchmore 1973).

A distinctive feature of *C. cancroides* is the presence of a latero-ventral process on the tarsal claws of adults (Fig. 26) with the exception of leg I in males, which is modified to assist in mating (Figs. 24, 25). Several other cheliferid genera also possess such processes including the cheliferins *Cubachelifer* Hoff 1946, *Mesochelifer* Vachon 1940, *Parachelifer* Chamberlin 1932 and *Tyrannochelifer* Chamberlin 1932 (e.g., Beier 1932a; Chamberlin 1932; Hoff 1946, 1956; Mahnert 1981; Zaragoza 2009), and the dactylocheliferins *Lissochelifer* Chamberlin 1932, *Lophochernes* Simon 1878, *Mucrochelifer* Beier 1932 and *Stenochelifer* Beier 1967 (Chamberlin 1932; Beier 1932a, 1967a). This process was first reported to be lacking in the nymphal stages of *C. cancroides* by Hoff (1949), an observation that is confirmed in the present study (Figs. 27, 28). It is also absent in nymphs of two different species attributable to the genus *Lissochelifer* from northern Australia, and a species of *Lophochernes* from Vanuatu (Figs. 35, 36). Hoff (1964) reported simple claws in nymphs of three species of *Parachelifer*, in which the adults possessed claws with ventral processes. This observation is also here confirmed in a species of *Parachelifer* from Florida (Figs. 37, 38) and has been observed by J. Zaragoza (in litt., 21 June 2012) in nymphs of *Mesochelifer fradei* Vachon 1940. It is not known whether the nymphs of the other cheliferid genera listed above also lack such processes, but the pattern observed so far suggests that they are completely restricted to adults.

Most cheliferids have five setae on the cheliceral hand. All of the specimens of *C. cancroides* examined in this study have four cheliceral setae. This state is not, however, unique within the family. Species of the monotypic cheliferin genera *Kashimachelifer* Morikawa 1957 and *Mexichelifer*, and the monotypic dactylocheliferin genera *Pugnochelifer* Hoff 1964,

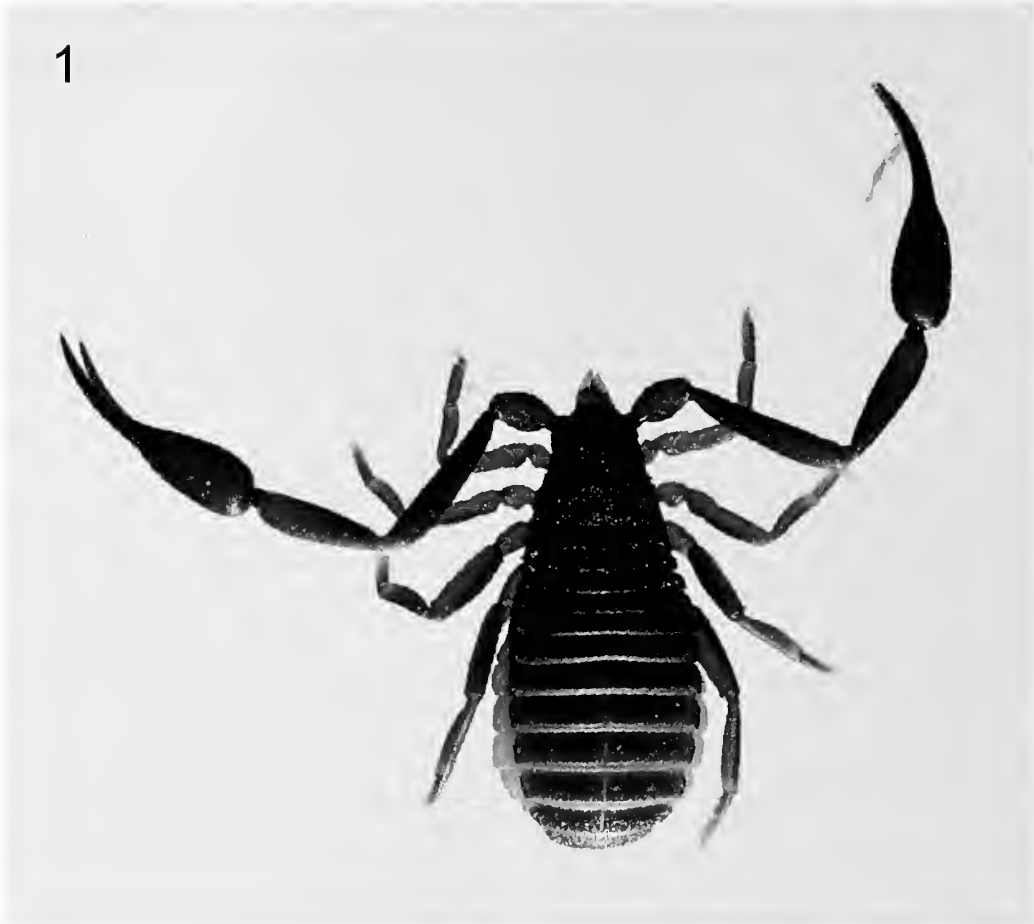


Figure 1.—*Chelifer cancroides* (Linnaeus), living male from near Prebbleton, New Zealand (image courtesy of B. Donovan).

Sinochelifer Beier 1967 and *Tetrachelifer* Beier 1967 also bear only four setae (Morikawa 1957; Hoff 1964; Beier 1967a; Muchmore 1973). Some species of *Rhacochelifer* Beier 1932 have only four cheliceral setae (Mahnert 1980; Callaini 1983; Dashdamirov & Schawaller 1995), but most have five setae. Some species of *Hysterochelifer* Chamberlin 1932 and *Paisochelifer* Hoff 1946 are reported to have either four or five cheliceral setae, and some specimens also rarely have a sixth seta located between setae *sbs* and *bs* (Hoff 1950, 1956). The missing seta in *Chelifer*, *Pugnochelifer*, *Rhacochelifer* and *Tetrachelifer* is *sbs*, which can be readily determined by comparison with other cheliferids with a full complement of five setae. The seta *ls* appears to be absent in *Kashimachelifer* based on illustrations of the chelicera by Morikawa (1957, 1960), but the missing seta of *Mexichelifer* and *Sinochelifer* has not yet been determined (Beier 1967a; Muchmore 1973).

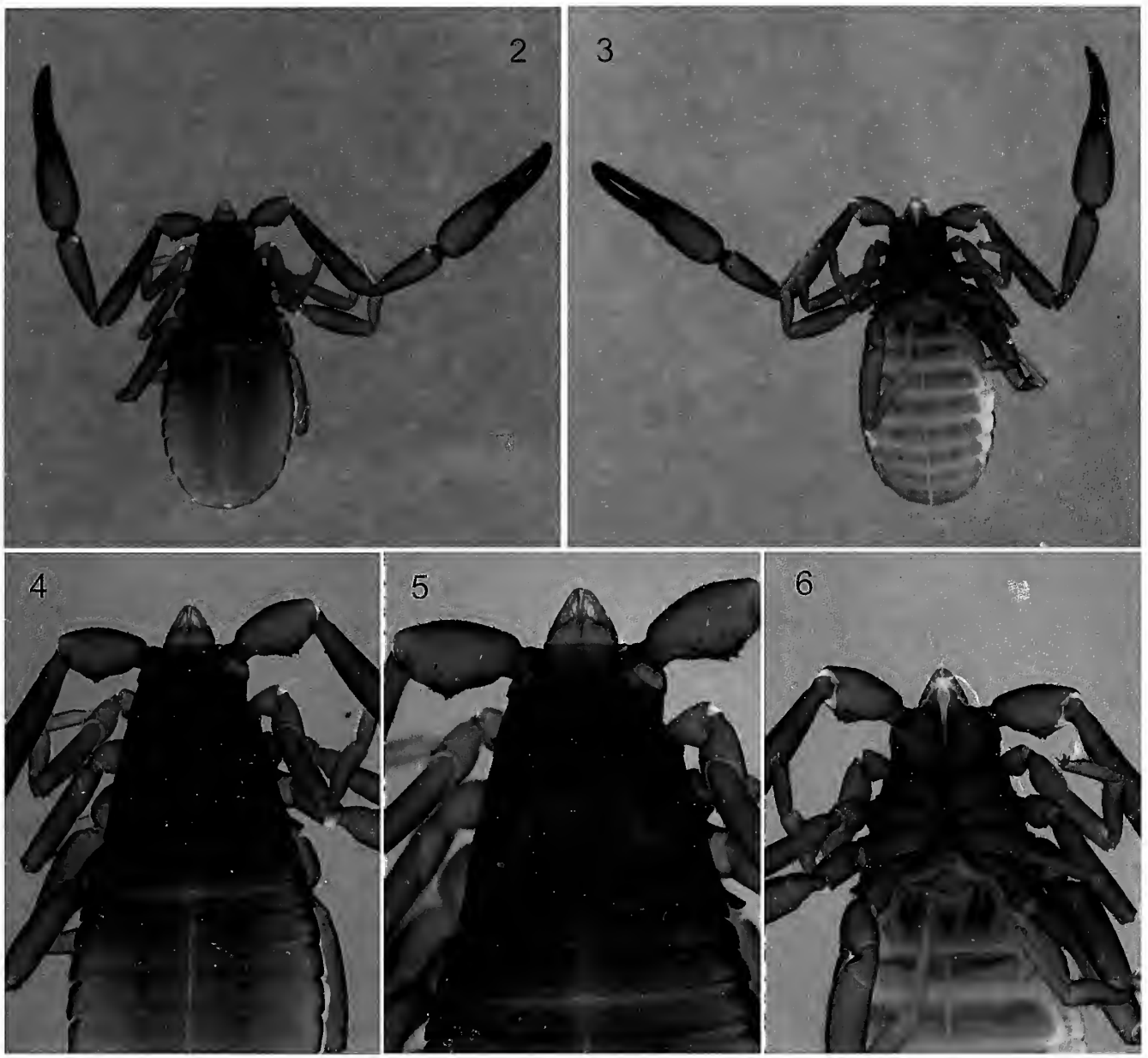
Chelifer cancroides (Linnaeus 1758)
(Figs. 1–34, 39–44)

Acarus cancroides Linnaeus 1758:616.
Chelifer europaeus de Geer 1778:355–357, plate 19, Figs. 14, 15.
Chelifer hermanni Leach 1817:49, plate 142, Fig. 3.
Chelifer sesamoides Audouin 1826:174–175, plate 8, Fig. 4.
Chelifer ixoides Hahn 1834:53, Fig. 140 (as *Chelifer ixioides* [sic]).
Chelifer granulatus C.L. Koch 1843:37, Fig. 777.
Chelifer grandimanus C.L. Koch 1843:38–39, Fig. 778.
Chelifer rhododactylus Menge 1855:32, plate 4, Fig. 6.
Chelifer serratus Stecker 1874:235–236.
Chelifer cancroides dentatus Ewing 1911:73.
Chelifer cancroides orientalis Morikawa 1954:73–75, Figs. 2a–e.
New synonymy.
For a full bibliographic treatment, see Harvey (2013).

Material examined. See Appendix 1.

Diagnosis.—Adults of the genus *Chelifer* and the sole included species *C. cancroides* can be distinguished from all other cheliferids by the following combination of morphological features, none of which, however, are unique to the genus: cheliceral hand with 4 setae, with seta *sbs* absent (Fig. 14); tarsal claws of all legs, with the exception of leg I of males, with latero-ventral process (Fig. 26); subterminal tarsal setae denticulate (Figs. 24, 26); carapace with large setose tubercles (Figs. 9, 10); carapace and tergites I–VII or VIII of male with distinct lateral keels (Figs. 7, 9); coxa IV of males strongly arcuate and with a large lateral process (Figs. 11, 12); coxa IV of males with coxal sac, which lacks a differentiated atrium (Fig. 12); male genitalia with rams horn organs and an anteriorly invaginated lateral rod forming a median depression, in which lies a sclerotic rod (Figs. 32, 33); and female genitalia with paired spermathecae and median cribriform plates (Fig. 34).

Description.—*Adults from near Pittsburg, Kansas, USA (AMNH Hoff slides S–4186.2, S–4186.4): Color*: sclerotized portions generally dark red-brown, males generally darker than females.

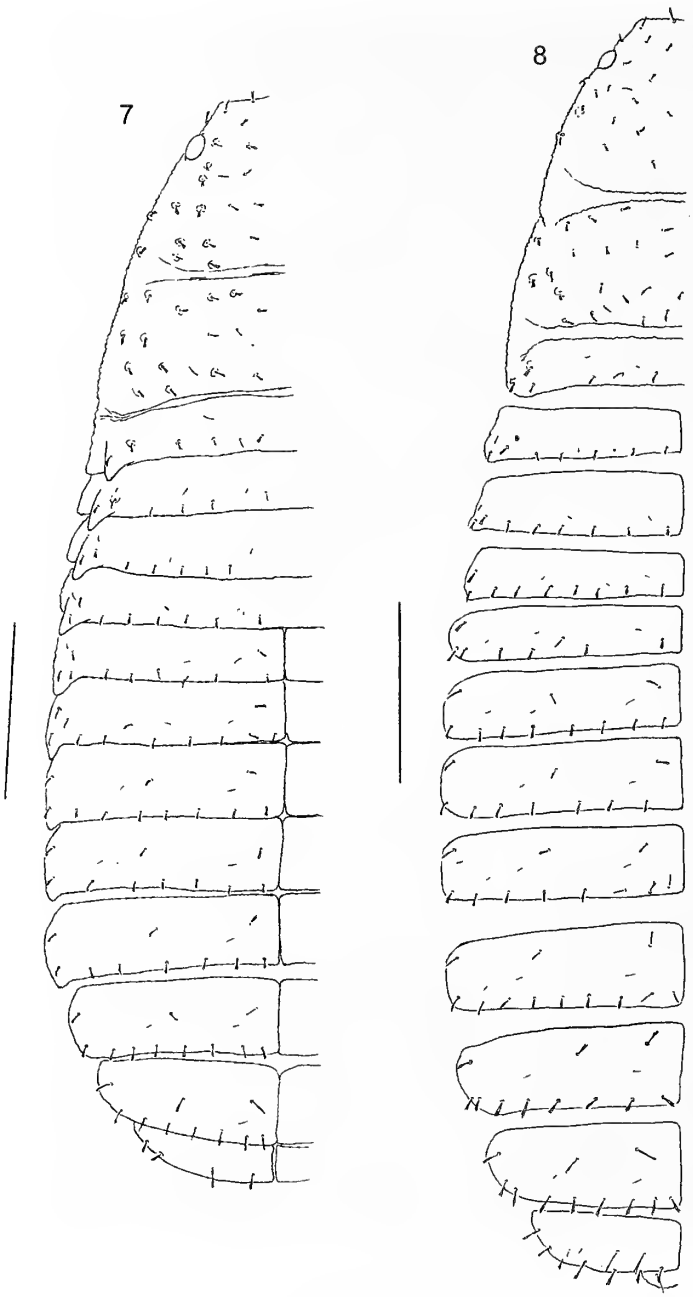


Figures 2–6.—*Chelifer cancroides* (Linnaeus), male from Tasmania, Australia (QVMAG 13_53185): 2. Dorsal; 3. Ventral; 4. Detail of carapace and abdomen, dorsal; 5. Carapace, dorsal; 6. Detail of coxae and abdomen, ventral.

Chelicera: With 4 setae on hand and 1 subdistal seta on movable finger (Fig. 14); seta *sbs* absent; seta *bs* dentate, remaining setae acuminate; seta *bs* shorter than others; with 2 dorsal lyrifissures and 1 ventral lyrifissure; galea of ♂ and ♀ with 5 terminal rami (Figs. 16, 17); rallum of 3 blades, the most distal blade with several serrations on leading edge, other blades smooth (Fig. 15); serrula exterior with 17 (♂, ♀) blades; lamina exterior present (Fig. 14).

Pedipalp (Fig. 18): Surfaces of trochanter, femur and patella coarsely granulate, chela including fingers mostly smooth, prolateral face very lightly granulate; patella with 2 small subbasal lyrifissures; trochanter 2.25 (♂), 1.97 (♀), femur 5.67 (♂), 5.18 (♀), patella 4.20 (♂), 3.87 (♀), chela (with pedicel) 4.61 (♂), 4.30 (♀), chela (without pedicel) 4.39 (♂), 4.09 (♀), hand (without pedicel) 2.07 (♂), 1.91 (♀) × longer than broad, movable finger 1.16 (♂), 1.20 (♀) × longer than hand. Fixed chelal finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 21): *eb* and *esb* situated basally, *ib* and *ist* sub-basally, *est* and *isb* sub-medially, *et* and *it* subdistally, *est* situated slightly distal to *isb*, and *et* slightly distal to *it*; *t* situated subdistally, *st* situated midway between *sb* and *t*, and *sb* situated much closer to *b* than to *st*; patch of microsetae present on external margin of fixed chelal finger near *et*. Venom apparatus present in both chelal fingers, venom ducts long, terminating in nodus ramosus midway between *et* and *est* in fixed finger and between *t* and *st* in movable finger (Fig. 21). Chelal teeth (Fig. 21) slightly retrorse, becoming rounded basally; fixed finger with ca. 49 (♂), 50 (♀) teeth; movable finger with ca. 52 (♂), 48 (♀) teeth; accessory teeth absent.

Carapace (Figs. 9, 10): 0.98 (♂), 1.00 (♀) × longer than broad; with 1 pair of rounded corneate eyes, which lack a



Figures 7, 8.—*Chelifer cancroides* (Linnaeus), carapace and abdomen, left side: 7. Male (AMNH S–4186.3); 8. Female (AMNH S–2707). Scale lines = 0.5 mm.

tapetum; with 91 (♂), 98 (♀) setae, arranged with 42 (♂), 45 (♀) (including 4 near anterior margin) in anterior zone, 37 (♂), 40 (♀) in median zone, and 12 (♂), 11 (♀) in posterior zone; postero-lateral corner of ♂ with triangular protuberance surmounted by 1 seta; with numerous lyrifissures; with 2 deep furrows, posterior furrow situated closer to posterior carapace margin than to anterior furrow; anterior furrow situated ca. 0.43 (♂), 0.55 (♀) mm from anterior margin, and posterior furrow situated ca. 0.15 (♂), 0.10 (♀) mm from posterior margin.

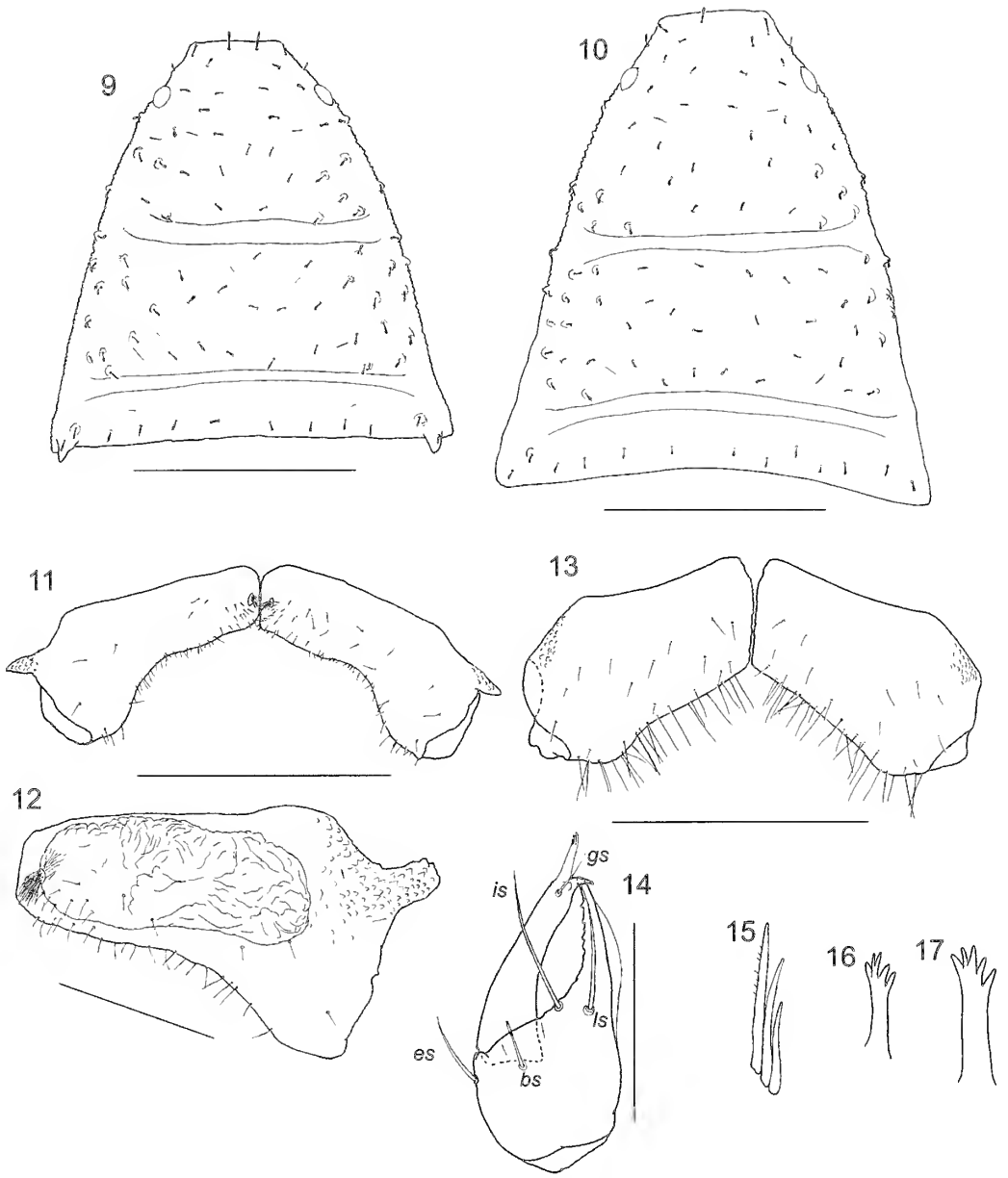
Coxal region: Maxillae and coxae slightly granulate; manducatory process rounded, with 2 apical acuminate setae, median seta much smaller than lateral seta, with 1 small sub-oral seta, and 19 (♂), 20 (♀) additional setae; median maxillary lyrifissure rounded and situated submedially; posterior maxillary lyrifissure rounded. Coxa IV of male (Figs. 11, 12) strongly arcuate and with large lateral processes; large coxal sac without atrium; coxal sac glandular setae long. Chaetotaxy of coxae I–IV: ♂, 11: 13: 43: ca. 70; ♀, 13: 11: 15: ca. 60.

Legs: Trochanter and patella of leg III and IV with dorsal setiferous granules; junction between femora and patellae I and II strongly oblique to long axis; junction between femora and patellae III and IV very angulate; femora III and IV much smaller than patellae III and IV; femur + patella of leg IV 3.17 (♂), 3.34 (♀) × longer than broad; tarsus IV with sub-distal tactile seta, TS ratio = 0.74 (♂), 0.78 (♀); subterminal tarsal setae dentate (Figs. 24, 26); claws of legs (except legs I of ♂) with latero-ventral process (Fig. 26); leg I of ♂ with prolateral claw curved and unmodified, and retrolateral claw slender with dorso-medial sharp process (Figs. 24, 25); tarsus I of ♂ not thickened and without dorsal process or spur (Fig. 24); arolium shorter than claws, not divided (Figs. 24, 26).

Abdomen: Tergites IV–XI of male and I–XI of female with median suture line fully dividing each tergite (Figs. 7, 8); tergites I–III without suture line (Fig. 7); sternites V–XI with medial suture line fully dividing each sternite. Tergal chaetotaxy: ♂, 11: 14: 16: 16: 19: 21: 20: 20: 19: 19: 14: 2; ♀, 15: 17: 17: 19: 20: 22: 20: 22: 20: 19: 14: 2; tergites IV–X biseriate, remainder uniseriate; all setae thickened and strongly dentate; tergites I–VIII of ♂ with lateral triangular keel surmounted with 1 or 2 seta (Fig. 7). Sternal chaetotaxy: ♂, ca. 100: (0) 20 [2+3] (0): (1) 6 (1): 15: 17: 16: 15: 14: 14: 12: 2; ♀, 20: (0) 11 (0): (1) 10 (1): 18: 17: 17: 18: 17: 18: 8: 2; uniseriate, except for sternites II and III, and the lateral discal seta on sternites IV–XI; most setae acicular, but setae on last two tergites becoming slightly clavate and denticulate; ♂ sternite II with arcuate posterior margin (Fig. 29) and with numerous setae, some bifurcate; ♂ sternite III enlarged with arcuate anterior margin and with scattered setae (Fig. 29), some bifurcate (Fig. 30); glandular setae on ♂ sternite III strongly bifurcate; ♀ sternite II with pair of median setae and numerous pairs of posterior setae (Fig. 31). Spiracles with helix. Anal plates (tergite XII and sternite XII) situated between tergite XI and sternite XI. Pleural membrane finely wrinkled-plicate; without any setae.

Genitalia: Male (Figs. 32, 33): lateral apodemes extending laterally; rams horn organs present; lateral rods medially joined and anteriorly invaginated forming a median depression with a sclerotic rod. Female (Fig. 34): with one pair of lateral cribriform plates and 2 pairs of median cribriform plates; with paired thin-walled spermathecae.

Variation: pedipalp: trochanter 1.82–2.07 (♂), 1.67–2.18 (♀), femur 4.78–5.85 (♂), 4.78–5.67 (♀), patella 3.42–4.20 (♂), 3.61–4.29 (♀), chela (with pedicel) 3.86–5.07 (♂), 3.56–5.10 (♀), chela (without pedicel) 3.88–4.41 (♂), 3.35–4.85 (♀), hand (without pedicel) 1.85–2.25 (♂), 1.66–2.31 (♀) × longer than broad, movable finger 0.89–1.20 (♂), 0.96–1.30 (♀) × longer than hand (without pedicel). Femur length mean = 1.201, standard deviation (SD) = 0.087 (♂), mean = 1.222, SD = 0.081 (♀); femur width mean = 0.224, SD = 0.018 (♂), mean = 0.236, SD = 0.017 (♀); chela (with pedicel) length mean = 1.752, SD = 0.139 (♂), mean = 1.795, SD = 0.116 (♀); chela width mean = 0.398, SD = 0.043 (♂), mean = 0.413, SD = 0.040 (♀).

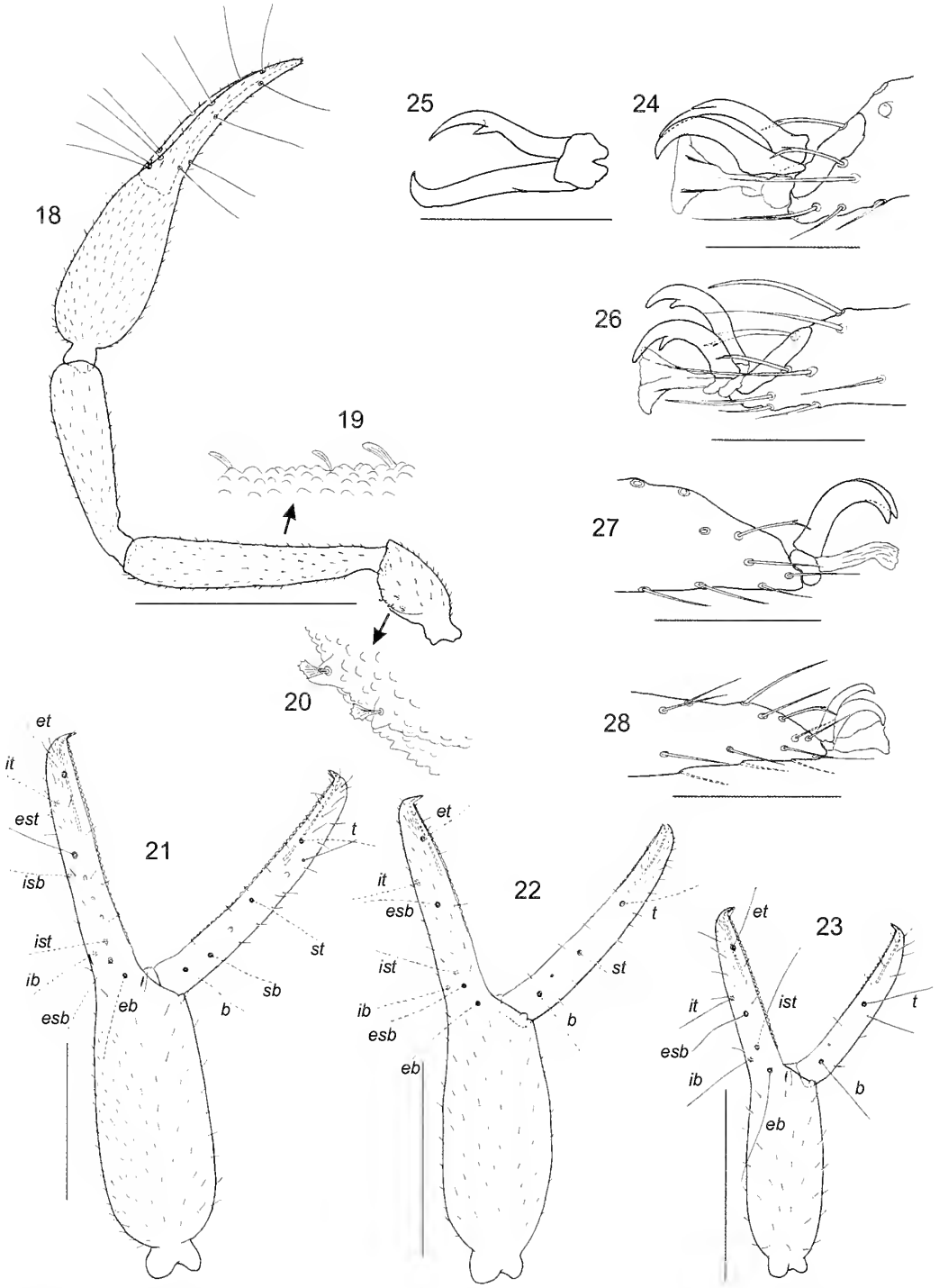


Figures 9–17.—*Chelifer cancroides* (Linnaeus): 9. Carapace, dorsal, male (AMNH S–4186.3); 10. Carapace, dorsal, female (CAS JC–1598.01002); 11. Coxae IV, ventral, male (AMNH S–2706); 12. Left coxa, IV, showing coxal sac, ventral, male (AMNH S–4186.3); 13. Coxae IV, ventral, female (CAS JC–1598.01002); 14. Left chelicera, dorsal, male (AMNH S–4080.2); 15. Rallum, male (AMNH S–4186.4); 15. Galea, male (AMNH S–3509); 16. Galea, female (AMNH S–4186.2). Scale lines = 0.1 mm (Figs. 15–17), 0.2 mm (Figs. 12, 14), 0.5 mm (Figs. 9–11, 13).

Carapace 0.91–1.04 (♂), 0.95–1.09 (♀) × longer than broad; posterior margin with 11–18 (♂), 10–14 (♀) setae; keels usually very prominent, but smaller specimens with keels barely noticeable. Legs: femur + patella IV 2.72–3.84 (♂), 3.07–3.75 (♀) × longer than broad. Abdomen: tergites II and III of male sometimes divided; tergal chaetotaxy: ♂, 12–16: 14–17: 14–20: 15–21: 16–26: 18–26: 19–26: 18–25: 18–23: 17–24: 12–20: 2; ♀, 12–17: 14–18: 14–19: 14–20: 16–22: 17–23: 16–24: 17–22: 17–22: 16–21: 12–16: 2; ♂ sternite III with glandular setae ranging from [3 + 1] to [4 + 5].

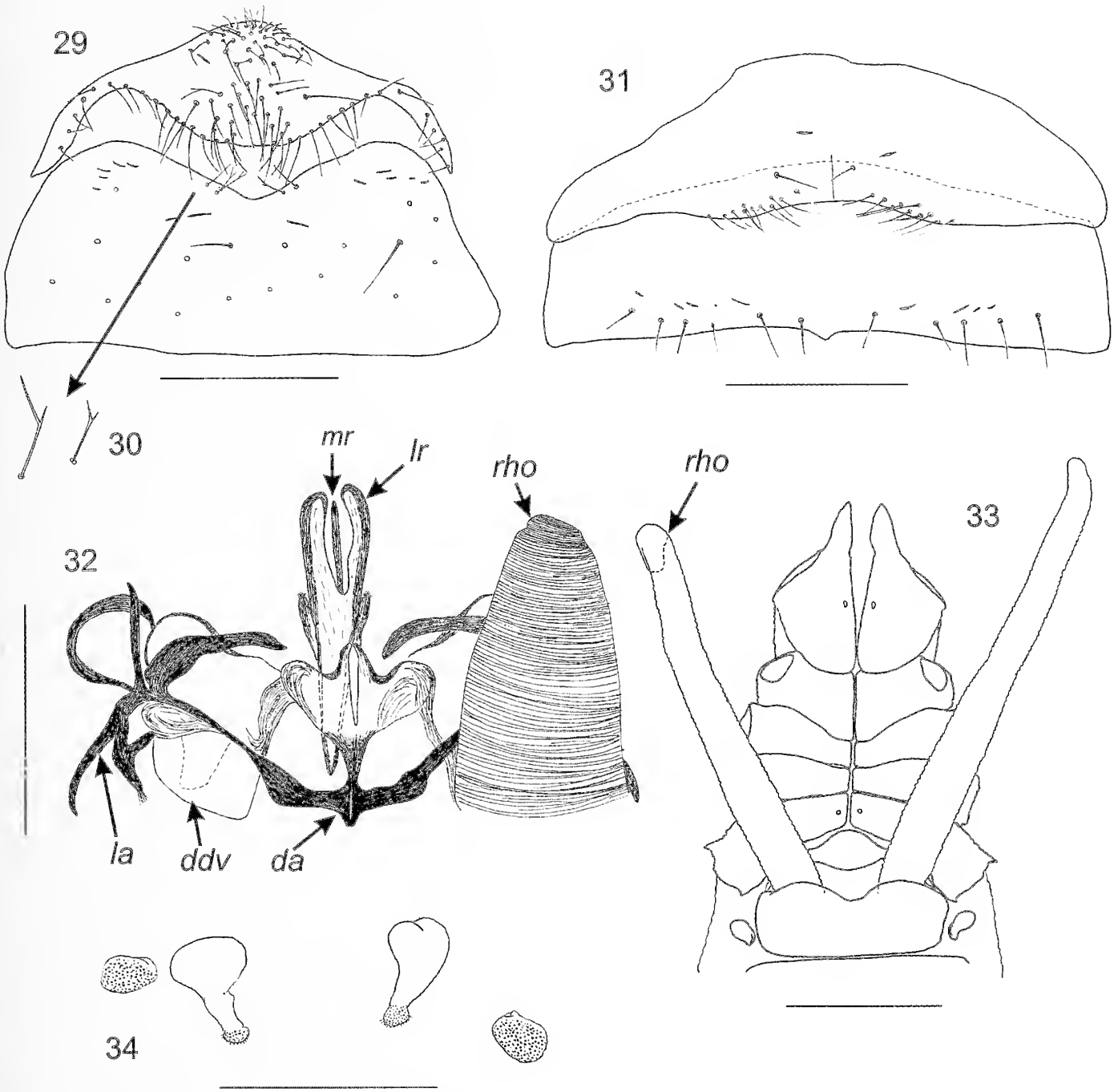
Dimensions: Male from near Pittsburg, Kansas (AMNH, Hoff slide S–4186.4) followed by all other males (where applicable): Body length 2.78 (2.38–3.50). Pedipalps: trochanter 0.505/0.225 (0.405–0.615/0.21–0.315), femur 1.105/0.195 (0.935–1.355/0.185–0.265), patella 0.925/0.22 (0.79–1.15/0.21–0.285), chela (with pedicel) 1.590/0.345 (1.29–2.01/0.30–0.48), chela (without pedicel) 1.515 (1.24–1.875), hand (without pedicel) length 0.715 (0.635–0.895), movable finger length 0.830 (0.60–1.04). Chelicera 0.270/0.130, movable finger length 0.165. Carapace 0.895/0.910 (0.81–1.09/0.85–1.14); eye diameter 0.060. Leg I: femur 0.305/0.175, patella 0.450/0.140, tibia 0.44/0.10, tarsus 0.39/0.085. Leg IV: femur + patella 0.84/0.265 (0.68–1.04/0.185–0.34), tibia 0.665/0.130, tarsus 0.480/0.085, TS = 0.355.

Female from near Pittsburg, Kansas (AMNH, Hoff slide S–4186.2) followed by all other females (where applicable): Body length 3.63 (2.18–3.71). Pedipalps: trochanter 0.570/0.290 (0.445–0.64/0.215–0.335), femur 1.270/0.245 (1.005–1.42/



Figures 18–28.—*Chelifer cancroides* (Linnaeus): 18. Left pedipalp, dorsal, male (AMNH S–4186.3); 19. Left pedipalp, detail of prolateral faee of femur, male (AMNH S–4186.3); 20. Left pedipalp, detail of retrolateral face of trochanter, male (AMNH S–4186.3); 21. Right chela, lateral, male (AMNH S–4186.3); 22. Left chela (reversed), lateral, tritonymph (CAS JC–257.01002); 23. Right chela, lateral, deutonymph (AMNH S–1995.3); 24. Right tarsus I, lateral, male (AMNH S–4186.3); 25. Claws of left tarsus I, ventral, male (AMNH S–4186.3); 26. Right tarsus I, lateral, female (AMNH S–4186.2); 27. Left tarsus I, lateral, tritonymph (CAS JC–257.01002); 28. Right tarsus I, lateral, deutonymph (AMNH S–1995.3). Scale lines = 0.1 mm (Figs. 19, 20, 24–28), 0.5 mm (Figs. 21–23), 1.0 mm (Fig. 18).

0.19–0.285), patella 1.045/0.270 (0.835–1.21/0.21–0.315), chela (with pedicel) 1.870/0.435 (1.405–2.08/0.305–0.54), chela (without pedicel) 1.780 (1.36–1.94), hand (without pedicel) length 0.830 (0.705–0.90), movable finger length 1.000 (0.68–1.125). Chelicera 0.315/0.165, movable finger length 0.210. Carapace 1.070/1.075 (0.85–1.17/0.79–1.20); eye diameter 0.065. Leg I: femur 0.360/0.190, patella 0.540/0.165, tibia 0.505/0.100, tarsus 0.495/0.075. Leg IV: femur + patella 1.020/0.305 (0.80–1.20/0.225–0.355), tibia 0.800/0.150, tarsus 0.580/0.105, TS = 0.450.

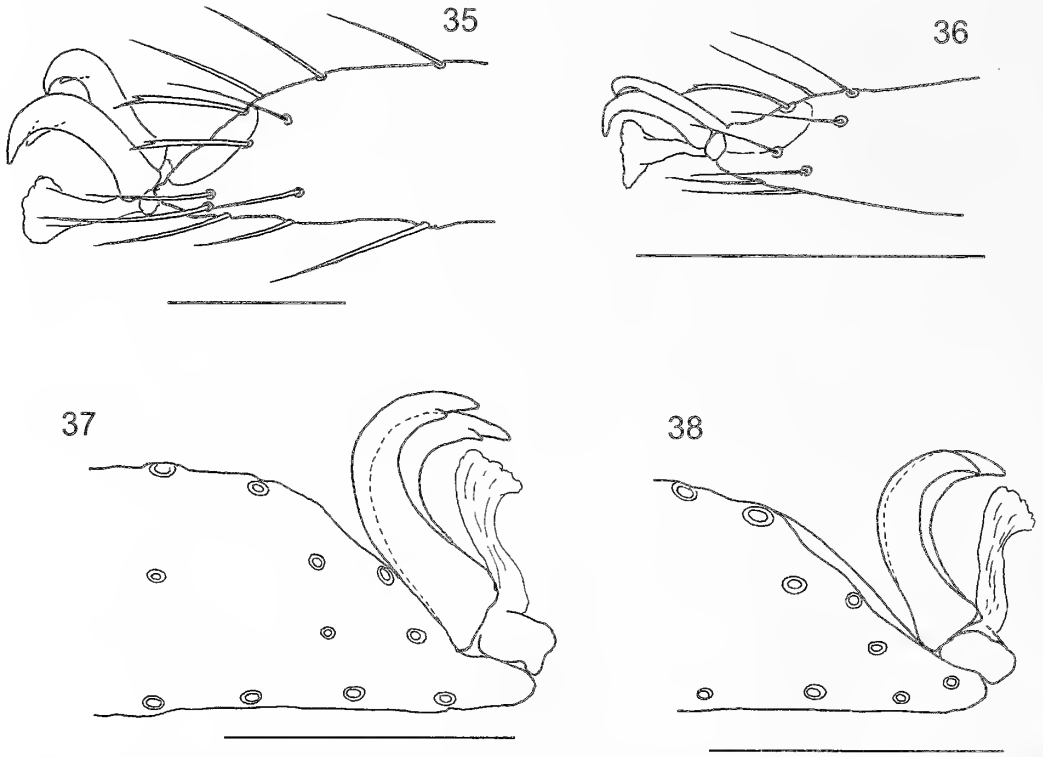


Figures 29–34.—*Chelifer cancroides* (Linnaeus): 29. Sternites III and IV, male (AMNH S–4186.4); 30. Detail of setae; 31. Sternites III and IV, female (AMNH S–4186.2); 32. Genitalia, ventral, male (AMNH S–2706); 33. Genital and coxal region showing distended rams horn organs, ventral, male (CAS JC–1523.01001); 34. Genital region, female (AMNH S–4186.2). Abbreviations: *da* = dorsal apodeme; *ddv* = dorsal diverticulum; *la* = lateral apodeme; *lr* = lateral rod; *mr* = median rod; *rho* = rams horn organs. Scale lines = 0.1 mm (Fig. 32), 0.2 mm (Figs. 29, 31, 34), 0.5 mm (Fig. 33).

Tritonymph from New York, USA. (CAS JC–257.01002): Color: sclerotized portions generally pale red-brown.

Chelicera: With 4 setae on hand and 1 subdistal seta on movable finger; seta *sbs* absent; seta *bs* dentate, remaining setae acuminate; seta *bs* shorter than others; galea broken, rami not visible; rallum with 3 blades, distal blade with spinules on anterior face, remaining blades smooth; serrula exterior with 14 blades.

Pedipalp: Trochanter 2.03, femur 4.98, patella 3.67, chela (with pedicel) 4.47, chela (without pedicel) 4.23, hand (without pedicel) 2.04 × longer than broad, movable finger 1.09 × longer than hand (without pedicel). Fixed chelal finger with 7 trichobothria, movable chelal finger with 3 trichobothria (Fig. 22): *eb*, *esb*, *ib* and *ist* situated sub-basally, *esb* situated closer to *et* than to *esb*, *et* situated closer to the end of the finger than to *it*, *t* situated subdistally, and *st* situated midway



Figures 35–38.—Tarsal claws showing lack of ventrolateral process in nymphs: 35, 36. *Lophochernes* sp. from Vanuatu (WAM T118590): 35. Left tarsus I, lateral, female; 36. Left tarsus I, lateral, tritonymph. 37, 38: *Parachelifer* sp. from Florida (CAS JC–209.02001–2): 37. Tarsus IV, lateral, male; 38. Left tarsus I, lateral, tritonymph. Scale lines = 0.05 mm (Fig. 35), 0.1 mm (Figs. 36–38).

between *b* and *t*; patch of microsetae present on external margin of fixed chelal finger near *et*. Venom apparatus present in both chelal fingers, venom ducts long, terminating in nodus ramosus midway between *et* and *est* in fixed finger and slightly distal to *t* in movable finger. Fixed finger with 36 teeth; movable finger with 40 teeth.

Carapace: 1.04 × longer than broad; with 1 pair of rounded corneate eyes; with 50 setae, arranged with 24 (including 4 near anterior margin) in anterior zone, 18 in median zone, and 8 in posterior zone; with 2 deep furrows, posterior furrow situated closer to posterior carapace margin than to anterior furrow.

Coxal region: Chaetotaxy of coxae I–IV: 4: 4: 6: 8.

Legs: Femur + patella of leg IV 2.74 × longer than broad; tarsus IV with sub-distal tactile seta, TS ratio = 0.72; subterminal tarsal setae dentate; claws of legs without latero-ventral process.

Abdomen: Tergal chaetotaxy: 10: 11: 11: 12: 11: 14: 13: 14: 12: 10: 4: 2; tergites without lateral keels. Sternal chaetotaxy: 2: (0) 6 (0): (1) 7 (1): 9: 10: 10: 9: 8: 6: 2: 2.

Dimensions: Body length 1.89. Pedipalps: trochanter 0.408/0.201, femur 0.861/0.173, patella 0.704/0.192, chela (with pedicel) 1.261/0.282, chela (without pedicel) 1.192, hand (without pedicel) length 0.575, movable finger length 0.625. Carapace 0.806/0.773. Leg I: femur 0.237/0.154, patella 0.352/0.141, tibia 0.326/0.099, tarsus 0.326/0.086. Leg IV: femur + patella 0.718/0.262, tibia 0.519/0.140, tarsus 0.384/0.090, TS = 0.275.

Deutonymph from Jensen, Utah, USA. (AMNH Hoff slide S–1995.3): Color: sclerotized portions generally pale red-brown.

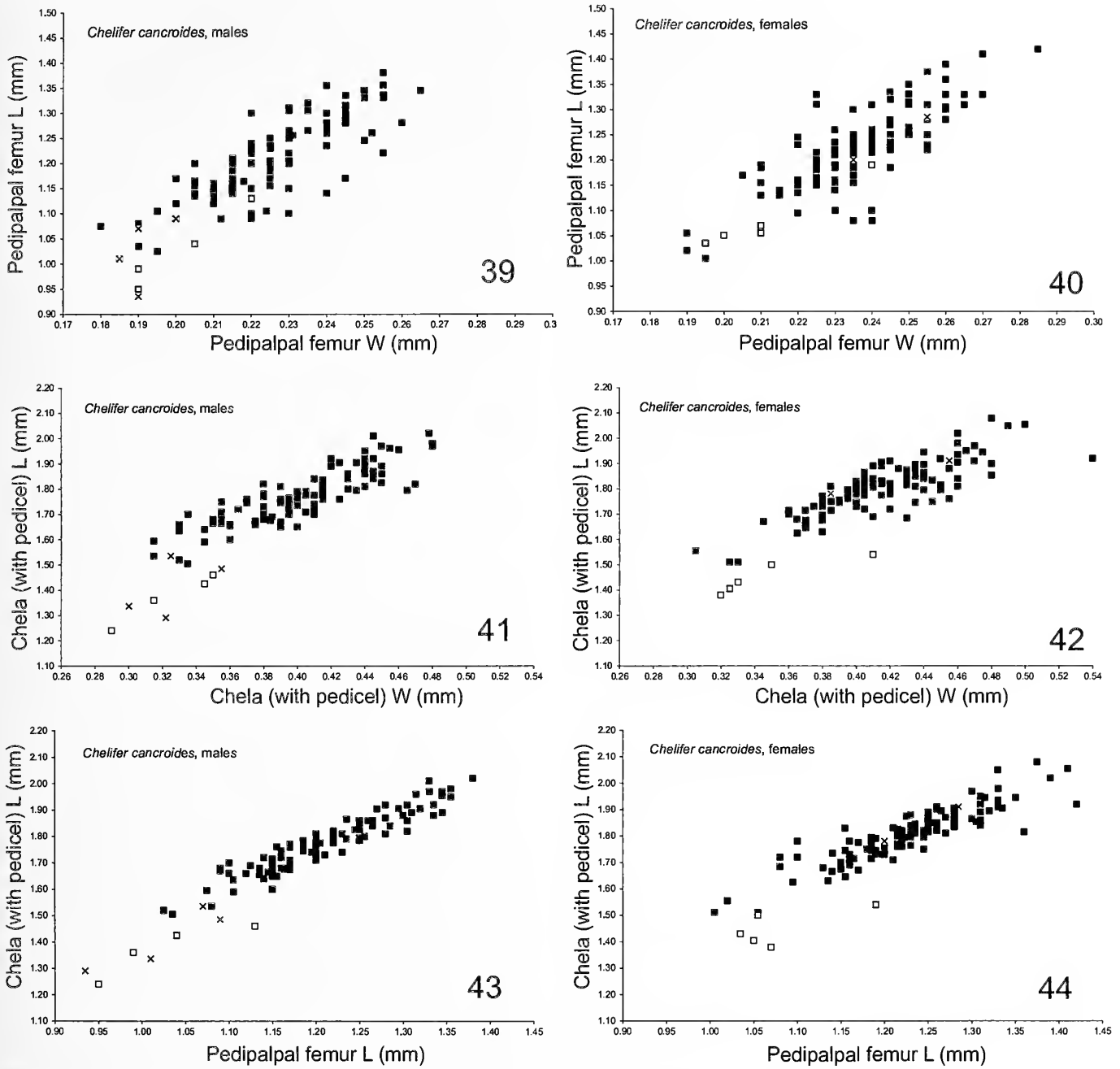
Chelicera: With 4 setae on hand and 1 subdistal seta on movable finger; seta *sbs* absent; seta *bs* dentate, remaining setae acuminate; seta *bs* shorter than others; galea with 4 distal rami; rallum with 3 blades, distal blade with spinules on anterior face, remaining blades smooth; serrula exterior with 13 blades.

Pedipalp: Trochanter 1.75, femur 4.83, patella 2.46, chela (with pedicel) 4.61, chela (without pedicel) 4.43, hand (without pedicel) 2.17 × longer than broad, movable finger 1.08 × longer than hand (without pedicel). Fixed chelal finger with 6 trichobothria, movable chelal finger with 2 trichobothria (Fig. 23): *eb* situated basally, *ib* and *ist* subbasally, *esb* and *it* submedially, *et* subdistally, *it* situated closer to *est* than to *et*; *b* situated basally and *t* situated subdistally; patch of microsetae present on external margin of fixed chelal finger near *et*. Venom apparatus present in both chelal fingers, venom ducts long, terminating in nodus ramosus midway between *et* and *it* in fixed finger and distal to *t* in movable finger. Fixed finger with 37 teeth; movable finger with 38 teeth.

Carapace: 0.96 × longer than broad; with 1 pair of rounded corneate eyes; with 39 setae, arranged with 21 (including 4 near anterior margin) in anterior zone, 14 in median zone, and 4 in posterior zone; with 2 deep furrows, posterior furrow situated closer to posterior carapace margin than to anterior furrow.

Coxal region: Chaetotaxy of coxae I–IV: 4: 5: 4: 4.

Legs: Femur + patella of leg IV 2.87 × longer than broad; tarsus IV with sub-distal tactile seta, TS ratio = 0.68; subterminal tarsal setae dentate; claws of legs without latero-ventral process.



Figures 39–44.—Size variation in adult specimens of *Chelifer cancroides* (Linnaeus): 39, 40. Pedipalpal femur length versus width; 41, 42. Pedipalpal chela (with pedicel) length versus width; 43, 44. Pedipalpal chela length (with pedicel) versus femur length. Asian specimens = open symbols; x = unknown locality; others = closed symbols. Measurements taken from Morikawa (1954) for specimens of *C. cancroides orientalis* (triangles).

Abdomen: Tergal chaetotaxy: 6: 6: 6: 6: 6: 7: 6: 7: 6: 6: 6: 2; tergites without lateral keels. Sternal chaetotaxy: 0: (0) 4 (0): (1) 4 (1): 6: 6: 6: 7: 6: 6: 5: 2.

Dimensions: Body length 2.07. Pedipalps: trochanter 0.285/0.163, femur 0.608/0.126, patella 0.505/0.205, chela (with pedicel) 0.945/0.205, chela (without pedicel) 0.945, hand (without pedicel) length 0.445, movable finger length 0.482. Carapace 0.635/0.661. Leg I: femur 0.176/0.108, patella 0.265/0.102, tibia 0.235/0.071, tarsus 0.260/0.065. Leg IV: femur + patella 0.513/0.179, tibia 0.371/0.100, tarsus 0.287/0.070, TS = 0.195.

Remarks.—*Type material of* Acarus cancroides*:* The original description of *Acarus cancroides* by Linnaeus (1758) was a few words of Latin: "A[carus]. antennis cheliformibus, abdomine ovato depresso" (i.e., an *Acarus* with chelate pedipalps, abdomen ovate, flat), which fails to provide any diagnostic features by which the species can be recognized. Apart from any specimens that Linnaeus (1758) may have had access to, the type series also consists of those specimens examined by the authors cited by Linnaeus (1758) listed under the name *A. cancroides* (International Commission on Zoological Nomenclature 1999, Article 72.4.1). These five

literature sources (Frisch 1730; Seba 1734; Linnaeus 1746; Rösel 1755; Clerck 1758) referring to a species he believed was *A. cancroides* are here discussed in chronological order.

Frisch (1730) contains two pages of text discussing 'Die Scorpion-Spinne', along with a very rudimentary drawing of a pseudoscorpion. The long pedipalps suggest that the species may indeed represent *C. cancroides*, but the provenance of the specimen is uncertain and the identification is somewhat tenuous. This citation was also listed by earlier Linnaeus volumes including Linnaeus (1746). The whereabouts of Frisch's collection – if it still exists – is unknown.

Seba (1734) presents a small illustration of an unidentifiable pseudoscorpion along with a Latin description and a Dutch figure caption that specifically mentions pseudoscorpions living in old walls and old wood. The habitat data suggests that he was referring to *C. cancroides*. Albertus Seba (1665–1736) resided in Amsterdam after moving there in 1696, and it is possible that the pseudoscorpion specimens were obtained locally. Much of Seba's early collections were purchased by Peter the Great in 1716 and transferred to Saint Petersburg, forming the basis for the Kunstkamera Museum (Engel 1937). Seba later developed a second collection, which may have included the pseudoscorpion illustrated in his 1734 volume. After his death the collection was auctioned to a variety of parties, with only a small proportion of it nowadays traceable (Boeseman 1970). The fate of any pseudoscorpions that might have been present in Seba's collection is unknown.

Linnaeus' *Fauna Svecica* (1746) was an early companion volume for *Systema Naturae*, the first edition of which was printed in 1735 (Linnaeus 1735). Pseudoscorpions were listed in his group "Acarus" as "Scorpio-araneus" (Linnaeus 1735). Under *Acarus cancroides*, Linnaeus (1758) refers to Species 1187 of *Fauna Svecica* which is "ACARUS pedibus primi paris cheliformibus" (= "a mite with first pair of legs cheliform"), and the Latin text accompanying the entry translates as "lives in houses that have been closed for a long time, not exposed to air, in chests and cellars" and "easily recognized from the rest by the crablike claws of the first legs and by the backwards gait, living on book lice" (H.D. Cameron, in litt. March 2012). The reference to book lice can be traced through his original Latin expression "pediculo ligni antiqui" which is the name applied to Species 1168 in Linnaeus (1746). This species was later described as *Termes pulsatorium* Linnaeus 1758, which is nowadays known as *Trogium pulsatorium* (Linnaeus 1758), a small psocopteran, which is a well-known minor pest in houses and other human facilities where they feed on fungal hyphae (e.g., Hall 1988; Smithers 1996; Turner & Ali 1996; New 2005).

Rösel (1755) provided nearly three pages of text describing the habits of a pseudoscorpion, which was depicted in superbly detailed color paintings (Tab. LXIV) of a male, female and a brood-sac. This pseudoscorpion has all the hallmarks of *C. cancroides*, including the general habitus and the proportions of the pedipalpal segments, and there is little doubt of its identity. The illustrations are included in a single plate entitled "Scorpio Minimus", but this Latin name does not appear in the text. After Rösel's death in 1759, the book series was updated and translated into Dutch by C.F.C. Kleeman. These volumes lack any mention of a publication date, but the entire four-volume series was published between 1764 and 1768. The precise publication date of the third volume, which contains the section on pseudoscorpions, is not known. This volume (Rösel von Rosenhof & Kleemann [1764–1768?]) reprinted the same figure (Tab. LXIV) that was originally printed by Rösel (1755). This figure was once again captioned "Scorpio Minimus" and like the earlier volume this name is not mentioned in the text. Although Rösel's original publication (Rösel 1755) predates the starting point of zoological nomenclature in 1758 (International Commission on Zoological Nomenclature 1999), the Dutch version (Rösel von Rosenhof & Kleemann [1764–1768?]) may be deemed an available work and the name "Scorpio Minimus" may be deemed to be an available species-group name. However, there is ample evidence elsewhere in the volume indicating that the figure headings were simply Latin translations of Dutch vernacular names, with "Scorpio Minimus" arising from the Dutch expression "Den Kleinsten Seorpioen" (Rösel von Rosenhof & Kleemann [1764–1768?], p. 317). Elsewhere in the same volume there are various different freshwater crustaceans depicted by Rösel (1755) and Rösel von Rosenhof & Kleemann ([1764–1768?]) under the name "Astacus Fluviatilis". It is clear that these names are simply Latin translations of vernacular names and should not be treated as available species-group names. Indeed, I can find no instance of Rösel von Rosenhof & Kleemann being used as the author of any animal species. The specimens that may have formed the basis for Rösel's plates cannot be traced, and it is assumed that Rösel's collections are lost, as are those of Kleemann (Horn et al. 1990).

Linnaeus (1758) listed Clerck (1758) as the fifth and final bibliographic citation for *Acarus cancroides*. Clerck (1758) published small paintings of a pseudoscorpion and a harvestman that he included in the Swedish and Latin text under an entry on "the so-called two-eyed spiders" (translation). Neither the pseudoscorpion nor the harvestmen were scientifically named (Holm 1978), unlike the spiders treated elsewhere in the volume which are deemed by the International Commission on Zoological Nomenclature to be the only animal names that predate Linnaeus (1758). The pseudoscorpion is not easily recognizable, but it may be the neobisiid *Neobisium carcinoides* (Hermann 1804), which occurs in Sweden (e.g., Tullgren 1899; Lohmander 1939; Harvey 2013). There are no pseudoscorpions in the Clerck collection lodged in the Swedish Museum of Natural History, Stockholm (Dr T. Kronestedt, in litt. 15 May 2012), and any specimens examined by Clerck are regarded as lost.

With the loss or unavailability of specimens used by Frisch (1730), Seba (1734), Rösel (1755) and Clerck (1758), some of which are unlikely to represent *C. cancroides*, the only possible type specimens are those in Linnaeus' own collection. The only surviving known speeimens apparently examined by Linnaeus are lodged in the collection of the Linnean Society of London, bearing the numbers 7004 and 7005. Images of these specimens are provided on the Linnean Soeiety's website (http://www.linnean-online.org/24329/ and http://linnean-online.org/24330/, accessed 27 February 2013). Two images are provided of specimen 7004, one of the body and the other of the right pedipalp and labels. The specimen lacks the left pedipalp, and is pinned with a standard entomological pin through the middle of the body, damaging or distorting much of the speeimen. Dr

M. Judson (in litt., 7 August 2013) kindly informed me that he has examined this specimen, which is a nymph. The two labels include an old hand-written label "cancroides" and a more recent printed or typed label "6 Chelifer cancroides (L.)". Specimen 7005 is also pinned through the middle of the body, and is accompanied only by a single printed or typed label "7 Chelifer cancroides (L.)". It lacks both pedipalps apart from the left trochanter and femur which are attached to the body. Dr Judson confirms this specimen is a female. Little morphological data can be obtained from the images of the two specimens to ascertain whether these specimens conform to modern diagnoses of the species.

One of these specimens was apparently examined by O.P.–Cambridge (1892), who compared it to specimens collected from human edifices in Britain and deemed them to be conspecific with *C. cancroides*. O.P.–Cambridge (1892, p. 221) clearly referred to a single specimen in the Linnean Society collection but it is impossible to ascertain to which specimen he was referring. O.P.-Cambridge (1892) regarded *Chelifer hermanni* Leach 1817 to be distinct from *C. cancroides*, citing its slightly smaller size, more slender pedipalps and different habitat, occurring under tree bark rather than associated with humans. *Chelifer hermanni* has since been treated as a synonym of *C. cancroides* (e.g., Kew 1911; Beier 1932a). These specimens are considered to represent the only surviving syntypes of *Acarus cancroides*, and further examination is required to obtain more accurate measurements and observations on their morphology.

The provenance of Linnaeus' specimens of *Acarus cancroides* is not certain, as he only stated "Habitat in Europae umbrosis suffocatis", which can be translated as "lives in dark constricted places of Europe" (H.D Cameron, in litt. March 2012). Linnaeus (1746) specifically mentioned these pseudoscorpions living in houses and feeding on psocopterans, and it is possible that his material was found inside buildings. Linnaeus resided for most of his life in Sweden but spent some time abroad, principally in Harderwijk, nowadays located within the Netherlands (Blunt 1971). *Chelifer cancroides* is commonly found in or near human dwellings (e.g., Beier 1963; Mahnert 1981; Zaragoza 2009), and it is not uncommon in southern Sweden where it has been frequently recorded from houses (e.g., Tullgren 1899, 1906; Lohmander 1939). However, the provenance of the specimens in the Linnean collection is uncertain, although Linnaeus clearly stated they came from a European location.

The identities of the other described species of *Chelifer* traditionally treated as synonyms of *C. cancroides* (listed above) are slightly doubtful, as in many cases the type material is lost or has not been examined by recent authorities on the group. Indeed, some may actually represent specimens of *Mesochelifer ressli* Mahnert 1981 rather than *C. cancroides* (see Mahnert 1981; Zaragoza 2009). Exceptions include the type material of *C. hermanni*, which was examined by O.P.–Cambridge (1892) and who, as mentioned above, suggested it represented a distinct species. Kew (1911) and later authors treated *C. hermanni* as a synonym of *C. cancroides*.

Chelifer europaeus is traditionally listed as a synonym of *C. cancroides* and appears to have been introduced as a synonym of the latter species (de Geer 1778). However, Welter-Schultes & Wieland (2012) have recently proposed that volumes 3–7 of *Mémoires pour servir à l'histoire des insectes* and the companion publication by Retzius (1783) be treated as available works, but that the majority of the polynominal names proposed in them be suppressed. *Chelifer europaeus* was not listed amongst the names to be suppressed.

Variation and the identity of Chelifer cancroides orientalis: Substantial size variation was found amongst the adults examined for this study, with the chela (with pedicel) ranging from 1.24–2.02 mm in males and 1.38–2.08 mm in females (Figs. 39–44); the largest specimens are approximately 50% larger than the smallest specimens. The smallest adults include all of the specimens from East Asia (China and specimens intercepted in Florida but originally from South Korea), three males intercepted on ships in Australia, five specimens from North America (one each from Canada, Indiana, New York, Oregon and Tennessee) and one with no collection data (Figs. 39–44). There are, however, no other apparent morphological features that unequivocally separate these specimens from the others. Males of *C. cancroides* typically have triangular keels on the postero-lateral corners of the carapace and the anterior tergites (Fig. 7). Two of the smallest male specimens examined have highly reduced carapaceal and tergal keels. Both specimens have the process on the claw of leg I in a more dorsal position but this may be an artifact of the slide preparation. Curiously, these small specimens (CAS JC–2234.01001 and ANIC) were both taken in quarantine samples. Other small male specimens taken at quarantine (other ANIC specimens) have normal shaped keels and tarsal claws, as do the other small males observed in this study.

The smaller specimens from Asia coincide with the description and dimensions provided by Morikawa (1954) for the specimens he used to describe *C. cancroides orientalis* (Figs. 39–44). This subspecies was based on specimens collected from Sapporo City, Hokkaidō Prefecture, and Mukaijima Island near Onomichi, Hiroshima Prefecture (Morikawa 1954), and was later recorded from Asahikawa, Hokkaidō Prefecture (Morikawa 1960). The specimens from Sapporo were taken from a honey-bee hive and those from Mukaijima Island were collected "at a cliff by the seashore" and "in the books" (Morikawa 1954, 1960). Unfortunately these specimens are not lodged in a public institution and have not been available for study (Dr H. Sato, pers. comm.). Morikawa (1954) suggested that the specimens differed from the nominate subspecies in several features including smaller body length, carapace broader than long, and the pedipalps very long in comparison with the body length. Distinguishing taxa using body length meristics is extremely inadvisable, as this measurement is easily affected by a variety of factors including whether the specimen was gravid, how well fed the specimen was, the mode of preservation and whether or not is has been treated for permanent slide-mounting. Concentrated preservatives will contract the abdominal membranes and artificially foreshorten the length of the specimen. Comparative measurements of complete structures such as the carapace or individual pedipalpal segments provide more reliable data to discriminate between species. Such measurements are, however, also subject to alteration if the structure is flattened or spread during the slide preparation process. The flattening is especially noticeable if the coverslip is not supported in some fashion such as by thin glass rods, glass beads or small strands

of fishing line. Such distortion of the carapace will produce slightly altered carapaceal ratios.

The few specimens examined for this study from East Asia, as well as the type specimens reported by Morikawa (1954), have slightly smaller and narrower pedipalps than most of the remaining specimens from Europe, Russia, North America and Australasia (Figs. 39–44). As there are no other detectable differences between the Asian and non-Asian populations, *C. cancroides orientalis* is here regarded as a junior synonym of *C. cancroides*. Further specimens of the Asian populations are required to better document the intraspecific variation observed in this study.

Postembryonic development.—Two of the three nymphal stages are represented in the material examined for this study, including several tritonymphs and deutonymphs. The number and position of the individual trichobothria of these nymphs and the adults (Figs. 22, 23) are identical to those presented by Vachon (1934c), who also documented the protonymph. Other morphological features are also similar including the number of setae on the posterior zone of the carapace, 4 in deutonymphs (n = 1), 8 in tritonymphs (n = 1), and 10–18 in adults; Vachon (1934c) reported 6–9 in deutonymphs, 8–12 in the tritonymph, and 11–16 in adults.

Distribution.—*Chelifer cancroides* is widely distributed and frequently recorded in Europe, Central Asia, North Africa and North America. There are, however, far fewer records in other regions of the world and documentation of specimens from the southern temperate regions are even scarcer. The few sub-Saharan African records include the Democratic Republic of Congo (Zaire) (e.g., Beier 1955; Beier 1959), Ethiopia (Simon 1904), Kenya (e.g., Beier 1944, 1967b; Mahnert 1988), Malawi (Beier 1944), South Africa (e.g., Ellingsen 1910; Beier 1929; Hewitt & Godfrey 1929) and Tanzania (Beier 1944). Millot (1948) recorded a species from Madagascar that was claimed to be very close to *C. cancroides*, which Legendre (1972) appears to have accepted as *C. cancroides*. The actual identity of this material should be checked to ascertain its status. Similarly, there are few records from Central and South America with specimens only reported from Argentina (Simon 1895; Ceballos & Ferradas 2008), Brazil (Ellingsen 1910), Chile (Simon 1887, 1895; Cekalovic K. 1976;), Cuba (Banks 1909; Franganillo Balboa 1936) and Mexico (Villegas-Guzmán & Pérez 2005).

The only East Asian records are from the Kamchatka Peninsula, Russia (Redikorzev 1935), Japan (Morikawa 1954, 1960), Mongolia (Krumpál & Kiefer 1982) and Vietnam (Beier 1951, 1967a), but among the specimens examined for this study were two females collected in 'Tsinan' (now Jinan), in Shandong Province, China, which represents the first record of *C. cancroides* from China. The specimens from Kamchatka identified by Redikorzev (1935) were examined and can be confirmed to be correctly identified as *C. cancroides*. Also, a pair collected in Florida in "straw scuffs from South Korea" represents the first record of *C. cancroides* from that country.

The only undisputed published records from the Australasian region are of several specimens collected in New Zealand, which were taken from "timber in insectary" at Owairaka (North Island) in 1945 and from a "nest of *Sturnus vulgaris*" at Kaikoura (South Island) in 1971 (Beier 1976). A newly identified female from New Plymouth (North Island) collected in 1924 from an ants' nest (CAS JC–544.01001) represents the earliest recorded specimen from the Australasian region. Recently, large numbers of *C. cancroides* were obtained from nests of the lucerne leafcutting bee *Megachile rotundata* (Fabricius 1787) in Christchurch (South Island) (B. Donovan, in litt. December 2012), some of whieh were examined for this study. The bee is native to Eurasia and was deliberately introduced into New Zealand in 1971 to assist in the pollination of lucerne (Howlett & Donovan 2010).

An early record of *C. cancroides* from Mount Lofty, near Adelaide, South Australia, by Beier (1930) was discounted by Harvey (1981) who regarded the identification of the single, apparently juvenile, specimen ("1 semiad. ♀") as doubtful. Harvey (1981) noted that *C. cancroides* was not included in subsequent synopses of the Australian fauna by Beier (1948b, 1966), and it is likely that the specimen from Mount Lofty was a misidentified member of the cheliferid genus *Protochelifer* Beier, which is common in southern and eastern Australia. The four male specimens of *C. cancroides* reported here from the Launceston region in northern Tasmania represent the first undisputed occurrence of *C. cancroides* from Australia. The specimens were collected inside a house in 1930, from straw in 1977 and without habitat data in 1985, indicating that the species is established in the area and has persisted for several decades.

Chelifer cancroides has been reported from a variety of states of the USA and provinces of Canada, and among the specimens examined for this study are several new state or provincial records: Connecticut, New Jersey, South Carolina, Tennessee and Washington in the USA, and New Brunswick and Saskatchewan in Canada.

The male specimen from near Baker, Oregon listed as *C. cancroides* by Benedict & Malcolm (1979) has been reexamined and found to belong to the genus *Parachelifer*. However, it was not possible to identify the specimen to species due to the poor condition of the slide preparation.

Harvey (1991) listed distribution records from Ghana and India, which were repeated in subsequent on-line catalogs (e.g., Harvey 2011). This Ghana record cannot now be verified and is presumed to be an error by Harvey (1991). The Indian record was based on Sharma & Sharma (1975), who reported many specimens of a speeies suggested to represent a new species of *Chelifer* from Jammu and Kashmir State in northern India, principally from buildings. Until further specimens are examined to verify the identification of these populations, it seems prudent to remove *C. cancroides* from the list of recorded Indian species.

Habitat.—*Chelifer cancroides* has been frequently reported from houses and associated human edifices such as barns, stables, bee hives and chicken coops (e.g., Vachon 1935; Beier 1939, 1948a, 1963; Hoff 1949; Levi 1953; Legg & Jones 1988), where they feed on a variety of small invertebrates (e.g., Kästner 1931; Vachon 1932; Schlottke 1933, 1940; Levi 1953).

Records of *Chelifer cancroides* from caves appear to be nonexistent, but Mr J. Zaragoza (in litt., 6 August 2013) has informed me of a sample of seven males, five females, and a tritonymph obtained by Berlese funnel extraction of dried bat guano from Cova dels Moseguellos cave, Vallada, Valencia, Spain (UTM 696400 4308700). The specimens do not exhibit any particular adaptations to cave life, although some are in the extreme range of the measurements given above.

Specimens have been very occasionally found on humans (Hermann 1804; André 1908, 1909a, 1909b; Artault de Vevey 1901; Vachon 1938a), and some have been observed to feed on the bed bug *Cimex lectularius* Linnaeus 1758 (Hemiptera: Cimicidae). Frickhinger (1920) reported observations made by a German prisoner of war held in 1915–1918 near Yekaterinburg, Sverdlovskaya oblast, Russia, who watched specimens of *C. cancroides* feeding on bed bugs. The pseudoscorpions emerged at night from the walls of the dwelling, but were never encountered in beds. Kaisila (1949) reported that *C. cancroides* has been observed feeding on bed bugs in Finland. A male *C. cancroides* collected in Wageningen, Netherlands in 1927 (CAS JC–281.01001) was taken from a "*Cimex* infested house", with an adult bug mounted on the slide alongside the pseudoscorpion.

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APPENDIX 1
The specimens studied in this study.

Chelifer cancroides (Linnaeus 1758)

AUSTRALIA: *Tasmania*, 2 ♂, Cressy, 6 December 1985, A. Harker (QVMAG); 1 ♂, Launceston, 5 July 1930, in house (bath), collector not stated (TMAG, J3110); 1 ♂, Launceston, 18 March 1977, in straw (one of many), A. Colvill (QVMAG, 13:6864).

CANADA: *New Brunswick*, 1 ♀, Saint Johns, no date, ex R.V. Chamberlin (CAS JC–1166.01001); *Ontario*, 2 ♂, 2 ♀, Westport, 29 September 1973, guano, S. Peck (FSCA WM3482.01–2); 1 ♀, Lake of the Woods, Sioux Narrows, April 2001, W. Kobel (FSCA WM8512.01001); *Quebec*, 1 ♀, Montreal, 30 September 2005, in house, C. Cloutier (WAM T67083); *Saskatchewan*, 1 ♂, 1 ♀, Lady Lake, 13 October 1931, D.J. Buckle (FSCA WM2050).

CHINA: *Shandong*, 1 ♀, Tsinan [=Jinan], 7 December 1922, on table, 2nd floor of house, Jacot (CAS JC–1418.02001); 1 ♀, Tsinan [=Jinan], 19 February 1925, in house, Jacot (CAS JC–1418.01001); *Province unknown*, 1 ♂, from China, at quarantine in New York, 2 November 1949, alive in shipment, dried orange peel, collector not stated (CAS JC–2234.01001).

CZECH REPUBLIC: *Hlavní mesto Praha*, 1 ♀, Praha, Leopoldova Street, 12 May 1998, inside building, F. Štáhlavský (WAM T78673).

"CZECHOSLOVAKIA": 1 ♀, at quarantine in Miami, Florida, 26 July 1948, in mail shipment, dry mushrooms, B.P. Stewart & R.C. Watson (CAS JC–2177.01001).

DENMARK: 1 ♂, no further locality, no date, collector not stated (CAS JC–655.01001) (det. by Schiödte as *Chelifer granulatus*).

FRANCE: 1 ♂, 2 ♀, 1 tritonymph, no further data (WAM T78672).

GERMANY: 1 ♀, from Germany at Cleveland, Ohio, 3 May 1945, in hay packing material, U.R. Kuhn (CAS JC–2022.01001); 1 ♂, 1 ♀, Germany, no further data, no date, A. Walcsuch (CAS JC–1481.01001–2); 1 ♂, in wood wool in packing case from Germany; intercepted in Australia, 30 September 1936 (QM W674).

ITALY: 1 ♂, ex Italy, at quarantine in New York, 15 September 1947, alive in garlic bulb, Fonner & Hidalgo (CAS JC–2155.01001); 2 ♀, 1 tritonymph, ex Italy, at quarantine in New York, 29 October 1948, in basket of grapes, Inspector Plummer (CAS JC–2181.01001–3).

NETHERLANDS: *Gelderland*, 1 ♂, Wageningen, 20 December 1927, in *Cimex* infested house, F. Spruyt (CAS JC–281.01001); 1 ♂, 1 ♀, Wageningen, 10 July 1928, from covers and straw of bee hives, F. Spruyt (CAS JC–1474.01001–2).

NEW ZEALAND: *Canterbury*, 5 ♂, 1 km S. of Prebbleton, 19 November–20 December 2012, in nests of the lucerne leafcutting bee *Megachile rotundata* in shed, B. Donovan (WAM T120948, T130746); *Taranaki*: 1 ♀, New Plymouth, July 1924, in ant's nest, W. Smith (CAS JC–544.01001).

POLAND: 20 ♂, 10 females, 1 tritonymph, laboratory colony in Wrocław, originally from Lower Silesia, 7 March 2013, I. Jędrzejowska (WAM T130754–130755).

SOUTH KOREA: 1 ♂, 1 ♀, Florida: Palm Beach County: Farmer's Market, 12 March 1962, in straw scuffs from South Korea, R.A. Long (FSCA WM3185.01001–2).

RUSSIA: *Kamchatka* Krai, 1 ♂, 2 ♀, Petropavlosk, 26 January 1921 (NHRS, GULI00000403).

SWEDEN: *Blekinge*, 1 ♂, Baggeboda, 19 June 1940, Butovitsch (NHRS, GULI000004105); *Gotland*, 1 ♂, Gotska Sandön, A. Jansson (NHRS, GULI000004104); *Småland*, 1 ♀, locality label not legible, 9 June 1946 (NHRS, GULI000004106); *Province not determined*, 2 ♂, 4 ♀, without precise data, 3 July 1946 (NHRS, GULI000004107); 1 tritonymph, without precise data (NHRS, GULI000004100); 2 ♂, 2 ♀, without precise data (NHRS, GULI0000040101, T. Thorell collection).

USA: *Alabama*, 1 ♀, Lee County: Auburn, no date, R.V. Chamberlin (CAS JC–657.01001); *Alaska*, 1 ♂, Denali Borough: Mount McKinley, [Denali] National Park, 21 February 1958, from mouse feces in hotel, Corson (AMNH, Hoff S–3587); *California*, 1 ♀, El Dorado County: Herus, Placerville, 16 October 1912 (CAS JC–662.01001); 1 ♂, Santa Clara County: Mayfield, no date, F. Sproyt (CAS JC–279.01001); 1 ♀, Santa Clara County: Stanford University, no date, J.C. Chamberlin (CAS JC–1791.01001); 1 ♂, Tulare County: Visalia, no date, ex R.V. Chamberlin (CAS JC–1760.01001); *Connecticut*, 1 ♀, Fairfield County: Stamford, Bartlett Tree Research Laboratory, 2 October 1945, S.W. Bromley (CAS JC–2151.01001); 1 ♂, Tolland County: Storrs, University of Connecticut, herbarium, November 1945 (AMNH, Hoff S–2634); *District of Columbia*, 1 ♀, Washington D.C., no date, ex R.V. Chamberlin (CAS JC–286.03001); *Florida*, 1 ♀, Miami-Dade County: Miami, International Airport, 27 May 1960, on stable floor, J.L. Weaver (FSCA Hoff S–4126); *Idaho*: 4 ♂, 3 ♀, Bear Lake County: Fish Haven, Bear Lake, 5 Sept. 1921, 'under bark of balsam log', 'under bark of balsam post' or 'bark of logs or slabs of fir', J.C. Chamberlin, B. Cain (CAS JC–676.01001–2, 5, JC–676.02001–4); 2 ♂, 1 ♀, Canyon County: Notus, 25 August 1932, W. Ivie (CAS JC–1350.01001–3); 1 ♂, Twin Falls County: Castleford Plot, 1932, debris, site of old barn, D.E. Fox and R.L. Piemeisel (CAS JC–1178.02001–2); 1 ♀, Twin Falls County: Twin Falls, 28 May 1935, from house (CAS JC–919.01001); *Illinois*, 1 ♀, Champaign County: Champaign, 29 July 1940, attacking people, R. Lehman (CAS JC–995.01001); 1 ♀, Chicago, 12 October 1910, A.W. Slocom (CAS, Hoff 6051–S–533); 1 ♀, Cook County: Glencoe, 22 June 1942, E. Best (CAS, Hoff 6052–S–534); 1 ♂, Cook County: Glencoe, 12 September 1941, M. Best (CAS, Hoff 6049–S–531); 1 tritonymph, Effingham County: Shumway, 1 November 1937, L.E. Richter (CAS, Hoff 6047–S–529); 1 ♂, Greene County: Roodhouse, 1938, from cattle, F.W. Helm (CAS JC–1057.01001); 1 ♀, Lake County: Waukegan, May 1940, H. Sorensen (CAS, Hoff 6055–S–536); 1 ♂, 1 ♀, north–central Illinois, 24 May 1938, in dwelling, C.L. Metcalf (CAS JC–1051.01001–2); *Indiana*, 1 ♂, Kosciusko County: Winond Lake, 3 July 1927, on weeds, Nester (CAS JC–423.01001); 1 ♂, LaGrange Country: Shipshewana, no date, under tin of chicken coop, Nester (CAS JC–410.01001); 1 ♂, Monroe County: Bloomington, 27 May 1927, oak fence rail, Nester (CAS JC–419.01001); 1 ♀, Monroe County: Bloomington, 1 June 1927, in house, Nester (CAS JC–421.01001); *Kansas*, 1 ♂, Crawford County: 4 miles E. and ½ mile S. of Pittsburg, 2 June 1963, B. Branson (AMNH, Hoff S–4187.1); 2 ♂, 1 ♀, Crawford County: 5 miles E. and 3 miles S. of Pittsburg, 22 May 1964, in barn, B. Branson (AMNH, Hoff S–4186.2–4); 1 ♂, McPherson County: Mound Ridge, 24 August 1934, in flour mill, N.E. Good (CAS JC–832.02001); 1 ♂, 1 ♀, Riley County: Manhattan, 3 July 1933, bottom poles of haystack, R.C. Smith (CAS JC–1201.01001–2); Kentucky, 1 ♂, Breathitt County: Quicksand, 25 June 1925, ex C.R. Crosby (CAS JC–1523.01001); *Maine*, 1 ♀, Hancock County: Brookline, no date, ex R.V. Chamberlin (CAS JC–1506.01001); *Massachusetts*, 2 ♀, Middlesex County: Groton, 8 June 1937, in barn swallow nest, E.A. Mason (CAS JC–1040.01001–2); *Michigan*, 1 ♂, Ingham County: East Lansing, July 1925, in house, R.H. Pettit (CAS JC–542.01001); *Missouri*, 1 ♂, Greene County: Springfield, no date, ex R.V. Chamberlin (CAS JC–1202.01001); *Montana*, 1 ♂, Garfield County: Jordan, 15 May 1927, collector not stated (CAS JC–632.01001); 1 ♂, Missoula County: Missoula, 1 May 1958, H.F. Pollmann (AMNH, Hoff S–4256); 1 ♂, Ravalli County: Hamilton, 1 March 1934, W.L. Jellison (CAS JC–1096.01001); 1 ♀, Ravalli County: Hamilton, 12 September 1932, laboratory, C.B. Philip (CAS JC–1112.01001); 1 ♀, Ravalli County, 20 May 1934, W.L.

Jellison (CAS JC–1121.01001); 1 ♂, no locality or date, Brunson (AMNH, Hoff S–2530); 1 ♂, no locality or date, Brunson (AMNH, Hoff S–2533); Nevada, 1 ♂, Elko County: Elko, 21 May 1935, in flour, M.W. Menke (CAS JC–829.01001); *New Hampshire*, 2 ♀, Grafton County: New Franconia, no date, ex R.V. Chamberlin (CAS JC–1487.01001–2); 1 ♂, Grafton County: Wentworth, 20 April 1957, H.M. van Deusen (AMNH, Hoff S–3509); *New Jersey*, 1 ♂, Bergen County: Ramsey, Gertsch (AMNH, Hoff S–1482); 1 ♀, Bergen County: Ramsey, September 1948, Gertsch (AMNH, Hoff S–1518); 1 ♀, Bergen County: Ramsey, June 1941, Gertsch (AMNH, Hoff S–1480); 1 ♂, Ocean County: Lakehurst, 2 October 1909 (AMNH, Hoff S–2648.1); *New Mexico*, 1 ♂, Bernalillo County: Albuquerque, 20 April 1961, from milk filter testing equipment (AMNH, Hoff S–41.70); *New York*, 1 ♀, Albany County: Albany, 6 August 1914, sandy woods, ex S.C. Bishop (CAS JC–1592.01001); 1 ♀, Albany County: Lincoln Pond, Huyck Preserve, Rensselaerville, 6 July 1949, J.C. Bishop (AMNH, Hoff S–2655); 1 ♀, Albany County: Voorheesville, 27 June 1934, barn swallow nest, D. Stoner (CAS JC–824.01001); 1 ♀, Chenango County: New Berlin, 22 August 1916, collector not stated (CAS JC–241.01001); 1 ♀, Dutchess County: Poughkeepsie, 19 August 1939, J.H. Fulton (CAS JC–991.01001); 3 ♂, 4 ♀, Genesee County: Bergen Swamp, 6 September 1965, collector not stated (FSCA WM827.01001–6, 8); 1 ♀, Monroe County: Penfield, July 1960, in house, collector not stated (FSCA WM359.01001); 1 ♀, Nassau County: Sea Cliff, no date, ex R.V. Chamberlin (CAS JC–298.07002); 1 ♂, Nassau County: Sea Cliff, no date, ex R.V. Chamberlin (CAS JC–298.04001); 1 ♀, Nassau County: Sea Cliff, no date, ex R.V. Chamberlin (CAS JC–298.07001); 1 ♀, 1 tritonymph, Nassau County: Sea Cliff, no date (CAS JC–257.01001–2); 1 ♀, Nassau County: Westbury, Long Island, June 1949, D.G. Nichols (AMNH, Hoff S–2656); 1 ♀, New York County: New York (AMNH, Hoff S–1541); 1 ♀, Oneida County: New Hartford, 12 March 1901, S.C. Bishop (CAS JC–1688.01001); 1 ♀, Steuben / Yates Counties: Lake Keuka, May 1904, June 1904, ex C.R. Crosby (CAS JC–1656.01001); 1 ♂, 1 ♀, Suffolk County: Babylon, 14 September 1930, F. Spruyt (CAS JC–1598.01001–2); 1 ♂, Sullivan County: Beaver Kill, 25 August 1947, R.B. Fischer (AMNH, Hoff S–2657); 1 ♂, Sullivan County: Beaver Kill, 14 July 1946, R.B. Fischer (AMNH, Hoff S–2654); 1 ♂, 1 ♀, Tompkins County: Groton, February 1960, in hay barn, H. Dietrich (AMNH, Hoff S–4080.1–2); 2 ♂, 1 ♀, Tompkins County: Ithaca, 1 August 1887 (CAS JC–661.01001–3); 1 ♀, New York, Tompkins County: Ithaca, April 1966, collector not stated (FSCA WM899.01001); 1 ♂, Tompkins County: Slaterville (now Slaterville Springs), 6 July 1929, P.R. Needham (CAS JC–1572.01001); *North Carolina*, 1 ♂, Currituck County: Shawboro, 14 April 1937, J.K. Duncan (CAS JC–1027.01001); *Ohio*, 1 ♂, Lawrence County: South Point,20 April 1934, in poultry house, H.C. Mason (CAS JC–855.01001); 1 ♂, Columbus, no date, in cellar under seed, C.M. Weed (CAS JC–1541.01001); *Oregon*, 1 ♂, Baker County: 10 miles W. of Baker, 7 August 1963, J.S. Buckett (CAS, EB.E–581.01001); 1 ♀, Baker County: Huntington, 5 May 1934, W.L. Jellison (CAS JC–1109.01001); 1 ♀, Benton County: Alsea, November 1920, J.E. Davis (CAS JC–1186.01001); 1 ♂, Benton County: Corvallis, 13 May 1936, on human being, in bathroom, Wheeler (CAS JC–831.01001); 1 ♀, Benton County: Corvallis, 14 May 1936, on human being, in bathroom, N. Larson (CAS JC–831.02001); 1 ♀, Benton County: Corvallis, 20 April 1940, from house, S.J. Couper (CAS JC–1087.01001); 1 ♀, Benton County: Corvallis, May 1936, Prof. Scullen (CAS JC–1185.01001); 1 ♀, Benton County: Corvallis, 20 April 1940, from house, S.J. Couper (CAS JC–1087.01002); 1 ♀, Benton County: Corvallis, 18 April 1938, E. Crumb (CAS JC–1082.01001); 1 ♂, Benton County: Corvallis, spring 1935, J.M. Pierson (CAS JC–1875.01001); 1 ♀, Benton County: Corvallis, 26 May 1935, C.E. Cody (CAS JC–1872.01001); 1 ♂, Benton County: Corvallis, 14 June 1939, in college building, V. Shattuck (CAS JC–915.02001); 1 ♀, Benton County: Corvallis, 20 March 1937, in shaving soap container in bathroom, D. Edwards (CAS JC–898.02001); 1 ♂, Clackamas County: Wilsonville, no date, in house, G. Danforth (CAS JC–1062.01001); 1 ♀, Clackamas County: Wilsonville, May 1938, from house, G. Danforth (CAS JC–1062.01002); 1 ♂, Deschutes County: Redmond, 28 April 1939 (AMNH, Hoff S–2025.1); 1 ♀, Deschutes County: Redmond, 28 April 1939 (AMNH, Hoff S–2025.2); 1 ♂, Harney County: P Ranch, 1 mile E. of Frenchglen, 12 May 1972, dung & hay, E.M. Benedict (CAS, EB.E–32.01001); 1 ♀, Jackson County: Medford, 7 July 1935, L.G. Gentner (CAS JC–1454.01001); 1 ♂, Lane County: 2.5 miles E. of Cheshire, 4 December 1971, shed, hay, nests and swallow debris, E.M. Benedict (CAS, EB–195.01001); 1 ♂, Marion County: Salem, 15 September 1945, from dead peach limb, J. Schuh (CAS JC–2246.01001); 1 ♀, Multnomah County: Gresham, 10 June 1944, found in bed in house, J. Schuh (CAS JC–2051.01001); 1 ♀, Wasco County: The Dalles, 23 June 1939, in house, presented by D.C. Mote (CAS JC–850.02001); 1 ♂, no further data, summer 1922, in sugar bowl, B.C. Cain (CAS JC–680.01001); *South Carolina*, 1 ♀, Pickens County: Clemson, 21 October 1962, J.A. Payne (FSCA WM528.01001); *Tennessee*, 1 ♀, Anderson County: Oak Ridge, 11 July 1960, A. Lawler (FSCA WM360.01001); *Utah*, 1 ♀, Box Elder County: Park Valley, 9 September 1932, R.V. Chamberlin (CAS JC–1605.01001); 1 ♂, Cache County: Logan, 8 August 1940, in chinchilla nest, G.F. Knowlton (CAS JC–996.01001); 1 ♀, Cache County: Logan, 20 March 1913, H.R. Hagen (CAS JC–627.01001); 1 ♀, Salt Lake County: East Millcreek, no date, under board, old hen coop, J.C. and O.W. Chamberlin (CAS JC–1209.01001); 1 ♂, Salt Lake County: Salt Lake City, no date, R.V. Chamberlin (CAS JC–1212.01001); 9 ♂, 5 ♀, Salt Lake County: Salt Lake City, September 1921, in ruins of old hen coop, B.C. Cain (CAS JC–228.01001, 2, 11–19, 24–26); 1 ♂, Salt Lake County: Salt Lake City, 14 April 1923, laboratory, ex R.V. Chamberlin (CAS JC–1234.01001); 1 ♂, Salt Lake County: Salt Lake City, September 1921, in old hen coop, B.C. Cain (CAS, no number); 1 ♂ (genitalia & chelicerae only), Salt Lake County: Salt Lake City, no date, collector not stated [CAS JC–258.01020 (2 slides)]; 1 ♂, Salt Lake County: Salt Lake City, no date, ex R.V. Chamberlin (CAS JC–1723.01001); 1 ♀, 1 deutonymph, Uintah County: Jensen, 7 November 1952, ex nest of *Peromyscus maniculatus*, D.E. Beck (AMNH, Hoff S–1995.1–3); 1 ♀, Utah County: Goshen, no date, R.V. Chamberlin (CAS JC–282.01001); 1 ♀, Washington County: Saint George, 1926, ex R.V. Chamberlin (CAS JC–1226.02001); 1 ♂, Emery County: San Rafael, 20 April 1928, A.M. Woodbury (ex R.V. Chamberlin) (CAS JC–1230.01001); 1 ♂, 1 ♀, Emery County: San Rafael River, 20 April 1928, A.M. Woodbury (ex R.V. Chamberlin) (CAS JC–1215.02001–2); *Virginia*, 1 ♂, Falls Church City: Falls Church, no date, R.V. Chamberlin (CAS JC–3.04001); *Washington*, 1 ♀, Pierce County: Puyallup, 27 March 1934, W.W. Baker (CAS JC–1812.01001); 1 ♂, Pierce County: Puyallup, 20 June 1932, C.W. Geteendaner (CAS JC–1923.01001); 1 ♀, Pierce County: Puyallup, no date, L.L. Stitt (CAS JC–2101.01001); 1 ♀, no further data, no date, ex R.V. Chamberlin (CAS JC–1560.01001); *Wisconsin*, 1 ♂, Crawford County: Prairie du Chien, June 1949, Levi and Smethurst (AMNH, Hoff S–2706); 1 ♂, 1 ♀, Dane County: Madison, Levi (AMNH, Hoff S–2717.1–2); 1 ♀, Dane County: Verona, February 1947 (AMNH, Hoff S–2715); 1 ♂, Marathon County: Wausau, 27 January 1950, in kitchen, Levi (AMNH, Hoff S–2674); 1 ♀, Marathon County: Wausau, May 1952, in house, Levi (AMNH, Hoff S–2707); 1 ♀, Oneida County: Hazelhurst, 19 August 1949, Levi (AMNH, Hoff S–2679); 1 ♀, Sauk County: Badger, April 1944, in house, Levi (AMNH, Hoff S–2675).

UNITED KINGDOM: *England*, 1 ♂, 1 ♀, Seabrook, Essex, no date, C. Warburton (CAS JC–660.01001–2).

NO DATA: 1 ♀, ex R.V. Chamberlin (CAS JC–1536.02002); 2 ♂ (CAS JC–29.02001–2); 1 ♀, ex R.V. Chamberlin (CAS

JC–1528.01001); 1 ♂, from the ship 'Mararita' at Newcastle, New South Wales, Australia, 31 August 1964, R.G. Winks (ANIC); 1 ♂, from the ship 'Alexandros' at Newcastle, New South Wales, Australia, 5 September 1964, R.G. Winks (ANIC); 1 ♂, from the ship 'Alexandros' at Geelong, Victoria, Australia, 18 September 1965, H. Caple (Department of Primary Industries Inspector) (ANIC).

Lissochelifer sp. ex Western Australia

AUSTRALIA: *Western Australia*, 3 ♂, 2 ♀, Mt Trafalgar, 15°16′50″S, 125°04′05″E, 12 June 1988, under bark, B.Y. Main (WAM T78668); 1 tritonymph, Mt Trafalgar, 15°17′S, 125°04′E, June 1988, litter, J. Majer (WAM T78669).

Lissochelifer sp. ex Queensland

AUSTRALIA: *Queensland*, 11 ♂, 7 ♀, 2 tritonymphs, Palmerville Station, 16°00′S, 144°05′E, 30 June 1997, under bark of rotting logs, F.D. Stone (WAM T118592).

Lophochernes sp. ex Vanuatu

VANUATU: 1 ♀, 2 tritonymphs, Vanuatu, 28 August 2000 (quarantine intercept in Australia) (WAM T118590); 1 ♂, Vanuatu, 17 July 2000 (quarantine intercept in Australia) (WAM T118591).

Parachelifer sp.

USA: *Florida*, 1 ♂, 1 tritonymph, Ormond [probably Ormond Beach], no date, R.V. Chamberlin (CAS JC–209.02001–2).

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A new troglobitic ideoroncid pseudoscorpion (Pseudoscorpiones: Ideoroncidae) from southern Africa

Mark S. Harvey: Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia; Research Associate: Division of Invertebrate Zoology, American Museum of Natural History, 79th Street at Central Park West, New York, New York 10024–5192, USA; Research Associate: Department of Entomology, California Academy of Sciences, Golden Gate Park, San Francisco, California 94103–3009 USA; Adjunct Professor: School of Animal Biology, University of Western Australia, Crawley, Western Australia 6009, Australia; Adjunct Professor: School of Natural Sciences, Edith Cowan University, Joondalup, Western Australia 6027, Australia. E-mail: mark.harvey@museum.wa.gov.au

Gerhard Du Preez: Unit for Environmental Sciences and Management, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

Abstract. The first blind African species of Ideoroncidae is described from a cave in northwestern Botswana, *Botswanoncus ellisi*, representing a new genus and a new species. Apart from the complete lack of eyes, it is also unusual in having the lowest recorded number of trichobothria of any adult ideoroncid with 17 on the fixed finger and nine on the movable finger.

Keywords: Taxonomy, morphology, Botswana, new genus

The pseudoscorpion family Ideoroncidae comprises 13 genera and 70 species found in Africa, Asia and the Americas (Harvey 2013; Harvey & Muchmore 2013). The majority occur in tropical ecosystems, but some occur in drier regions such as the southwestern USA and Mexico, Chile and Argentina, and the deserts of the Middle East. Ideoroncids have the combination of two unique morphological features, supernumerary trichobothria and the sub-basal position of the median maxillary lyrifissure (Harvey 1992; Harvey & Muchmore 2013). Another unusual feature, the division of the median genital sac of the male genitalia into two distinct parts, is also found in some species currently attributed to the family Syarinidae (e.g., Vachon 1938, 1954, 1969; Chamberlin 1952; Mahnert 1980; Harvey 1992).

The African ideoroncid fauna (Fig. 3) consists of four genera, *Afroroncus* Mahnert 1981, *Dhanus* Chamberlin 1930, *Nannoroncus* Beier 1955 and *Negroroncus* Beier 1931 (Harvey 2013). The genera *Nannoroncus* and *Afroroncus* are restricted to forested habitats in Kenya (Mahnert 1981). Three species of the genus *Dhanus* are known from the island of Socotra located off the Somali coast (Mahnert 2007). This genus is otherwise known from the Middle East (Afghanistan and Iran), India and southeast Asia (Harvey 2013). *Negroroncus* is the most widespread African genus, being found throughout Kenya, eastern Democratic Republic of Congo and northern Tanzania, as well as individual outlying species from Zimbabwe and the Republic of Congo (Mahnert 1981; Harvey 2013).

Among a small collection of pseudoscorpions taken from a cave in the Gcwihaba region of northwestern Botswana, the junior author found a small ideoroncid that completely lacks eyes (Fig. 4). Eyeless ideoroncids are elsewhere only known from the New World: *Albiorix anophthalmus* Muchmore 1999 from a cave in Arizona, USA (Muchmore & Pape 1999), *Ideoroncus anophthalmus* Mahnert 1984 and *I. cavicola* Mahnert 2001 from Brazil (Mahnert 1984, 2001) and five species of *Typhloroncus* 1979 from caves in Mexico and epigean ecotypes in the US Virgin Islands (Muchmore 1979, 1982, 1986; Harvey & Muchmore 2013). The only cave-dwelling ideoroncids from Africa are *Negroroncus aelleni* Vachon 1958 from the Republic of Congo and *N. jeanneli* Vachon 1958 from Tanzania (Vachon 1958), but neither species is completely eyeless, even though the eyes are small (Vachon 1958).

An initiative by the Botswana government to discover and explore unknown caves with the aim to promote caving in the tourism industry led to the discovery of several caves with no natural openings in the northwestern Gcwihaba region of Botswana. These caves were discovered through gravimetrical surveys of dolomitic outcrops. This technique identifies isolated subterranean cavities that are subsequently penetrated by means of drilling 700 mm vertical shafts. In 2010 efforts led to the discovery of a cave system named Diviners Cave. The drilled shaft, with a surface entrance altitude of 1056 m a.s.l., penetrates through 50 meters of rock into the cave. Exploration revealed an extensive cave system (Fig. 1) at several levels, with chambers of up to up 180 meters in diameter. Within the sealed cave we found areas where the roots of wild fig trees (*Ficus cordata*) penetrate the caves associated with sand and water drips. In these areas we found a diversity of invertebrates including diplurans, centipedes and termites. Although the cave does not reach the water table, the relative humidity exceeds 95% with a fairly constant temperature of 27°C (±2°C). Prior to drilling to open the cave, the system was under a high CO_2 pressurized atmosphere, making the circumstances in which the cavernicoles survived quite different from other natural caves in southern Africa.

Our study of the specimens revealed a number of unusual features, including the lowest number of trichobothria thus far recorded for an adult ideoroncid (Table 1), and a short arolium that lacks a ventral hook-shaped process. Apart from being a distinctive new species, we also suggest that it represents a previously undescribed genus. Therefore, we here provide a description and name the species to highlight its distinctive morphology and habitat.

Figures 1, 2.—1. The type locality of *Botswanoncus ellisi*, Diviners Cave, Botswana (Photo: Gerhard Jacobs); 2. Roots of the fig tree (*Ficus cordata*): the specimens were collected from the soil below the roots.

METHODS

The specimens examined in this study were mainly sampled with pitfall traps. Each trap was neatly buried in the soft sand associated with wild fig tree roots (Fig. 2) and half-filled with 75% ethanol. Also, some specimens were caught by hand or extracted from soil samples using Berlese funnels. These specimens are lodged in the Western Australian Museum, Perth (WAM) and the KwaZulu-Natal Museum, Pietermaritzburg (NMSA), and were studied using temporary slide mounts prepared by immersion of the specimen in lactic acid at room temperature for several days. They were then mounted on microscope slides with a 10 mm coverslip supported by small sections of 0.25 or 0.35 mm diameter nylon fishing line. After study the specimens were returned to 75% ethanol with the dissected portions plaeed in 12 × 3 mm glass genitalia microvials (BioQuip Products, Inc.). Specimens were examined with a Leica MZ–16A dissecting microscope and a Leica DM2500 compound microscope, the latter fitted with interference contrast, and illustrated with the aid of a drawing tube attached to the compound microscope.

Measurements were taken at the highest possible magnification using an ocular graticule. Terminology and mensuration mostly follow Chamberlin (1931), with the exception of the nomenclature of the pedipalps and legs, and with some minor modifications to the terminology of the trichobothria (Harvey 1992), chelicera (Judson 2007) and faces of the appendages (Harvey et al. 2012).

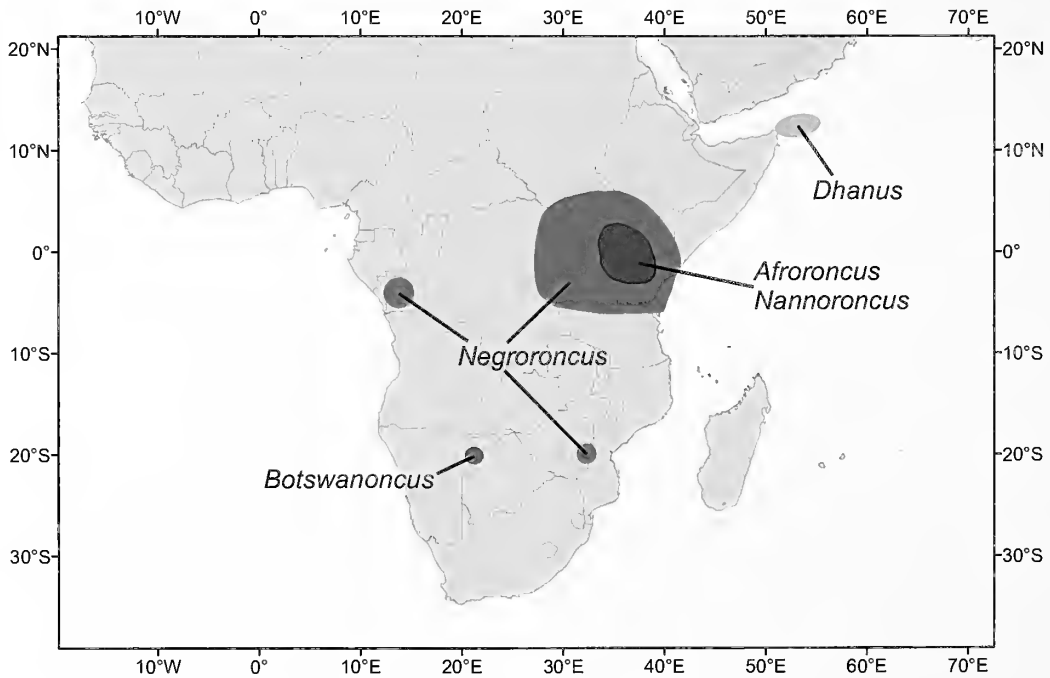


Figure 3.—Distribution of ideoroncid genera in Africa.

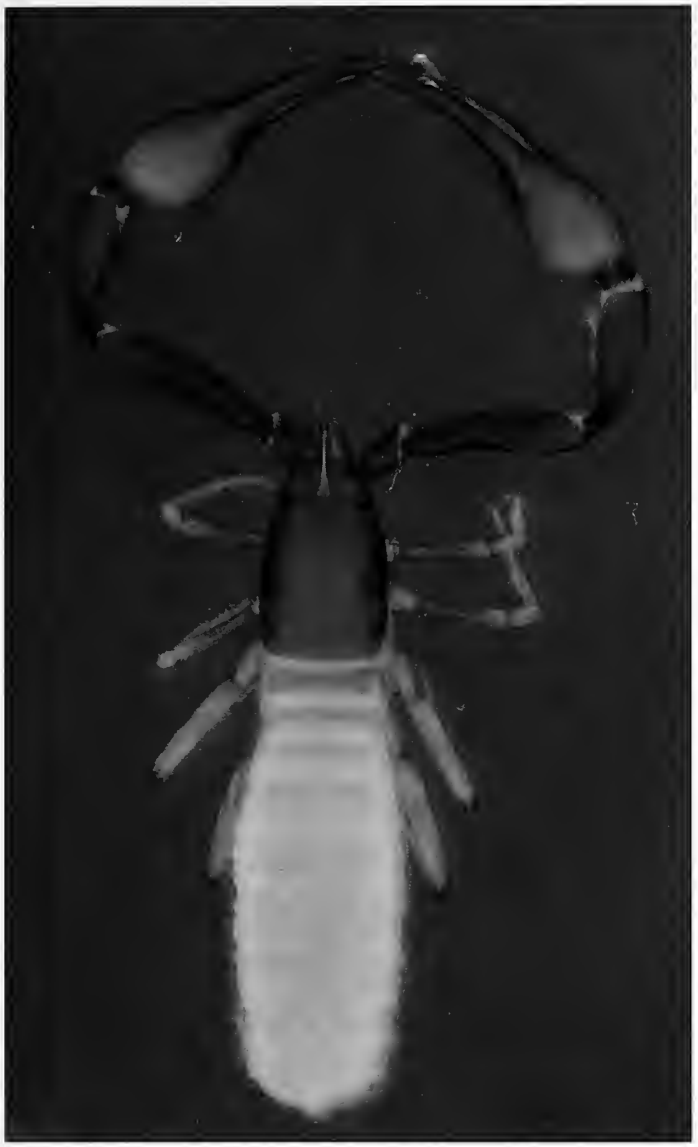


Figure 4.—*Botswanoncus ellisi*, new species, female holotype, prior to dissection, dorsal aspect.

The description was compiled using the DELTA (DEscription Language for Taxonomy) Editor computer program, version 1.0-Beta (available at http://code.google.com/p/open-delta/) (Dallwitz et al. 1999).

SYSTEMATICS

Family Ideoroncidae Chamberlin 1930
Genus ***Botswanoncus*** gen. nov.

Type species.—*Botswanoncus ellisi* new species

Diagnosis.—*Botswanoncus* is the only ideoroncid genus with a short arolium that lacks a ventral hook on the arolium. It also differs from other ideoroncids by the presence of only 17 trichobothria on the fixed chelal finger and nine trichobothria on the movable chelal finger (Table 1) (Figs. 11, 12), and from all other African ideoroncids by the complete lack of eyes (Fig. 5).

Description.—*Adult female (male unknown)*: Chelicera (Fig. 9): hand with 5 setae; movable finger with 1 sub-distal seta; all setae acuminate; galea present, simple, long and slender; lamina exterior absent; serrula exterior connected to chelicera finger for only part of length; not modified to form velum; rallum (Fig. 10) with 4 blades, all with anterior spinules, basal and sub-basal blades shorter than others.

Pedipalp (Fig. 8): long and slender. Fixed finger with 17 trichobothria (Figs 11, 12): *eb*, *esb*, *et*, *isb* and *it* regions each with 1 trichobothrium; *est* region with 6 trichobothria; *ib* region with 3 trichobothria; *ist* region with 3 trichobothria; *et* slightly distal to *it*. Movable finger with 9 trichobothria (Figs. 11, 12): *b*, *sb* and *st* regions each with 1 trichobothrium; *t* region with 6 trichobothria. Chelal teeth (Fig. 11) juxtadentate, with fixed finger chelal teeth low and flattened, and movable finger chelal teeth low; venom apparatus present in both chelal fingers; venom ducts of medium length, terminating midway between *it* and *est* in fixed finger and basal to *t* in movable finger; nodus ramosus not inflated.

Cephalothorax: carapace (Fig. 5) sub-rectangular; anterior margin slightly convex; with 4 setae on anterior margin and 2 on posterior margin; furrows absent; eyes completely absent. Manducatory process somewhat pointed, with 2 apical setae; median maxillary lyrifissure situated sub-basally.

Legs: femora I and II much longer than patellae I and II, respectively; femora III and IV about same size as patellae III and IV, respectively; metatarsi shorter than tarsi; subterminal tarsal seta acuminate; arolium about same length as elaws, with slight medial division (Figs. 6, 7); ventral hook-shaped process absent.

Abdomen: tergite I with 2 setae; spiracular plates each with 1 seta; medial sternites without suture line; pleural membrane uniformly longitudinally striate; stigmatic helix present; anus situated between tergite XI and sternite XI.

Genitalia: female: with gonosac covered in small acetabula.

Remarks.—The arolium of *B. ellisi* is about the same length as the claws (Figs. 6, 7), and therefore resembles the New World genera *Typhloroncus* and *Xorilbia* Harvey and Mahnert 2006, the African *Negroroncus aelleni* Vachon 1958, and the Asian *Dhanns siamensis* (With 1906), which have arolia that are slightly shorter than the claws. The remaining ideoroneid genera have arolia that are clearly longer than the claws. It differs from these genera with short arolia by lacking the ventral hook of the arolium. It further differs from all other ideoroncids by the reduced number of trichobothria, with only 17 on the fixed finger and nine on the movable finger (Table 1, Figs. 11, 12).

Etymology.—The genus is named for its occurrence in Botswana, combined with the last five letters of *Roncus*, a common pseudoscorpion stem which has been thought to be derived from the Latin *rmco*, "living in weeds" (Parker 1982). It is to be treated as masculine.

Botswanoncus ellisi sp. nov.
Figs. 4–12

Material examined.—Botswana: *North-western District*: holotype female, Diviners Cave, Gcwihaba region, 20°08′32.2″S, 21°12′36.6″E, 19 October 2011, G. Du Preez (WAM T125604). Paratypes: 1 female, same data as holotype except 13 March 2012 (WAM T130804); 1 female, same data as paratype (NMSA-Pse 026870).

Diagnosis.—As for genus.

Table 1.—The recorded number of trichobothria found in adults of genera of Ideoroncidae. Variant numbers are shown in brackets.

	eb	*esb*	*est*	*et*	*ib*	*isb*	*ist*	*it*	*b*	*sb*	*st*	*t*	Fixed finger, total	Movable finger, total	Reference
Afroroncus	1	1	6	1	4	1	5	1	2	1	1	6	20	10	Mahnert (1981)
Albiorix	1	1	6	1	4–5	1	5–6 (4)	1	2	1	1	6	20–22	10	Harvey & Muchmore (2013)
Botswanoncus	1	1	6	1	3	1	3	1	1	1	1	6	17	9	This study
Dhanus	1–3	1	5–9	1	3–6	1	5–12	1	2–4	1	1	6–8	23–31	11–14	Mahnert (1984, 2007)
Ideoroncus	1	1	6	1	4 (5)	1	4–6	1	2	1	1	6	20–21 (22)	10	Mahnert (1984, 2001); Harvey & Muchmore (2013)
Malmertius	1	1	6	1	5	1	6	1	2	1	1	6	22	10	Harvey & Muchmore (2013)
Muchmoreus	1	1	6	1	4	1	5	1	2	1	1	6	20	10	Harvey & Muchmore (2013)
Nannoroncus	1	1	6	1	4	1	5	1	2	1	1	6	20	10	Mahnert (1981)
Negroroncus	1–2	1	6–10	1	4	1	5–6	1	2–3	1	1	6–7	20–26	10–12	Mahnert (1981); Vachon (1958)
Nhatrangia	2	1	9	1	6	1	9–10	1	4	1	1	8	30–31	14	Mahnert (1984)
Pseudalbiorix	1	1	6	1	4	1	5	1	2	1	1	6	20	10	Harvey et al. (2007)
Shravana	1	1	6	1	5	1	7	1	3	1	1	7	23	12	Mahnert (1984)
Typhloroncus	1	1	6	1	4–5	1	6–7	1	2	1	1	6	22	10 (11)	Muchmore (1986); Harvey & Muchmore (2013)
Xorilbia	1	1	6	1	5	1	6	1	2	1	1	6	22	10	Mahnert (1984, 1985)

Description.—*Adult*: Color: pedipalps, carapace, chelicerae and coxae light red-brown, tergite I and legs pale brown and remainder light yellow-brown (Fig. 4).

Setae and cuticle: setae long, mostly straight and acicular; most cuticular surfaces smooth and glossy, with exception of pedipalps and chelicera, which are finely granulate.

Chelicera (Fig. 9): ca. 50% length of carapace; surface reticulate; cheliceral hand with 5 setae; movable finger with 1 sub-distal seta; all setae acuminate; galea present, simple, very long, slender and slightly curved; fixed finger ca. 7 teeth, each approximately same size, small; movable finger with ca. 8 teeth, each approximately same size, small; exterior and interior condylar lyrifissures present; serrula interior with 18 blades; lamina exterior absent; rallum (Fig. 10) with 4 blades, all with anterior spinules; basal and sub-basal blades shorter than others.

Pedipalp (Fig. 8): long and slender; trochanter and femur granulate, prolateral margin of patella and chelal hand granulate, retrolateral surfaces of fingers granulate, all other surfaces smooth; setae acicular, straight or nearly so; trochanter with anterior margin rounded, 2.85 x; femur cylindrical, without trichobothria, 4.57–5.01 x; patella cylindrical, with strongly pronounced pedicel, with 3 lyrifissures, 2 at distal end of pedicel and 1 in middle of pedicel, 2.99–3.25 x; chelal hand ovoid, external chelal condyle small and rounded, internal chelal condyle small and rounded, chela (with pedicel) 3.91–4.14 x, chela (without pedicel) 3.71–3.92 x, hand (without pedicel) 1.26–1.32 x, movable finger 1.93–2.54 x longer than hand (without pedicel). Fixed finger with 17 trichobothria; movable finger with 9 trichobothria (Figs. 11, 12); *eb*, *esb* and *isb* in straight row at base of finger; *ib* region situated dorsally in the middle of chelal hand; *eb*, *esb*, *et*, *isb* and *it* regions each with 1 trichobothrium; *est* region with 6 trichobothria; *ib* region with 3 trichobothria; *ist* region with 3 trichobothria; *et* slightly distal to *it*; *b*, *sb* and *st* regions each with 1 trichobothrium; *t* region with 6 trichobothria; not ventrally displaced, or *st* situated much closer to *b* than to *t*; trichobothrium *t* acuminate. Both fingers straight in lateral view; chelal teeth juxtadentate (Figs. 11, 12); fixed finger chelal teeth low and flattened; venom apparatus present in both chelal fingers, venom duct of medium length, terminating midway between *it* and *est* in fixed finger and basal to *t* in movable finger; nodus ramosus not inflated.

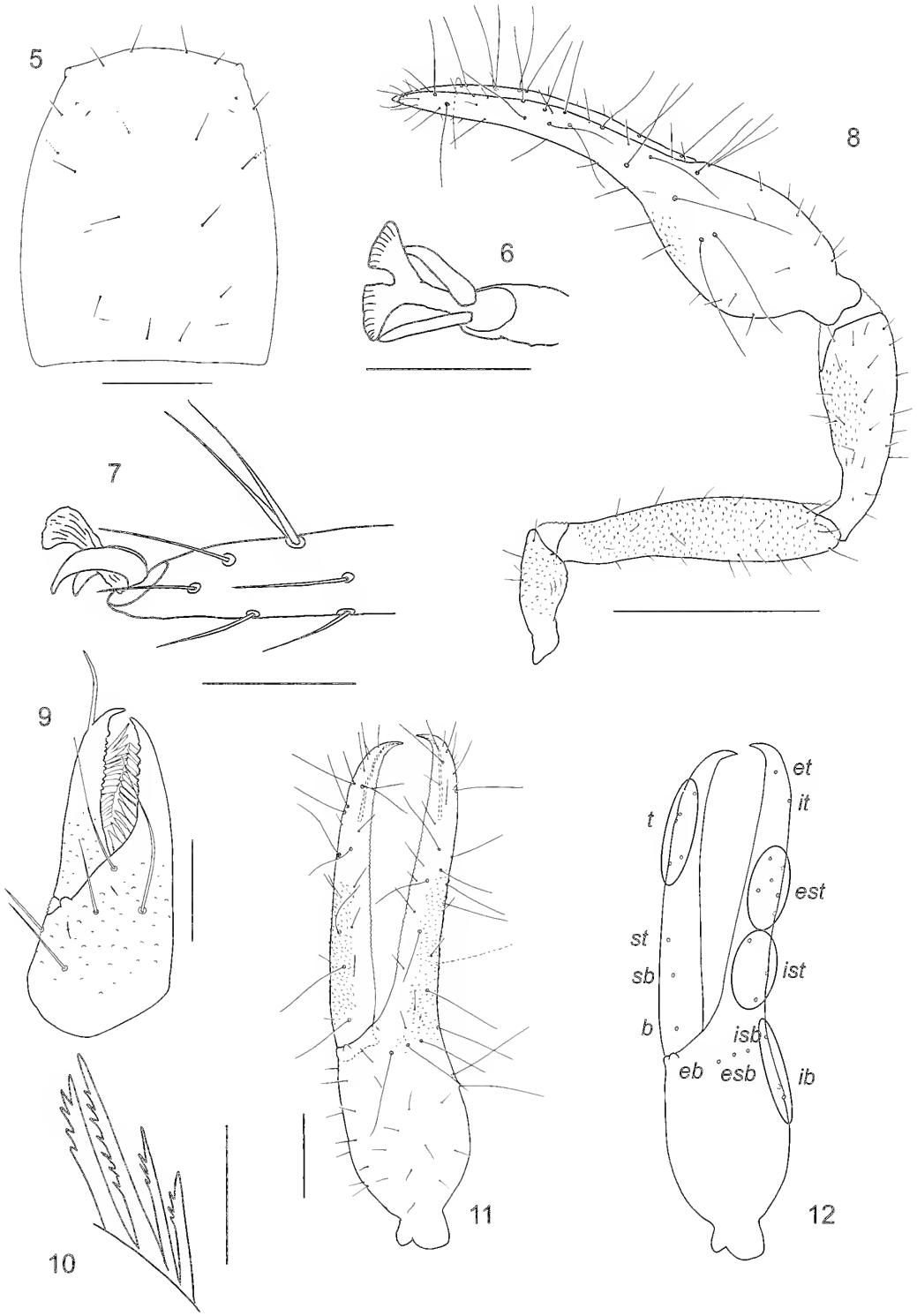
Cephalothorax: carapace (Fig. 5) sub-rectangular, 1.20–1.39 x longer than broad; anterior margin slightly convex; epistome absent; lateral margins slightly convex; posterior margin straight; with 18 setae, arranged 4: 4: 4: 2: 2: 2; setae subequal in length; furrows absent; eyes completely absent. Manducatory process somewhat pointed, with 2 apical setae, both setae approximately equal in length; maxilla with 5 additional setae; maxillary shoulder absent; median maxillary lyrifissure present, situated sub-basally, strongly curved, U-shaped; posterior maxillary lyrifissure present, strongly curved. Coxa I about same width as coxa IV; anterior margin of coxa I with process near foramen; coxae I–IV of ♀ with setae arranged 4: 5: 4: 4.

Legs: femora I and II much longer than patellae I and II, respectively; femur I and II without basal swelling; femora I and II with primary slit sensillum directed transversely; femora III and IV about same size as patellae III and IV, respectively; femur + patella IV 3.89 x longer than broad; tibiae III and IV without obvious tactile seta; metatarsi III and IV with long tactile seta, situated medially; tarsi III and IV without tactile seta; metatarsi and tarsi of all legs not fused; metatarsi shorter than tarsi; subterminal tarsal seta acuminate; claws smooth; arolium about same length as claws, with slight medial division (Figs. 6, 7); ventral hook-shaped process absent (Fig. 7).

Abdomen: tergites straight, without suture line, setal formula ♀, 2: 4: 4: 4: 5: 6: 6: 6: 6: 6: 6 (arranged T1TT1T): 2; arranged in single rows; sternites, without suture line, setal formula ♀, 6: (1) 2 (1): (1) 4 (1): 8: 8: 8: 8: 8: 8: 6 (arranged 1T2T1): 2; setae of anterior genital operculum (sternite II) of ♀ very small; posterior tergites and sternites with several tactile setae; glandular setae absent; pleural membrane uniformly longitudinally striate.

Genitalia: female: with gonosac covered in small acetabula.

Dimensions (mm): Female holotype (with paratypes in parentheses, where applicable). Body length 2.28 (2.16–2.25). Chelicera 0.313/0.132, movable finger length 0.200. Pedipalp:



Figures 5–12.—*Botswanoncus ellisi*, new species, female holotype: 5. Carapace, dorsal; 6. Tip of left tarsus IV showing claws and arolium, dorsal; 7. Distal end of left tarsus IV, lateral; 8. Right pedipalp, dorsal; 9. Left chelicera, dorsal; 10. Rallum; 11. Left chela, lateral; 12. Left chela, lateral, showing trichobothrial pattern. Scale lines = 0.05 mm (Figs. 6, 7), 0.1 mm (Figs. 9, 10), 0.2 mm (Figs. 5, 11, 12), 0.5 mm (Fig. 8).

trochanter 0.314/0.110, femur 0.685/0.150 (0.659–0.722/0.141–0.144), patella 0.545/0.182 (0.502–0.550/0.163–0.169), chela (with pedicel) 1.182/0.302 (1.136–1.250/0.295–0.302), chela (without pedicel) 1.12 (1.072–1.184), hand (without pedicel) 0.380 (0.390–0.397), movable finger length 0.732 (0.689–0.768). Carapace 0.564/0.471 (0.584–0.596/0.419–0.435). Leg I: femur 0.330/0.080, patella 0.160/0.070, tibia 0.250/0.049, metatarsus 0.152/0.039, tarsus 0.337/0.032. Leg IV: femur + patella 0.521/0.140, tibia 0.324/0.071, metatarsus 0.228/0.046, tarsus 0.328/0.040.

Remarks.—*Botswanoncus ellisi* exhibits some moderate modifications for cave existence, including the complete lack

of eyes (Figs. 4, 5) and pallid coloration (Fig. 4). The appendages, however, are not especially elongated as in the other troglobitic ideoroncids *Albiorix anophthalmus* Muchmore 1999 from Arizona, USA and several *Typhloroncus* species from Mexican caves, which have long, slender appendages consistent with a permanent cave-dwelling lifestyle (Muchmore 1982, 1986; Muchmore & Pape 1999; Harvey & Muchmore 2013).

Only two other pseudoscorpion species have been recorded from Botswana. *Nanolpium subgrande* (Tullgren 1908) and *Beierolpium deserticola* (Beier 1964) (Tullgren 1908; Beier 1964), both in the family Olpiidae, making it one of the least known countries for pseudoscorpion diversity (Harvey 2013).

Etymology.—This species is named for the renowned caver Roger Ellis, who facilitated GDP's trip to Botswana. Roger has devoted over 40 years to the discovering, surveying and conserving of caves in southern Africa.

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Comparison of scorpion behavioral responses to UV under sunset and nighttime irradiances

Douglas D. Gaffin and **Tristan N. Barker**: Department of Biology, University of Oklahoma, Norman, Oklahoma 73019 USA. E-mail: ddgaffin@ou.edu

Abstract. Scorpions are nocturnal arachnids that fluoresce a bright cyan-green when exposed to UV light. Although the function of this fluorescence remains unknown, some authors have suggested that it may aid the scorpions' light detection. Taking advantage of scorpions' negatively phototactic behavior, we tested the responses of desert grassland scorpions, *Paruroctonus utahensis* (Williams 1968), to 395 nm UV light at irradiances corresponding to an hour before sunset (0.15 μW/cm^2), sunset (0.01 μW/cm^2), and moonlight (0.0001 μW/cm^2), as well as no light. We found that animals showed the strongest responses to UV light levels equivalent to sunset. The animals moved more quickly and sporadically under the higher light levels. In addition, animals were less likely to complete a trial under highest light conditions, suggesting that UV light may inhibit normal scorpion locomotion. Finally, this study resulted in several methodological refinements, including automated tracking of the subjects' movements that should prove useful in future behavioral studies of scorpion phototactic behavior.

Keywords: Fluorescence, light, orientation, sensory, vision

Scorpions are nocturnal arachnids that fluoresce a bright green color when exposed to ultraviolet (UV) light due to the presence of beta-carboline and 4-methyl-7-hydroxycoumarin in their cuticle (Stachel et al. 1999; Frost et al. 2001). No functional reason behind their fluorescence has been determined. Some authors, including Frost et al. (2001) and Wankhede (2004), have suggested that scorpion fluorescence may serve no behavioral purpose, while others have proposed that fluorescence may help scorpions capture prey (Kloock 2005), attract mates, or ward off predators and territorial rivals (Kloock 2008). Other researchers hypothesize that fluorescence may play an active role in light detection, helping scorpions identify shelter or decide when to stay in their burrows (Camp & Gaffin 1999; Blass & Gaffin 2008; Gaffin et al. 2012; Kloock et al. 2010).

Scorpion cuticle fluoresces most strongly under 395 nm UV light, reemitting it as green (~ 500 nm: Fasel et al. 1997; Kloock 2009). Studies indicate that the medial eyes of scorpions are most sensitive to green light (peaking around 500 nm: Machan 1968; Fleissner & Fleissner 2001). Parts of the scorpion metasoma are also sensitive to green light (Zwicky 1968, 1970a,b; Rao & Rao 1973). It is therefore tempting to suggest that fluorescence may aid in light detection by transducing UV light to increase light intensity in the range of peak sensitivity of their visual system.

A few behavioral studies support this hypothesis. Blass & Gaffin (2008) showed that scorpions become most active when exposed to UV or green light, as compared to other wavelengths. Kloock et al. (2010) found a difference between fluorescent and fluorescence-reduced scorpions in the variance of time spent in light-exposed areas, as well as differences in activity levels under UV light. Gaffin et al. (2012) found that scorpions with medial and lateral eyes covered were far less likely to react to 505 nm light, but only slightly less likely to react to 395 nm light. This last study led to the hypothesis that the cuticle may act as a whole-body UV photon collector, transducing UV wavelengths to green wavelengths. This information may allow the scorpion to detect and turn toward shade when one part of the cuticle receives diminished light levels.

Taken together, the physiological and behavioral evidence suggest that UV light plays an important role in scorpion orientation. However, to better understand these effects, we first need to quantify the levels at which UV light becomes behaviorally relevant.

Our objective in the current study was to establish a dose response curve illustrating scorpions' reactions to irradiances of UV light corresponding to natural conditions ranging from early sunset to the middle of the night. Gaffin et al. (2012) observed significant phototactic behavior when scorpions were exposed to 0.15 μW/cm^2 UV light, slightly greater than the UV component of sunlight about an hour before sunset when the sun is 11.4° above the horizon (Johnsen et al. 2006). This light level is somewhat higher than what scorpions normally encounter; they become active shortly after sunset (Polis 1980) and are therefore most likely to encounter intensities of light corresponding to refracted sunlight, starlight, or moonlight.

We used 0.15 μW/cm^2 as the high light treatment on our dose response curve. We selected 0.01 μW/cm^2, the UV component of the sky's irradiance at sunset (when the sun is at the horizon: Johnsen et al. 2006), as the second treatment. We selected 0.0001 μW/cm^2, the UV component of full moonlight on a clear night (Johnsen et al. 2006), as the third and lowest light treatment. Finally, we used no light as the control and the final point on the curve, where we expected to see no phototactic behavior. Combined, these four points represented a relatively even distribution of celestial irradiance values that would be present from the early evening into the night.

We found that scorpions respond to UV light levels that correspond to irradiance values found around sunset. Since this assay uses a negative phototactic locomotor behavior as the response, the actual threshold of sensitivity is probably lower than these deterrence levels. Taken together, scorpions appear capable of detecting UV levels that are consistent with light levels during early evening.

An additional objective of this study was to improve the efficiency of the behavioral assay used to detect light avoidance behavior in scorpions. We have greatly improved

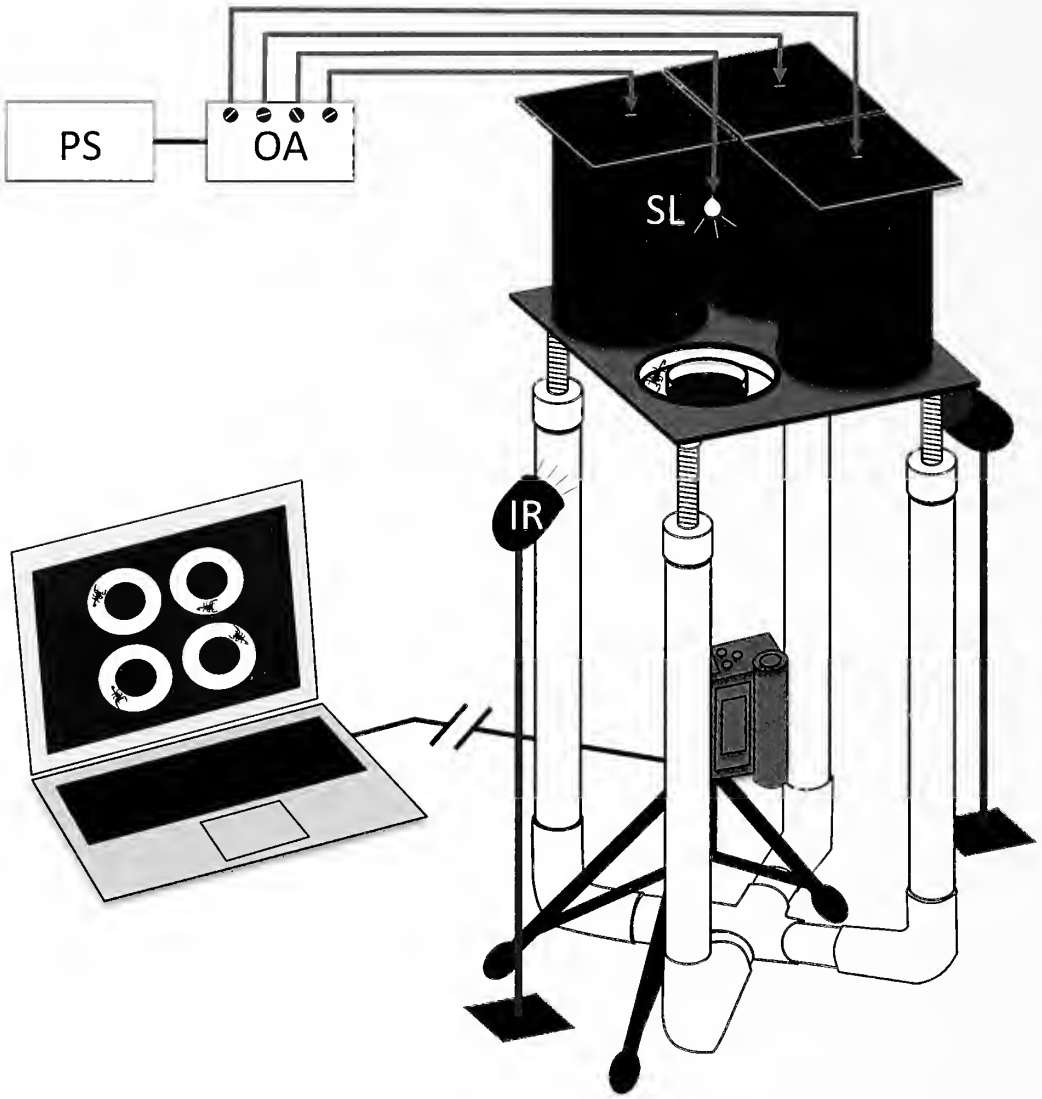

Figure 1.—Diagram of behavioral set-up. A power source (PS) powers four independently controllable operational amplifier circuits (OA), which connect to LEDs (SL) that extend through holes in the tops of dark PVC cylinders placed over Petri dish arena. An IR sensitive video camera below the stage monitors scorpion activity. Infrared light is direeted across the bottoms of the arenas from two sources (IR) placed at the side of the set-up. Video output is relayed to a computer for recording, processing, and analysis.

the visibility of the animals in the behavioral arenas and applied automated tracking software to assist in the scoring of behavioral trials and to reduce possible sources of human bias.

METHODS

Animals.—We used 12 male and 12 female adult *Paruroctonus utahensis* (Williams 1968) scorpions collected in late August and early September 2012 from sandy regions of the northern Chihuahua Desert. Collecting areas ranged from the Texas-New Mexico border between El Paso and Las Cruces to areas east of Socorro, New Mexico and near the Sevilleta field station in La Joya, New Mexico. We deposited a voucher specimen in the Sam Noble Oklahoma Museum of Natural History on the University of Oklahoma campus in Norman, Oklahoma. Animals were kept in the laboratory at the Sevilleta station and housed individually in plastic food storage containers (Great Value, 236 ml) that had four 5.6 mm air holes drilled in the corners of their lids. Each container also held 20 ml of sand from their native habitat (filtered through a #12 sieve) and a 4 cm × 4 cm square of paperboard folded into a tent for shelter. The animals were provided a few ml of water weekly by misting and a wax worm every other week. The animals were exposed to a 14:10 h light:dark cycle (on at 0530, off at 1930) using a white fluorescent bulb (General Electric "Energy Smart" 13 W bulb – 60 W equivalent) in a work light (Bayco clamp light, 21.6 cm) plugged into a timer switch. The light was placed 50 cm from the animals. The room temperature ranged from 20–21°C during the day. To increase animal activity on trial nights, a small heater (Sunbeam compact ceramic heater) was used to warm the room to 22–24°C.

Behavioral apparatus.—We used a modified version of the apparatus described in Gaffin et al. (2012). Figure 1 shows a diagram of the behavioral set-up. We created a circular arena for the animals by gluing a 5.40-cm diameter Petri dish upside down in the center of an 8.75-cm diameter Petri dish (15 mm deep). The larger Petri dish lids were used for covers and secured to the dish bottoms with small pieces of electrical tape.

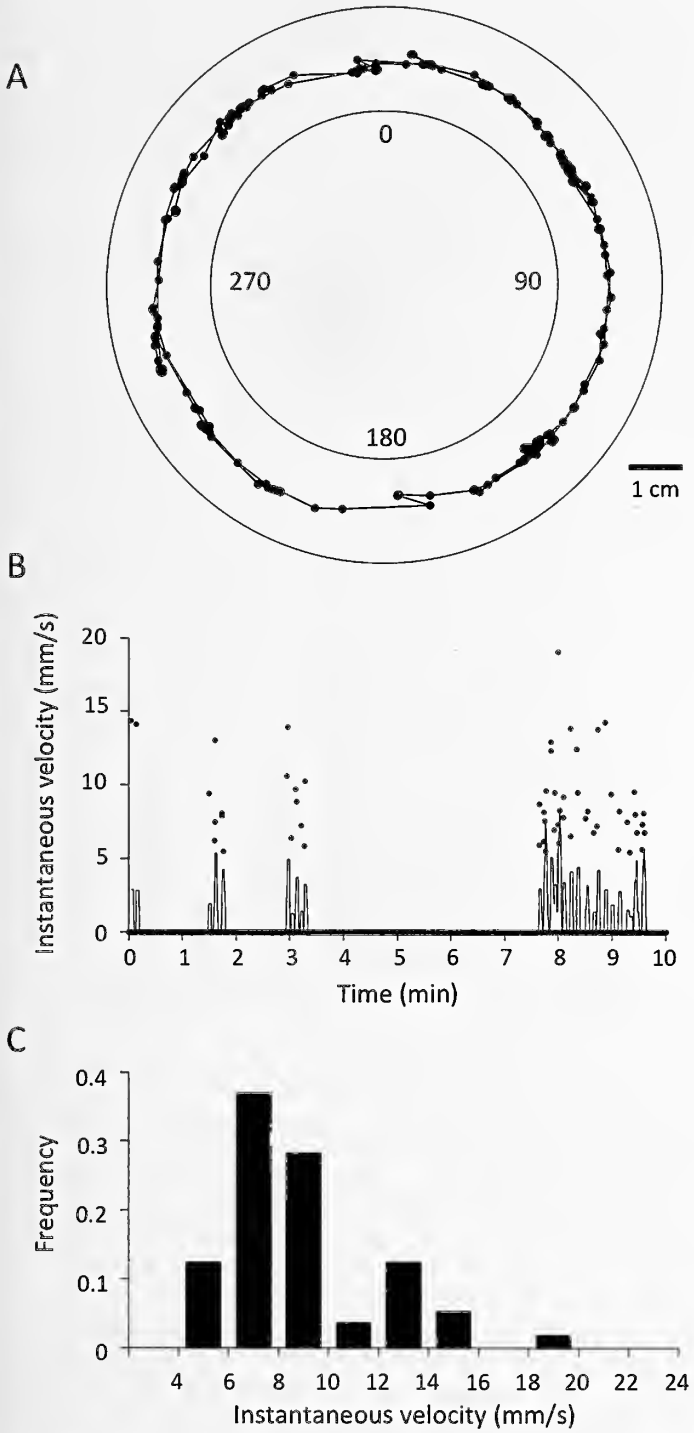

Figure 2.—Scorpion behavior in the behavioral test apparatus. **A.** Sample plot of animal movements during 10-minute trial under no light condition; the points are plotted at 0.67 s intervals. The numbers inside the circle indicate arena coordinates that are referenced in figure 3A. **B.** Plot of instantaneous velocity across duration of the trial shown in A. Instantaneous velocity is calculated as distance traveled in mm between each frame divided by 0.67 s (time between frames). The line is a five-point running average of instantaneous velocity. **C.** Frequency distribution of the instantaneous velocities for the trial shown in A.

The arenas were inserted into four holes in a 36 × 30 × 1.5 cm particleboard stage that was suspended by a PVC frame with adjustable supports for leveling. The outside upright walls of the larger Petri dish arenas were covered by black electrical tape to keep light from entering the sides of the arenas and to help the dishes fit snugly in the stage holes. The dishes were lowered into the holes until the arena lids rested flush against the top of the particleboard stage. We then placed a piece of PVC pipe (10 cm diameter and 15 cm tall) lined with black construction paper over each arena and topped this pipe with a black square of Plexiglas that had a 5-mm hole drilled in its center to accommodate an LED light. We fitted four such tubes with LEDs emitting UV light (395 nm, 15° viewing angle; Super Bright LEDs Inc.). The LEDs were fixed in place with black electrical tape and connected via patch cables with mini-hook clips to op-amp circuits to control the intensity of each LED and allow the light of each arena to be independently set, so that each trial could include four separate treatments. We filmed the arenas from below with an infrared-sensitive camera (Sony Handycam CCD-TRV16 with 'nightshot' feature) connected to a computer running a video capture program (Elgato Video Capture System). To reduce glare and improve the image, we covered the camera's IR light source with two layers of black electrical tape. We directed the IR light emitted from two surveillance cameras (Swann NightHawk Day/Night Security Cameras) at 45-degree angles onto the bottom of the arenas to provide the IR source for the camera. We taped some thin semi-transparent foam packing material over the lights to diffuse the IR. To reduce glare and light contamination of the video image from the trial LEDs, we taped a cirele of blaek construction paper to the bottom of the arena to cover the area occupied by the smaller Petri dish.

Light calibrations.—We used an Oeean Optics USB4000-UV-VIS-ES spectrophotometer (200 μm slit, 600 μm diameter optical fiber, 3900 μm diameter CC-3 cosine corrector) to calibrate each LED to the same relative irradiance for each trial. Since we exposed the animals to LEDs emitting only UV, we matched the irradiance of these LEDs to the isolated UV component of natural light levels such as moonlight and sunlight.

We tested the scorpions' responses to four light levels. The highest level, 0.15 $\mu W/cm^2$, is approximately equal to the ultraviolet component of sunlight when the sun is just over 11.4° above the horizon; this level is also about 1500 times the ultraviolet component of full moonlight (0.0001 $\mu W/cm^2$). This allowed us to verify that our scorpions demonstrated behavior similar to that observed under previously tested light conditions (Gaffin et al. 2012). We compared the scorpions' behavior under this light to their behavior under the UV irradiances of sunset (light emitted from the sky when the sun is at the horizon; 0.01 $\mu W/cm^2$), full moonlight and no light. We determined these intensities based on irradiance values given in Figure 2C of Johnsen et al. (2006) and conversion factors provided by Johnsen (2012).

Dose response trials.—We conducted these trials in a windowless room at the Sevilleta field station in September 2012. All animal manipulations were done under dim red light provided by a headlamp (Energizer Trailfinder 6 LED Headlight); previous studies showed no apparent behavioral sensitivity of scorpions to red light (Camp & Gaffin 1999;

Gaffin et al. 2012). Trials began around 2000 (30 min after the beginning of the dark cycle) and were completed by 2130. Trials were run four at a time, with each arena's light tuned to one of the four light levels: arena A, no light; arena B, 0.0001 μW/cm^2; arena C, 0.01 μW/cm^2; arena D, 0.15 μW/cm^2. Based on the activity levels of *P. utahensis* under these conditions in our pilot studies and as reported in previous studies (Blass & Gaffin 2008; Gaffin et al. 2012), 24 animals participated in two sets of trials separated by 15 days. Within each set of trials, each animal experienced a different intensity on four successive nights. The light intensity order was randomized so that the animals were exposed to neither ascending nor descending intensities; the sequence was reordered for the second set of trials. Each animal was therefore exposed to each light condition twice, separated by about 15 days. One animal died during the 15-day interval and was removed from all data analyses. Subtracting this animal, 184 trials formed the data set for these experiments (23*4 + 23*4). Each animal was fed a wax worm seven days before the start of the first set of trials and another worm one day after completing the first set of trials.

The protocol for all trials was identical. Each night, the 24 animals were lined up in their numbered containers on the counter in the dark room. Five minutes before the beginning of the each trial, the four arenas were cleaned with 70% ethanol and dried with a Kimwipe, to ensure that the animals could not detect pheromones or other clues from previous animal use. One animal was then put into each arena, and the lids were secured with two small strips of electrical tape. The arenas were then placed in the holes on the particleboard platform under the PVC pipe with the correct light intensity, but with the arena lights switched off. Once all four animals were in place, the video and the lights were turned on. The video was set to turn off automatically after 10 minutes. Halfway through one trial, we set up the arenas for the next trial. When the video turned off, the arena lights were switched off, the video was saved to the hard drive, and the four trial animals were returned to their home containers. The next video was readied and the four new animals were placed on the stage under the correct lights. This routine was completed for all 24 animals (six groups of four simultaneous trials per night).

Analysis.—We imported each video (saved in .mp4 by the Elgato system) to iMovie (Apple Corporation), used the speed function to increase the playback rate 10×, and saved to .mov format (Quicktime, medium band). These clips were imported into ImageJ (Rasband 2012), and the images were cropped to 100 × 100 pixel squares around each of the four arenas; each cropped image was saved to a separate animated .tif file. Each file was then imported to Fiji (Schindelin et al. 2012) and converted to 8-bit format and adjusted with the Image–Adjust–Threshold function to highlight and extract the scorpion from the background. Then the outside and the inner circle areas were cleared to isolate the image to the scorpion track. We further cleaned the image by digitally removing small video incongruities outside areas of animal movement. We used Fiji's Mtrack2 plugin to track scorpion movements (settings: minimum object size (pixels) = 50; maximum object size (pixels) = 99999; maximum velocity = 50; minimum track length (frames) = 2). The software marked the position of a centroid defined by the animal's outline. If there was too much glare contamination for accurate automated tracking (about 25% of the trials), we tracked the animals manually using Fiji's Manual Tracking plugin, marking each point based on a position just caudal to the medial eyes. After processing, the ten-minute videos parsed to 900 frames in Fiji. Therefore, each frame represented 0.67 seconds (600 s / 900 frames).

We imported each of the tracked files to Excel for further analysis. First, we applied the Pythagorean theorem to calculate the distance (d) moved between each frame in the record:

$$d = \sqrt{\left((x_2 - x_1)^2 + (y_2 - y_1)^2\right)}$$

(where $[x_2, y_2]$ and $[x_1, y_1]$ are the scorpion's coordinates within the current frame and the previous frame, respectively). We then removed all movements less than 4 pixels (3.6 mm; 1 pixel = 0.9 mm) between frames to avoid bias from some jitter introduced by the automatic tracking program. We then summed the remaining distances to obtain the total distance moved for the trial. We set a threshold of at least 100 total pixels (9 cm) moved for trials to be considered legitimate; this distance is approximately two-thirds of the way around the arena track and is roughly equivalent to the movement criterion used in Gaffin et al. 2012. Scorpions typically show bouts of movement interrupted by prolonged pauses. This minimum distance was important to filter random movements from potential stimulus-induced responses.

Next we calculated the instantaneous velocity of each movement by dividing each legitimate distance (those > 4.0 pixels between frames) by 0.67 s (the time between frames). We used these numbers to derive a frequency plot of all of the instantaneous velocities for each trial. A plot of all 5648 instantaneous velocities obtained during this study shows a positive skew (mean = 10.91, median = 9.58, mode = 5.54, standard deviation = 5.18; Pearson's first and second skewness coefficients: 1.04 and 0.77, respectively). Because of the skewed distributions, we calculated the median instantaneous velocity for each trial and used those numbers to determine the mean of each animal's scores for legitimate trials for each light level. For a given light level, if an animal had one trial that was legitimate and another that was not, the legitimate score was used as the animal's seore. No score was given if neither trial was legitimate; these trials were not included in our statistical analyses.

Note that the scorpions' behavior ean be described in terms of either the total distance traversed or the instantaneous velocity. In our experience, measuring the total distance traversed is unsatisfactory, as control animals often walk slowly and deliberately, covering the same distance overall as stimulated animals that "sprint" and rest. In this study, distances traveled by animals exposed to different light levels were not significantly different (repeated measures ANOVA: Fr = 1.000; P = 0.8438); we therefore estimated aetivity levels based on instantaneous velocity.

We used circular statistics to test for bias in the animal arena position in these trials. We first calculated the mean vector direction for animal positions in each legitimate trial. We then used these directions to calculate the overall mean vector direction and length (r). We calculated the z-statistic and used the Rayleigh test for randomness to determine the statistical significance of the mean vector.

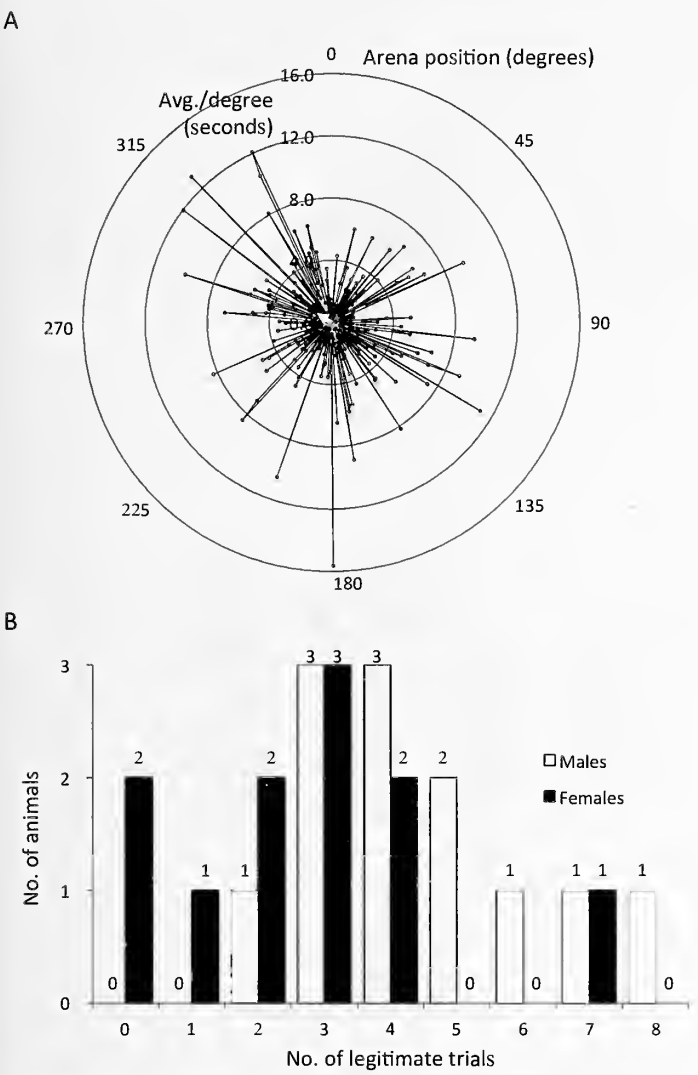

Figure 3.—Activity patterns in behavioral apparatus. **A**. Average animal arena position (in degrees) for all 83 legitimate trials shows no arena or global position bias (0 degrees is toward the top of the video screen; 290 degrees is geomagnetic north relative to this reference). **B**. Females and males show differences in the number of trials that met the legitimacy criterion.

We also looked at activity differences between males and females. We used a two-tailed Mann-Whitney U analysis to test statistical differenees in number of legitimate trials per animal among males and females.

We used a repeated-measures ANOVA and Dunn's multiple eomparisons post-hoc test to analyze the significance of differences among the scorpions' median instantaneous velocities under different light intensities. In this assay, scorpion responses appear as spurts of locomotor movement; as such, we predicted that stimulated scorpions would have higher median instantaneous velocities. We considered treatments significantly different if the P value was less than 0.05. Our null hypothesis was that there would be no significant differences between the scores at different irradiances. We used InStat 3 statistical software (Graph Pad Software, Inc., San Diego, CA, U.S.A.) for the Mann-Whitney U and ANOVA analyses.

Table 1.—Number of legitimate trials by experimental factor.

Factor	Condition	Legitimate trials	Total trials
Time of night	Early	48	96
	Late	35	88
Trial set	First	39	92
	Second	44	92
Night order	1	22	46
	2	21	46
	3	21	46
	4	19	46
Gender	Males	54	96
	Females	29	88

RESULTS

The new behavioral apparatus is different from the one used by Blass and Gaffin (2008) and Gaffin et al. (2012) in that the Petri dish arenas are suspended in holes in a particleboard stage rather than sitting atop a Plexiglas stage. The arrangement we used in this study, coupled with diffuse IR light directed from the side, provided clear images of the scorpions when filmed through the Petri dish from below. The scorpion images were distinct enough to use video detection software to automatically track animal movements, thereby removing the potential bias of a human observer. A sample plot of an animal moving under the "no light" condition is shown in Fig. 2A. The time course of this animal's instantaneous velocities is shown in Fig. 2B and these data are compiled in Fig. 2C as a frequency plot of the instantaneous velocities for the 10-min trial.

We made various checks of the new behavioral assay. Legitimacy (> 9 cm movement) was achieved in 45.1% of the trials (83 of 184). Although we recognize that considering all 83 legitimate trials includes non-independent observations, we feel that we can learn something about general patterns of behavior by examining these data. We found no bias in arena or global position among the legitimate trials (Fig. 3A: φ = 307°, r = 0.0369, z = 0.1132. P = 0.8935). Table 1 gives the number of legitimate trials by time of night (roughly, first half from 2000 to 2045, second half from 2045 to 2100), trial set, trial night and gender. Pooling across all light conditions, males had more legitimate trials than females (Fig. 3B: P = 0.0265, Mann-Whitney, two-tailed; U-statistic = 30.0).

Figure 4 shows an example of an animal with legitimate trials under all four light levels. This composite shows a general trend in behavior, with animals under no light (4A) or the lowest light (0.0001 $\mu W/cm^2$; 4B) conditions making shorter, steadier movements than the sporadic movements of animals under the highest light (0.15 $\mu W/cm^2$) condition (4D). The 0.15 $\mu W/cm^2$ trial contained the highest proportion of instantaneous velocities greater than 28 mm/s (right side of 4D). Likewise, the 0.01 $\mu W/cm^2$ trials occasionally contained examples of faster instantaneous velocities, as can be seen in the initial movements depicted in the middle plot of 4C.

The averages of instantaneous velocities for scorpions under each light level for all legitimate trials are shown in Fig. 5A. The graph shows similar patterns for the no light and 0.0001 $\mu W/cm^2$ trials and a flattening of the distribution pattern for the 0.15 $\mu W/cm^2$ trials. The pattern for the

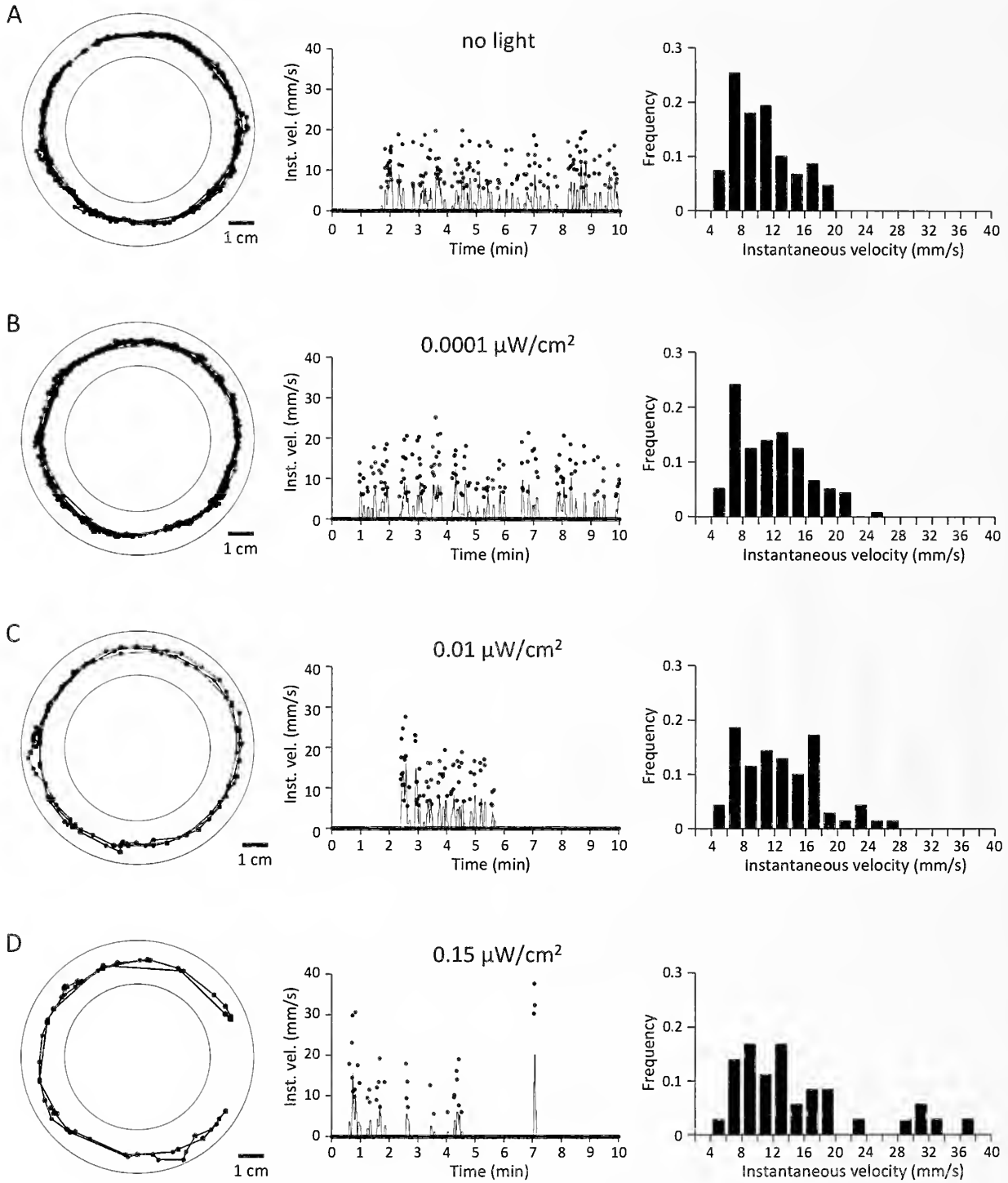

Figure 4.—Sample behavioral response of an animal to all four light levels. **A.–D.** Response to no light, 0.0001 µW/cm^2, 0.01 µW/cm^2 and 0.15 µW/cm^2, respectively. Left: plots of animal movements in arena; middle: plots of instantaneous velocities during the 10-min trials (lines = five-point running averages); right: frequency distributions of instantaneous velocities.

0.01 µW/cm^2 trials is similar to, but slightly below, the no light and 0.0001 µW/cm^2 trials curves.

Figure 5B shows the distribution of the 83 legitimate trials based on light treatment. We scored each animal as 0 if neither its first nor its second trial was legitimate, 0.5 if one of its two trials was legitimate, and 1 if both of its trials were legitimate. A Friedman test (nonparametric repeated measures ANOVA) across these data showed significant variation among the treatments (P = 0.0274); Dunn's multiple comparisons test showed no significant difference between pairs of treatments. Figure 5C compares the median instantaneous velocity scores among the light levels (scores were averaged for animals with two legitimate trials within a given treatment). Seven of the 23 animals had legitimate trials across all four treatments.

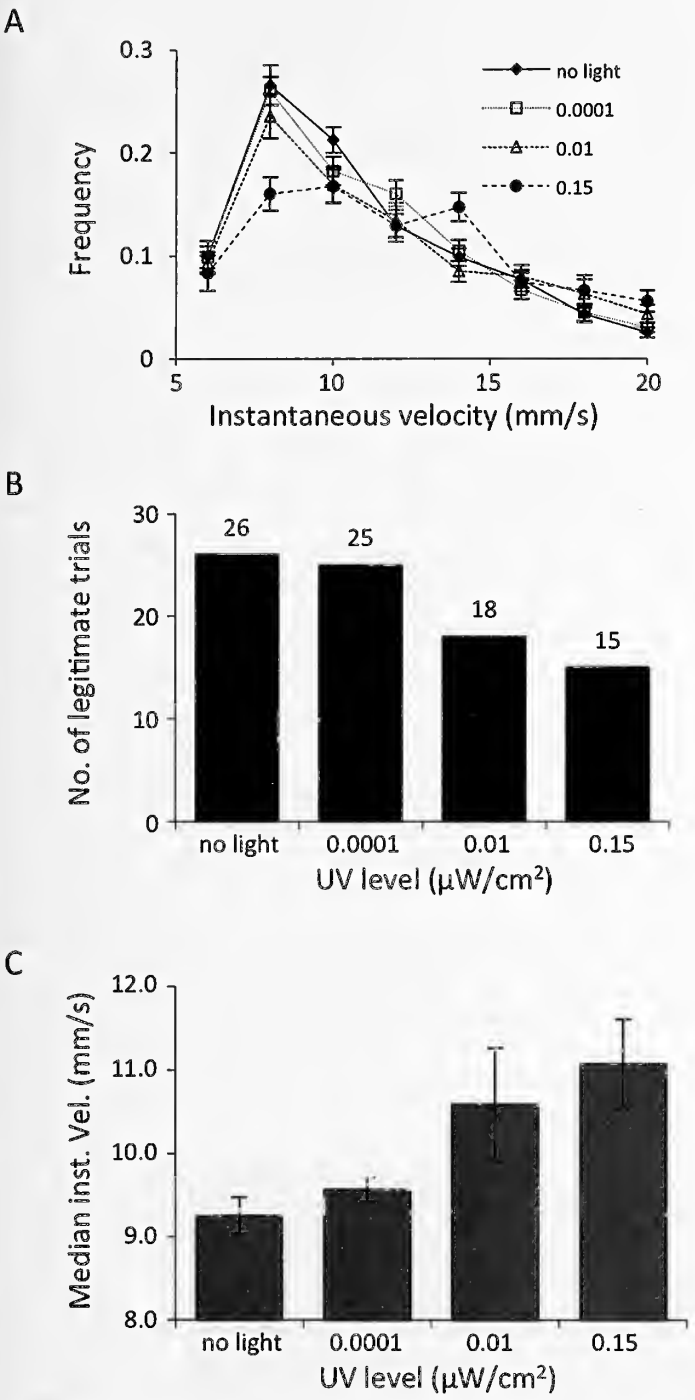

Figure 5.—Composite behavioral responses to various UV levels. **A**. Averaged instantaneous velocity distributions for the four experimental light levels (mean ± SE). **B**. Number of legitimate trials parsed by light level. **C**. Median instantaneous velocities by light level for the seven animals included in our repeated measures ANOVA analysis (mean ± SE).

However, the no-light values for one animal were more than two standard deviations greater than the mean; we therefore removed all trials for this animal from our analyses, reducing our sample size to six. A repeated measures ANOVA showed significant overall differences among the light levels (Fr = 9.000; $P = 0.0218$); there was also a pair-wise difference between the 0.15 μW/cm^2 and no-light treatments ($P < 0.05$).

DISCUSSION

The results of these studies are clear: using a negative phototactic behavioral assay, scorpion locomotor behavior is highest at UV irradiance levels that correspond to sunset. Adding additional indicators, such as the distribution of instantaneous velocities and the number of legitimate trials by light treatment, suggests that the UV response threshold detected by this assay is between the 0.15 μW/em^2 and 0.01 μW/cm^2 light responses. Pooling across all light treatments, we also found a difference in the activity of males and females, which is to be expected since these trials were conducted at the end of the mating season when males are more active on the surface and tracking females (Bradley 1988).

This study demonstrates that scorpions respond differently to different UV levels experienced during the normal activity time of *P. utahensis*, the time at sunset when they normally move to the thresholds of their burrows (Gaffin 2011). Since the assay used in these trials measures scorpion locomotor behavior, the absolute detection sensitivity to UV could be much lower. In addition, these studies suggest that relatively high UV inhibits normal scorpion locomotion, indicated by the low number of legitimate trials under 0.15 μW/cm^2 and 0.01 μW/cm^2 levels. This inhibition affects the sensitivity of the assay because many animals did not move at all during the 10-min trials at high UV irradiation. Those data were dropped from the analyses (59 of 92 trials = 64%).

Several recent studies suggest that scorpion cuticle plays a role in UV detection (Kloock et al. 2010; Camp & Gaffin 1999; Blass & Gaffin 2008; Gaffin et al. 2012). Kloock et al. (2010) found that scorpions that had their fluoreseence compromised by photo-bleaching made more transitions between UV light exposed and unexposed regions of Petri dish arenas than untreated scorpions; also, fluorescent scorpions reduced their activity under UV light at intensity levels similar to what we present here. The authors discuss the possibility that scorpion fluorescence is related to the detection of moonlight and the decision to avoid foraging on nights with high moon illumination; scorpions are less active on the surface during moonlit nights than during moonless nights (Skutelsky 1996). However, our results do not support this notion since animals under UV levels that match the UV composition of full moon nights showed no difference in behavior from animals under no-light conditions. This does not mean that the animals are not detecting and using UV at full moon levels; it simply suggests that it does not act as a deterrent.

Gaffin et al. (2012) found similar loeomotor responses of scorpions under UV and green wavelengths at the 0.15 μW/cm^2 intensity and differences in behavior under the two wavelengths when the eyes were covered. The behavior of eyes-blocked animals changed more when exposed to green light than to those animals exposed to UV light, suggesting a possible role for the fluorescent cuticle in UV detection. Gaffin et al. (2012) suggested the cuticle could serve as a whole-body light detector for purposes of finding shelter. That is, shading of any part of the cuticle stimulated by UV would represent overhead shelter (such as a twig or blade of grass), and a reflexive turning toward the shaded side would move the animal's body under the shelter.

We made several changes to earlier behavioral assay protocols to improve the efficiency of the trials. Most

significantly, we dramatically improved the scorpion image by removing interference from the Plexiglas stage and glare from direct IR projection of the recording camera. By using diffuse IR from the side, the image cleared to a point that we could easily detect scorpions using the public domain ImageJ image-processing program. Once resolved from the background, the animals were accurately tracked via Fiji's Mtrack2 plugin. Automated tracking greatly reduces scoring time and removes the potential for human bias. We think similar tracking will be useful for additional scorpion studies, including those behaviorally testing for and identifying chemicals that make up scorpion pheromone secretions (Taylor et al. 2012).

Additional steps need to be taken to determine whether scorpion fluorescence has an adaptive function in UV detection. Although our assay has been useful for detecting a response and a potential deterrence threshold, it is also laborious, time consuming and requires a large number of trials to register an effect. It would be helpful to develop a behavioral assay that measures individual responses to light of various intensities and wavelengths, perhaps focused on various body regions. Also, it could be useful to reduce the fluorescence through bleaching (Kloock 2009) or other means to see if the behaviors we observe can be compromised. Finally, some members of the family Chaerilidae Simon have been recently reported to lack the fluorescence phenomenon (Lourenço 2012). These animals could be useful in comparative light detection assays with normally fluorescent animals.

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SHORT COMMUNICATION

Fine structure of the stinger (aculeus) in *Euscorpius*

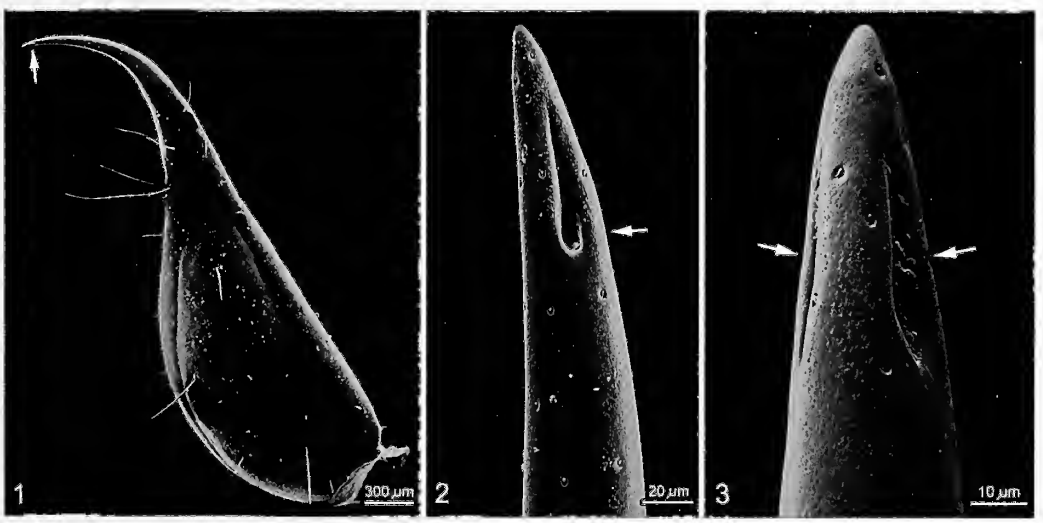
Rainer Foelix, Bruno Erb and **Matt Braunwalder**: Neue Kantonsschule Aarau, Biology Department, Electron Microscopy Unit, Zelgli, CH-5000 Aarau, Switzerland. E-mail: r.foelix@gmx.ch

Abstract. A scorpion's last metasomal segment (telson) consists of a bulbous base that contains two venom glands and a curved tip (aculeus) where two venom ducts open to the outside. These two openings lie laterally just before the very tip of the aculeus; to see both of them at the same time, the stinger has to be looked at "tail-on" from the dorsal side. The two venom ducts have a distinct cuticular lining, which can be recognized in a transparent exuvia as long tubes (1 mm) extending from the distal pores back to the venom glands. Whereas the proximal bulb has many long sensory hairs on its surface, the distal aculeus is very smooth but contains small pits with tiny club-shaped hairs. These are probably contact chemoreceptors. The advantage of such sunken sensory hairs is certainly that the stinger can penetrate into prey (or foe) but can still perceive mechanical or chemical stimuli. Additionally, the aculeus bears several slit sensilla and numerous fine pores of unknown function. The aculeus is thus not only a well-adapted injection device but also contains sensory structures, which provide information on mechanical and chemical input.

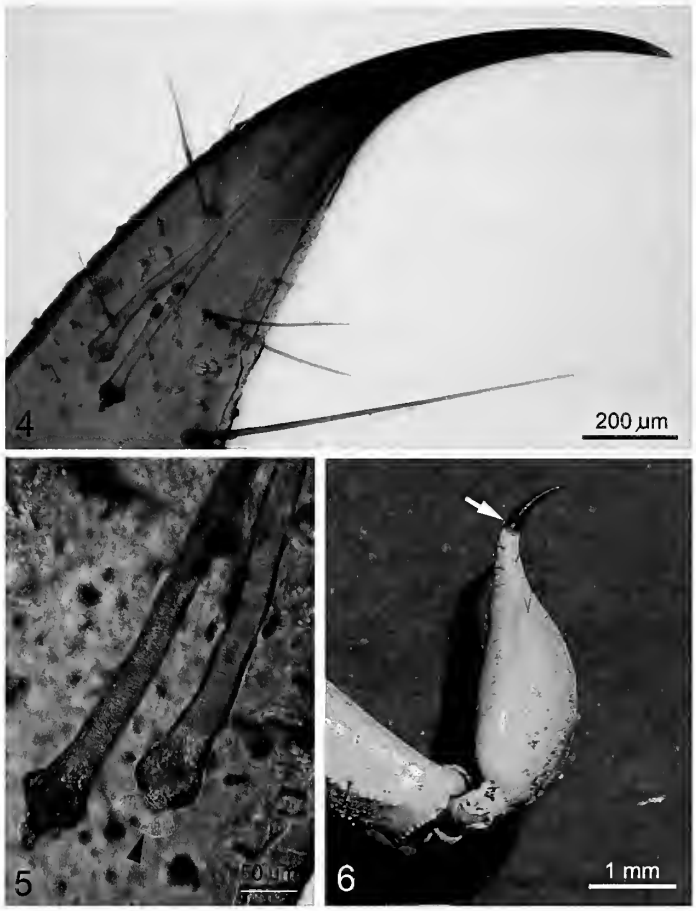
Keywords: Scorpions, stinger, aculeus, fine structure

It is common knowledge that a scorpion delivers its venomous sting with the tip of its tail (Hjelle 1990; Braunwalder 2005; Mahsberg et al. 2012). However, many arachnologists are unaware that there are in fact two small venom openings near the very tip. This is understandable because these pores are quite small (less than 10 µm in diameter) and hard to detect within the dark tip of the stinger. It is remarkable that some of the very early microscopists had already noted both openings. For instance, Antoni van Leeuwenhoek (1700) described in a letter to the Royal Society: "... *when we observe the sting through the magnifying glass, we find that the sting has an opening on either side close to the sharply pointed part* ...". A more detailed picture was given by Maupertuis (1733) by conducting a small experiment, namely squeezing the bulb of the stinger: "*Si l'on presse fortement la fiole, ...on voit la liqueur qu'elle contient, s' échapper à droite & à gauche, par ces deux trous.*" (If one presses the bulb strongly ... one can see the liquid that it contains, exiting on the left and right side, through two holes.") Later, Joyeux-Laffuie (1883) made histological sections of the stinger (telson) and provided illustrations of the tip (aculeus) in lateral and dorsal views. Modern microscopical techniques have rarely been used to examine a scorpion's stinger, perhaps with the exception of a SEM (scanning electron microscope) picture showing both venom openings with congealed venom oozing out (Farley 1999). We have looked at the stinger of several species of *Euscorpius* using light microscopy and SEM to illustrate the dual opening clearly, and to study the overall organization of the exterior and interior of the aculeus.

We used mostly exuviae from *Euscorpius flavicaudis* De Geer 1778, but also from the species *E. italicus* Herbst 1800, *E. germanus* Koch 1837, *E. alpha* Caporiacco 1950 *and E. tergestinus* Koch 1837. All specimens were from scorpions bred in captivity by one of the authors



Figures 1–3.—1, Lateral view of the last tail segment (telson) in *E. flavicaudis* showing a bulbous base proximally and the curved stinger (aculeus) distally. The venom exits near the tip of the aculeus (arrow). Several sensory hairs cover the bulb, but are lacking on the smooth aculeus. 2, Aculeus tip laterally (*E. flavicaudis*), showing a subterminal venom opening (arrow). A corresponding opening would be visible on the other side. 3, A dorsal view of the aculeus tip (*E. flavicaudis*) shows two elongated venom openings side by side (arrows). Note several small dimples, containing very short sensory hairs.



Figures 4–6.—4, A bleached stinger (*E. flavicaudis*) reveals a rather solid tip and two cuticular tubes inside, which originate from the two venom glands. 5, Higher magnification of the initial part of the venom ducts. A very delicate collar (arrowhead) makes the connection to the distal end of the venom glands lying inside the bulb of the telson. 6, UV illumination of the telson (*E. italicus*) causes a bright green fluorescence, except for the distal stinger, which remains black. Note the sharp borderline (arrow) between the fluorescent and the non-fluorescent part of the stinger. (Photo by Bastian Rast).

(MB). For light microscopy, stingers were bleached in lactic acid for several hours, washed, and then immersed in 70% alcohol for bright field, dark field and phase contrast examination. Photographs were taken with a digital camera (Canon 600D) attached to a Leitz Diaplan microscope. For scanning electron microscopy (SEM) stingers were dissected under 70% alcohol, then dehydrated in acetone, and after air-drying, earefully mounted and oriented on aluminum stubs. After sputter coating with gold they were examined in a Zeiss DSM 950; digital photographs were taken at 20–5000x.

The last tail segment, the telson, measures 5–6 mm in length in *Euscorpius* species. Its base is bulbous and contains the two venom glands; its distal end, the aculeus, is curved and narrows into a needle-like tip. Microscopic examination shows that the bulb is covered with sensory hairs, mostly on the ventral side. In contrast, the distal aculeus appears smooth and does not bear long sensory hairs (Fig. 1). However, at high magnification small dimples containing tiny club-shaped sensilla become visible (Figs. 2, 3); they are surrounded by numerous tiny pores (ca. 0.1 µm diameter; Figs. 3, 7, 9), which can only be discerned under the electron microscope. These nanopores occur only on the distal half of the aculeus and may be related to an equally restricted network of nanometer canals, which are involved in the deposition of heavy metals (Schofield et al. 2003; see below).

The most conspicuous features of the aculeus are the venom openings, which are located laterally, about 0.1 mm away from the very tip of the stinger. Normally, only one opening is visible, because the stinger is usually seen from the side (Fig. 2). Only if the telson is viewed from behind ("tail-on") can both venom openings be observed in one picture. Although this can be convincingly demonstrated with the SEM (Figs. 3, 7), it is almost impossible with the light microscope. Firstly, the diameter of these pores is only 6–7 µm, and secondly, the cuticle of the aculeus tip is rather dark and almost solid, thereby obscuring fine structural details. We were partly successful, however, if we used preparations that had been bleached for several hours in lactic acid. In those cases one can vaguely see the actual venom openings near the stinger's tip and, much more clearly, the two venom ducts inside the aculeus, leading back to venom glands (Figs. 4, 5). This distinct visibility is due to a cuticular lining of the venom ducts, which is preserved during ecdysis. These ducts are about 1 mm long and 30 µm in diameter; the wall thickness is about 1 µm. It appears that each duct is connected to the distal end of the venom gland by a fine cuticular collar (Fig. 5).

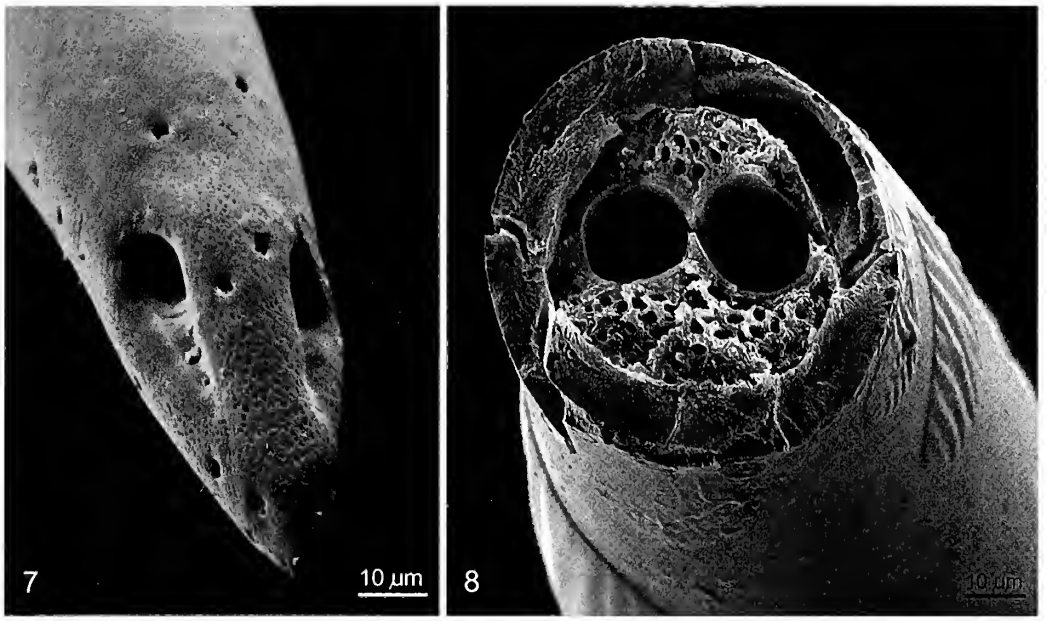
Because we did not know whether the two venom ducts would eventually fuse distally into one channel, we cut off the very end of the aculeus tip with fine scissors and looked at the cross-sectioned stinger under the SEM (Fig. 8). All our preparations exhibited two separate venom ducts, where the medial walls touched but never fused. Both tubes were embedded in a slightly porous cuticle, which was peripherally surrounded by the solid wall of the stinger. Thus, despite its seemingly delicate nature, the aculeus tip must be rather rigid and resistant to mechanieal stress.

As mentioned above, the distal-most mm of the aculeus bears no projecting sensory hairs, but only tiny sunken sensilla and very small pores – both of which are not present on the proximal telson (bulb). However, we did observe a few larger pores that are most likely openings of dermal glands (Fig. 11). And we also found a few slit sensilla with their slits oriented perpendicular to the long axis of the aculeus (Fig. 13). A few short hair sensilla (40 µm long, 4 µm in diameter; Fig. 12) were detected in the proximal part of the aculeus. Based on their morphology (blunt tip, distinct socket), they could be contact chemoreceptors (Foelix & Schabronath 1983; Foelix 1985; Gaffin & Brownell 2001).

A remarkable property of scorpion cuticle is its bright green fluorescence in response to longwave ultraviolet (UV) illumination (Pavan 1954). Although this is true for the entire body cuticle, there is one exception: the distal (black) end of the aculeus does not fluoresee. Under daylight illumination the transition into the dark aculeus tip appears as a gradual change, but under UV light there is a sharp borderline between the proximal fluorescent telson and the completely black, non-fluorescent aculeus tip (Fig. 6). We assume that the cuticle of that distal region lacks the fluorescent substanees (β-carboline and coumarin) that are normally present in the body cuticle (Stachel et al. 1999; Frost et al. 2001). Unfortunately, hardly anything is known about the possible biological significance of fluorescence in scorpion cuticle (Gaffin et al. 2012).

A conspieuous feature of the aculeus is the smooth surface of the last millimeter of its tip. This is certainly advantageous for an easy and relatively deep penetration into the prey's tissues. Any hair sensilla projecting from the surface are restricted to the proximal part of the telson. However, several tiny club-shaped sensilla do occur in the tip region, but they are hidden in tiny dimples. It is quite likely that they represent contact chemoreceptors. Very similar sunken sensilla were found on the jaws (maxillae) of ant lions, which are also used for venom injection into prey (R. Foelix unpublished results).

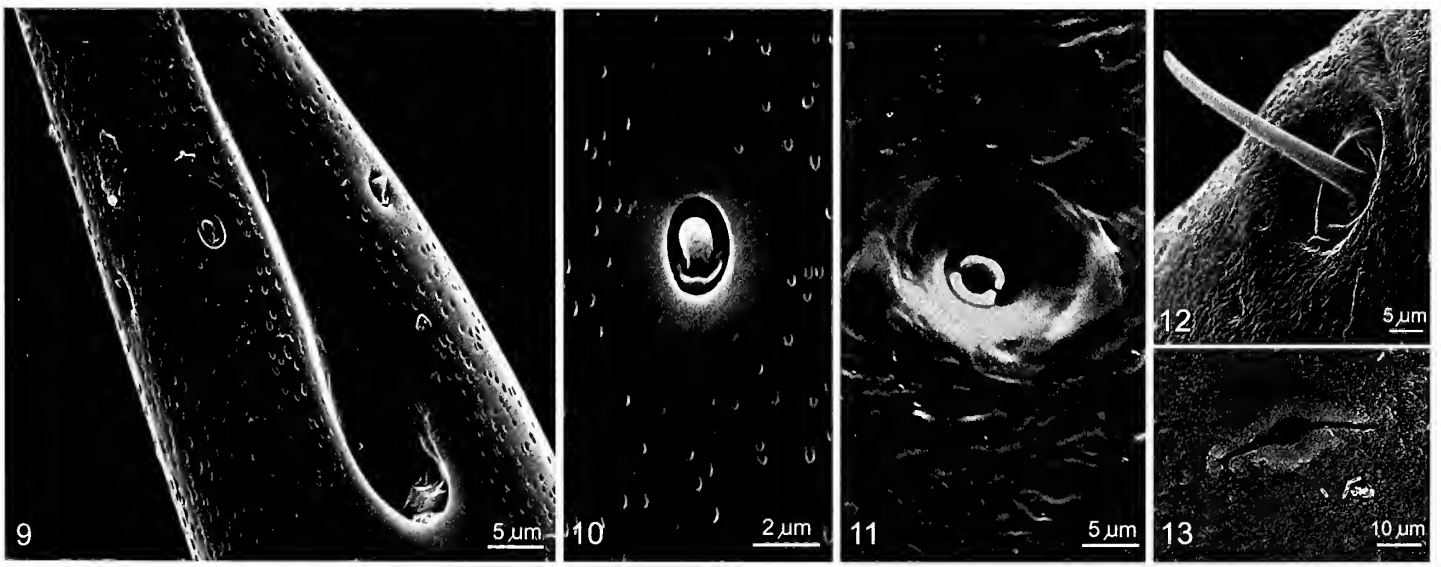
Additionally, there are several single slit sensilla embedded in the cuticle of the aculeus, which provide information on mechanical strain. The aculeus is thus not merely an injection device, but is also able to integrate mechanical and chemical stimuli. This is also indicated by the presence of a telson nerve running between the two venom ducts (Farley 1999).



Figures 7, 8.—7, A posterior view of the aculear tip (*E. flavicaudis*) showing the two venom openings and small dimples containing sunken sensory hairs. 8, If the very tip of the aeuleus (*E. italicus*) is snipped off with scissors, the two venom ducts are seen in cross-section; they are surrounded by a porous cuticle inside and a solid cuticle wall in the periphery.

The funetion of the dermal glands on the telson is not really known, but it is possible that they play a role in courtship. During early courtship the male scorpion often stings the female into her joint membranes, leaving the aculeus there for up to 10 min (Francke 1979). It is not clear whether this implies an actual venom injection or a transfer of other chemical substances, which may originate from the aculear dermal glands. Another possibility is that these dermal glands produce a sex pheromone in females; it has been reported that female cuticle extracts induce courtship patterns (tail wagging and pedipalp reaching) in male scorpions (Gaffin & Brownell 1992).

The venom ducts are lined by a thin cuticle and are firmly enclosed by an inner porous and an outer solid cuticle. Thus, the aculeus tip is rather solid and hence resistant to mechanieal damage—a feature that was already pointed out in early descriptions ("*fort dur*"; Maupertuis 1733). On the other hand it was also claimed that this hardness makes the stinger brittle and that the very tip can easily break off (Joyeux-Laffuie 1883). However, our own observations on hundreds of scorpions showed only a few instances of broken tips and we therefore conclude that the aculeus tip is not that vulnerable. An increased hardness of certain cuticular parts in arthropods is often achieved by



Figures 9–13.—9, Close-up of one venom opening (*E. flavicaudis*), three sunken sensory hairs and many small cuticular pores. 10, High magnification of Fig. 9, showing a sunken sensory hair with a folded joint membrane at its base. 11, Relatively large pores with a split duct in the center most likely represent openings of dermal glands. 12, Relatively short sensory hairs projecting from a distinct socket occur on the proximal aculeus but not in the distal tip region. 13, Several slit sensc organs are found on the stinger's surfacc, lying perpendicular to the long axis of the telson.

an accumulation of heavy metals (zinc, manganese, iron); for example, in insect mandibles. This has also been demonstrated in scorpion mouthparts, tarsal claws and the stinger (Schofield 2001, 2005). Since it is known that zinc incorporation into ant mandibles increases their hardness about three-fold (Schofield et al. 2003), it is very likely that the high zinc or manganese concentration in the scorpion's aculeus will also render it more resistant to wear and tear. Interestingly, this distal region of heavy metal accumulation is exactly the same region that is non-fluorescent under UV illumination, yet so far, we do not know whether there is any causal relationship between those two phenomena.

It is noteworthy that the venom openings are loeated subterminally; that is, not at the very tip as in a pipette, but on both sides of the aculeus (almost 100 μm from the tip). This arrangement is well known from other injection devices, such as the cheliceral fangs of spiders, the venom teeth of vipers or in hypodermic needles (Foelix 2011). From a technical viewpoint this is a superior solution because a lateral pore opening is mechanically more stable and cannot be clogged by tissue when pushed into the prey.

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We are indebted to Samuel Furrer (Zoo Zürich) for pointing out the lack of fluorescence in the tip of the scorpion's stinger, and to Bastian Rast for taking excellent photographs of stingers under UV light. We are grateful to the Neue Kantonsschule Aarau for letting us use the facilities of their electron microscopy laboratory. And we thank Douglas Gaffin for help with the literature search, and Jerome Rovner and Benno Wullschleger for critically reading our manuscript.

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SHORT COMMUNICATION

Intense leg tapping behavior by the harvestman *Mischonyx cuspidatus* (Gonyleptidae): an undescribed defensive behavior in Opiliones?

Bárbara Crespo Dias, Elene da Silva Souza, Marcos Ryotaro Hara and **Rodrigo Hirata Willemart**[1]: Laboratório de Ecologia Sensorial e Comportamento de Artrópodes (LESCA), Escola de Artes Ciências e Humanidades, Universidade de São Paulo, Rua Arlindo Béttio, 1000 - Ermelino Matarazzo - 03828-000, São Paulo, SP, Brazil

Abstract. We describe for the first time the behavior "Intense Leg Tapping (ILT)" being used in a prey-predator context between the Neotropical harvestman *Mischonyx cuspidatus* (Roewer 1913) and the syntopic spider *Ctenus ornatus* (Keyserling 1877). Previously, the harvestman's repeated fast dorsoventral movements of legs II had only been described during conspecific male-male interactions. We suggest it has a defensive function.

Keywords: Ctenidae, defense, deimatic, harvestmen, spider

Prey species often produce signals toward predators according to the sensory modality these predators use. Several invertebrates and vertebrates use colors or body marks against visual diurnal predators (Eisner et al. 2005; Caro 2009; Stevens & Ruxton 2012); moths produce sounds against nocturnal predators that use echolocation to hunt, such as bats (Conner & Corcoran 2012), and squirrels increase the temperature of their tail specifically against predators sensitive to minimum temperature variation, such as vipers (Rundus et al. 2007). Such signals function either to warn the predator of a potentially dangerous defense or distastefulness (the case of aposematism) or to startle the predator (the case of deimatic behaviors), causing it to hesitate or give up attacking (Edmunds 1974).

Ctenid spiders are nocturnal hunters that feed on a variety of arthropods by quickly jumping onto them, biting and injecting venom (Höfer et al. 1994; Wullschleger & Nentwig 2002). Though they may detect visual stimuli, eyes are not necessary to find prey: they rely almost exclusively on substrate and air borne vibrations and air displacement to catch prey detected by the very sensitive metatarsal organs and trichobothria on their legs (Barth 2002). Therefore, if a prey was to use any deimatic behavior or send warning signals to such spiders, one could expect it to use air displacements or vibrations.

Harvestmen are known to defend themselves in several ways. The list includes anachoresis, the use of chemical deterrents from repugnatorial glands, fleeing, feigning death, leg autotomy and retaliation (Gnaspini & Hara 2007). A heavy armature has also been shown to be effective (Souza & Willemart 2011; Dias & Willemart 2013) and based on their colorful body, some species have been suggested to be aposematic (Gnaspini & Hara 2007; Pomini et al. 2010). In addition to these, the harvestman *Eumesosoma roeweri* (Goodnight & Goodnight 1943) (Sclerosomatidae) has been shown to avoid chemicals from predators (Chelini et al. 2009). Herein we describe a putative new defensive behavior in the order Opiliones, namely the intense dorso-ventral movements of legs II (Intense Leg Tapping – ILT, sensu Willemart et al. 2009a) of *Mischonyx cuspidatus* (Roewer 1913) against a predator sensitive to air displacements and vibrations, the large wandering spider *Ctenus ornatus* (Keyserling 1877) (Ctenidae) (Fig. 1).

Individuals of the harvestman *M. cuspidatus* and the spider *C. ornatus* were collected at the Reserva da Cidade Universitária Armando Salles de Oliveira, São Paulo State (23°33'S, 46°43'W). They were maintained individually in plastic containers (12 × 8 × 4 cm height for the harvestmen and 20 cm (diameter) × 8 cm height for spiders) with soil on the bottom and cotton rolls for humidity. The harvestmen were fed on wet dog food and the spiders on crickets. Temperature was ambient (20–25°C) and the light cycle was natural (approximately 12:12 light:dark cycle).

We made the behavioral observations from May to September 2009 (spiders: six subadult males and 36 adult females; harvestmen: 42 individuals, 23 adult males and 19 adult females) and again in January 2011 (spiders: 10 females, three adult males and two immature individuals; harvestmen: three adult females and 11 adult males) under dim light, between 1800 and 2300 h. All the spiders were starved for 25–30 days before the observations. Each individual spider and harvestman was used only once, and the sequence was determined in random order. The circular arena used for the tests (20 cm diameter × 8 cm height) had humid soil on the bottom. A spider was introduced into this arena 8 h before the trial to minimize stress, and the harvestman was introduced in a vial as far as possible from the spider, allowed to acclimate for 2 min and then released. We used a Sony Handycam DCR-TRV361 'nightshot' (hand held to allow recording from better angles, only one observer) to record the behaviors related to the approach between the two animals, the physical interaction and the 10 s subsequent to the interaction to determine if the spider would start eating the prey. Results are presented as "mean ± S.D."

We observed four female and eight male *M. cuspidatus* displaying ILT against adult females of *C. ornatus*, out of 56 harvestmen observed. Intense Leg Tapping consisted of very rapid dorso-ventral movements of legs II, with either one or both legs. Harvestmen performed one or several bouts (sensu Lehner 1998) of ILT. The total number of bouts, pooling across all individuals and single and double leg bouts, was 74, 43 pointing toward the spider and 31 not pointing toward the spider. Among harvestmen that displayed ILT, males displayed 1.67 ± 0.5 single leg bouts and 2.6 ± 2.2 double leg bouts per interaction with spiders. Females displayed 1.75 ± 0.95 single leg bouts and 1.25 ± 1.26 double leg bouts per interaction with spiders. There was no difference between males and females (Mann-Whitney test: single leg bouts: $U = 28.0$, $P = 0.938$, $n = 4$ and 9; double leg bouts: $U = 19.0$, $P = 0.283$, $n = 4$ and 8). The mean duration of bouts was 4.96 ± 0.57 s (min: 3.4; max: 6.3) (data combined for all 74 bouts). Six harvestmen did not displace while exhibiting ILT, four moved toward the spider and two moved in the opposite direction. Eight harvestmen made contact with the spider before displaying ILT (in four cases contact was established because the spider attacked the harvestmen) and the remaining four did not. The distance between the anterior portion of the prosoma of spiders and the tip of the closest

[1] Corresponding author. E-mail: willemart@usp.br

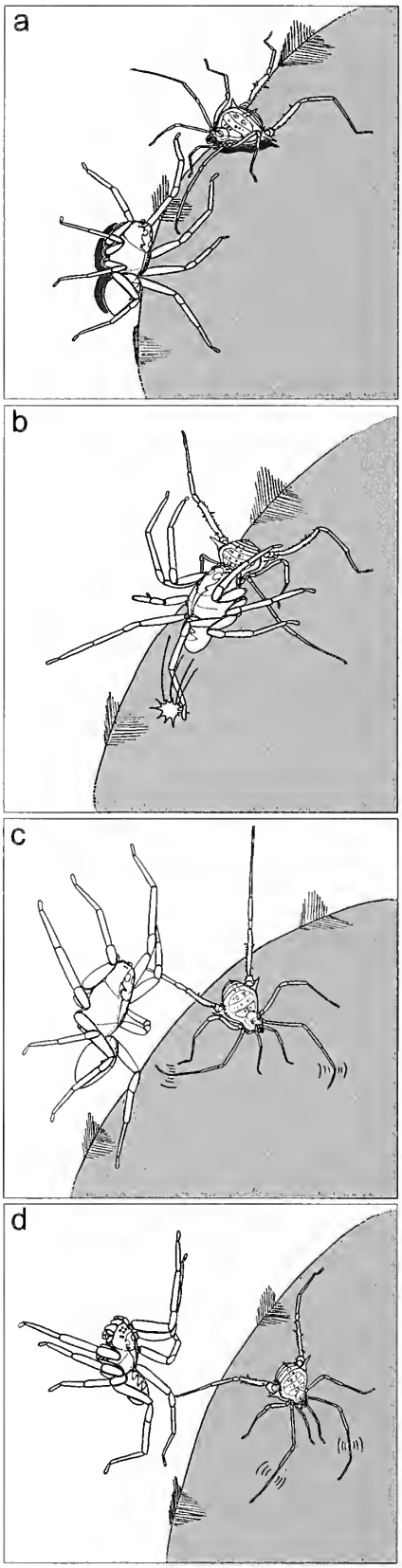

Figure 1.—a) The harvestman *Mischonyx cuspidatus* touches the spider *Ctenus ornatus* with its leg II; b) the spider jumps onto the harvestman; c) the spider retreats and the harvestman starts displaying intense leg-tapping (ILT); d) the spider walks away.

leg of the harvestmen when ILT started was 4.4 ± 2.3 cm (max: 9.3, min: 1.8 cm, n = 12). In six cases, the spider did not move after the harvestman started displaying ILT. Five spiders moved in the opposite direction and one spider attacked.

Intense Leg Tapping is performed during male-male fights in the species *Neosadocus maximus* Giltay 1928 (Gonyleptidae) (Willemart et al. 2009a). The function of the behavior in that context remains unknown, but it was clear that the males only started ILT when fighting with conspecifics. Now that we have observed the same behavior by harvestmen in a predator-prey interaction, a very different context, we suggest that ILT is also a defensive behavior. We propose two main pieces of evidence. First, we have accumulated approximately 340 hours of behavioral observations that were taped in distinct contexts (resting, walking, interacting with conspecifics of both sexes, interacting with heterospecifics, foraging, etc.), of several harvestmen species (at least 13), in addition to field observations since 1999 that were not taped (R.H. Willemart and members of our laboratory, personal observations). Intense leg tapping had never been observed except in the context of male-male fighting and after making contact with a predator (see Willemart et al. 2009a; this paper). Second, the sensory modality exploited by the harvestman when displaying ILT is exactly what these spiders use when hunting, namely air displacement and vibrations (Barth 2002 and references therein). It could therefore be analogous to animals that use color, sounds or variation in body heat as a deimatic behavior or when signaling to predators that use each of these specific sensory modalities to hunt (Stevens 2007; Rundus et al. 2007; Ruxton 2009).

In almost half of the bouts in which ILT was observed, the behavior was not directed toward the spider. We believe this reflects the limited sensory abilities of the harvestmen and its inability to accurately detect nearby arthropods (Willemart et al. 2009b). We unfortunately do not have solid data on how the predator is affeeted by ILT. This is also the case for several mechanisms that have been considered to be defensive in harvestmen, such as nipping behavior, pinching with chelicerae and pedipalps, tanathosis and aposematism (Gnaspini & Hara 2007 and references therein). The fact that ILT has also been observed in conspecifie fights is not evidence against the defensive hypothesis. Deer, antelope, and other mammals with horns or antlers use these weapons in male-male fights and for defense (Andersson 1994). Since both sexes of *M. cuspidatus* have been observed performing ILT, ILT in the sexual context (only males have been observed doing it) could be an exaptation (sensu Gould & Vrba 1982) of ILT in the defensive context. This would be more parsimonious than to believe that it first appeared in a sexual context among males, after which they also started using in a defensive context, after which it then appeared again in females for defensive purposes.

An alternative hypothesis could be that these movements have a sensory function and that harvestmen are actually trying to gain information about the predator. Based on previous studies on the sensory biology of harvestmen (e.g., Willemart & Chelini 2007; Willemart et al. 2009b), this would be very unusual since laniatorid harvestmen use slow waving movements of legs I and II in the air or gently tap the substrate when stimuli are provided. These are very different from ILT.

Another alternative hypothesis is that ILT against predators is a displaced, out of context behavior with its origin in male-male fights, but that females also exhibit this behavior. Further studies with observations of ILT in multiple contexts would weaken the defensive hypothesis and maybe strengthen the "displaced behavior" hypothesis.

We have documented that ILT occurs in the context of prey-predator interaetion. The defensive hypothesis could be further tested by examining exactly what stimuli trigger it or how it affects their predators, but alternative hypotheses should not be discarded at this point.

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SHORT COMMUNICATION

Seasonal patterns of microhabitat selection by a sub-tropical whip spider, *Phrynus longipes*, in the Luquillo Experimental Forest, Puerto Rico

Caroline A. Curtis[1] and **Christopher P. Bloch**[2]: [1]Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, Amherst, Massachusetts 01003 USA. E-mail: cacurtis@cns.umass.edu; [2]Department of Biological Sciences, Bridgewater State University, Bridgewater, Massachusetts 02325 USA

Abstract. *Phrynus longipes* (Pocock 1894) is a top predator among arboreal invertebrates in the Luquillo Mountains of Puerto Rico, but many aspects of its ecology remain poorly understood. We sampled four of the most abundant tree species in the Luquillo Mountains during the dry and wet seasons of 2008 to evaluate microhabitat preferences of this species. In the dry season, *P. longipes* occurred significantly less frequently on a palm, *Prestoea acuminata* var. *montana* (Arecaceae), than the other tree species. Carapace length and the diameter of the tree on which an individual was found were positively correlated, suggesting competition for substrates. Microhabitat selection shifted in the wet season. Individuals occurred as frequently on *P. acuminata* as on any other species. The seasonal shift in substrate use could result from altered distribution or abundance of prey, an ontogenetic shift in substrate preference or greater competition arising from an increased abundance of *P. longipes*.

Keywords: Amblypygi, resource availability, competition, fitness, arboreal predators

Habitat selection contributes to individual fitness by affecting the ability to acquire resources, survive and reproduce (Rosenzweig 1981; Huey 1991). Furthermore, intra- and interspecific competition influence the ability of individuals to occupy optimal habitats (Whitham 1980; Stamps 1991). Habitat selection is likely to be affected by temporal changes in food availability, reproductive activity and the emergence of young, all of which occur seasonally (Wolda 1978). Therefore, a comprehensive understanding of habitat selection requires comparisons among seasons.

Whip spiders (Amblypygi) are nocturnal ambush predators that use vertical substrates, such as trees or rocks, for hunting and nearby crevices as mating or retreat sites. Adults of many species defend a small home range near the structure they employ for hunting (Weygoldt 2000). Patterns of habitat use are poorly known for most amblypygid species, but some factors associated with amblypygids that inhabit tropical forests have been identified. In the central Amazon Basin, *Heterophrynus longicornis* (Butler 1873) occurs most often on large trees with buttressed roots (Dias & Machado 2007). In contrast, Porto & Peixoto (2013) found that individuals were more likely to maintain their territory on a tree if a burrow was present at its base, regardless of tree size. The presence of termite nests at a tree base is another indicator of higher *H. longicornis* abundance (Carvalho et al. 2012). In Costa Rica *Phrynus parvulus* Pocock 1902 selects habitats based on tree surface area, the presence of buttresses and moss cover (Hebets 2002). Large trees with buttresses might be preferable if they provide high prey density in the deep leaf litter between roots (Heyer & Berven 1973), or areas for mating or retreat sites.

Phrynus longipes (Pocock 1894) inhabits caves and forests on Hispaniola, Puerto Rico and the Virgin Islands (Quintero 1981), but relatively little is known about its ecology or behavior. A previous study of *P. longipes* in the Luquillo Mountains of northeastern Puerto Rico found individuals most often on the sierra palm, *Prestoea acuminata* var. *montana* (Arecaceae) (Bloch & Weiss 2002). This is unusual compared to the habits of other whip spiders, as *P. acuminata* has a relatively small diameter compared to other trees in this forest and lacks buttresses. Two explanations could account for this unexpected pattern of habitat use. First, neither the sampling methodology nor the statistical analyses of Bloch & Weiss (2002) explicitly accounted for tree species abundance, so *P. longipes* may have appeared to prefer *P. acuminata* simply because it was the most abundant substrate within the sampling plots. Second, the extensive anthropogenic and natural disturbance history of northeastern Puerto Rico might contribute to unique habitat characteristics and population dynamics. Until the 1930s, much of the land below 600m was used for farming, logging and coffee cultivation (Thompson et al. 2002). In addition, hurricanes of moderate to high intensity hit the forest every 50–60 years on average, and storms of lesser intensity occur more frequently (Scatena & Larson 1991). This disturbance regime would have necessitated adaptation by organisms to a heterogeneous, frequently changing environment, and could have affected patterns of habitat use.

This study evaluated whether *P. longipes* in the Luquillo Mountains actually selects substrates differently from other species of forest-dwelling whip spider, or if the unusual pattern of habitat use observed by Bloch & Weiss (2002) emerged simply beeause of a sampling bias. We compared use of *P. acuminata* to use of three other abundant trees in the Luquillo Mountains to determine whether *P. acuminata* was occupied more than expected by chance or was simply used in proportion to abundance. We also compared substrate use between dry and wet seasons to assess the effects of seasonality.

The Luquillo Experimental Forest (LEF) is an 11,300-ha reserve in the Luquillo Mountains of northeastern Puerto Rico, and is a site in the National Science Foundation's Long-Term Ecological Research Network (Hobbie et al. 2003). This study was conducted near the El Verde Field Station (18°19′16.83″N, 65°49′10.13″W), in the northwestern region of the LEF, where elevation ranges from approximately 300 to 400 m. Total annual precipitation at the El Verde Field Station averages 335 cm $year^{-1}$ (Heartsill-Scalley et al. 2007). Precipitation is mildly seasonal, with a relatively dry period from January to April (McDowell & Estrada Pinto 1988). For 1975–1989, average monthly precipitation for the dry (January–April) and wet (May–December) seasons was approximately 22 cm and 35 cm, respectively.

To determine the most dominant tree species in the area we sampled based on basal area (Thompson et al. 2002). From these common species, we selected four of the most abundant within our sampling area, each of which has a distinct root system. *Prestoea*

Table 1.—Data for each of the two sampling periods are presented. The number (n) of each tree species (Cs, *C. schreberiana*; Mb, *M. bidentata*; Pa, *Prestoea acuminata*; Sb, *Sloanea berteriana*) sampled, DBH for each of the four species and two seasons are included, as well as values combined for both sampling periods. The number of adult and juvenile *P. longipes* recorded on each species is also listed by tree species and season.

					Phrynus longipes	
		n	Average DBH (cm)	Maximum DBH (cm)	Adults	Juveniles
Dry Season	Cs	25	18.9	36	1	2
	Mb	55	19.5	59.6	3	1
	Pa	121	10.1	19.1	2	0
	Sb	84	7.2	44.6	8	5
	Total	285			14	8
Wet Season	Cs	56	20.3	41.3	5	7
	Mb	80	18.2	77	3	4
	Pa	214	13.6	46	25	36
	Sb	115	9.2	38.4	4	18
	Total	465			37	65
Combined	Cs	81	19.9		6	9
	Mb	135	18.7		6	5
	Pa	335	12.3		27	36
	Sb	199	8.4		12	23
	Total	750			51	73

acuminata has a tightly packed system of prop roots, *Cecropia schreberiana* (Urticaceae) has widely spaced branching roots that angle off from the main stem, *Sloanea berteriana* (Elaeocarpaceae) develops buttresses as it grows, and *Manilkara bidentata* (Sapotaceae) has a straight trunk devoid of buttresses (Thompson et al. 2002).

Surveys were conducted in March 2008 (dry season) and from June to August 2008 (wet season). Sampling was conducted by two researchers and occurred between 20:00 and 02:00. As many trees as possible of each target species were found, and each was visually inspected up to a height of 2m, including all visible crevices, buttresses, and exposed roots. Total body length (TBL, the length of the abdomen and carapace) of each whip spider was measured using dial calipers (Table 1). Diameter at breast height (DBH) was measured using a diameter tape for all trees, regardless of presence or absence of whip spiders (Table 1).

Currently, there is no documentation of the size at which *P. longipes* becomes sexually mature. A congener, *Phrynus marginemaculatus* Koch 1840, is sexually mature when it has reached approximately half of its average adult size (Weygoldt 2000). Typical adult size of *P. longipes* is roughly 35 mm (Weygoldt 2000), so individuals were considered adults if TBL $\geq$18 mm. All remaining individuals were considered juveniles. Although 18 mm is an arbitrary threshold, changing this value by a few mm did not substantively alter the results.

Chi-square contingency table analyses were used to test the null hypothesis that *P. longipes* occupied tree species randomly (i.e., in proportion to abundance). If there was a significant result, standardized residuals were examined to determine which species contributed most to the overall significant result. If a standardized residual exceeded two standard deviations (i.e., $-2.0 > z > 2.0$), the associated count was considered to be significantly different from the expected.

Pearson's Product-moment Correlation Coefficient was used to test for an assoeiation between TBL of *P. longipes* and the DBH of trees on which individuals were found. For each season, analyses were conducted for all individuals, as well as for adults and juveniles separately. Further analyses assessed the relationship between TBL and DBH separately for each tree species.

Microhabitat selection by *P. longipes* was non-random in the dry season ($\chi^2 = 14.24$, $df = 3$, $P = 0.003$; Fig. 1a). Standardized residuals indicated that whip spiders were observed more frequently than expected on *S. berteriana* ($z = 2.61$) and less frequently than expected on *P. acuminata* ($z = -2.38$). TBL of *P. longipes* was significantly and positively correlated with the DBH of trees on which it was found ($r = 0.68$, $P = 0.001$, $n = 22$) when all individuals were included in the analysis and for adults only ($r = 0.59$, $P = 0.026$, $n = 14$). Furthermore, the significant positive correlation between TBL of individuals and the DBH of *S. berteriana* on which they were found ($r = 0.72$, $P = 0.005$, $n = 13$) suggests that, as occurs in other amblypygid species, larger individuals were selecting and defending territories on larger trees. However, for the remaining tree species, or when only juveniles on all trees were considered, TBL was not significantly correlated with DBH, suggesting that some individuals were unable to acquire or defend territory on the preferred trees. This may also correspond to the relatively small size range observed for the other tree species, especially *P. acuminata*, which rarely exceeded 20 cm DBH.

In the wet season, substrate use was non-random among the four tree species ($\chi^2 = 11.01$, $df = 3$, $P = 0.012$; Fig. 1b). Whip spiders were observed less frequently than expected on *M. bidentata*, the species with the simplest above-ground component of the root system and the least surface area ($z = -2.21$). This was also true if the analysis was restricted to juvenile whip spiders ($z = -2.14$). Other species did not differ significantly in frequency of occupancy. TBL was not significantly correlated with DBH when all individuals and all trees were tested together ($r = -0.05$, $P = 0.642$, $n = 102$) or for adults ($r = 0.03$, $P = 0.857$, $n = 37$) and juveniles ($r = -0.17$, $P = 0.179$, $n = 65$) analyzed separately. However, individuals that occupied *S. berteriana* displayed a positive correlation between TBL and DBH ($r = 0.59$, $P = 0.004$, $n = 22$), while TBL was negatively correlated to DBH for individuals on *C. schreberiana* ($r = -0.63$, $P = 0.029$, $n = 12$). For individuals found on *M. bidentata*, a negative correlation approached significance ($r = -0.69$, $P = 0.085$, $n = 7$), but TBL of individuals found on *P. acuminata* was not significantly correlated to the DBH of the tree on which they were found ($r = 0.03$, $P = 0.828$, $n = 61$).

In the LEF, *S. berteriana* has similar characteristics (i.e., buttressed roots and large DBH) to those of trees that are selected by *H. longicornis* in northern Brazil (Dias and Machado 2007) and *P. parvulus* in Costa Rica (Hebets 2002) and may therefore provide the same putative benefits. Nevertheless, Bloch & Weiss (2002) report that a palm, *P. acuminata*, was the tree most often occupied by

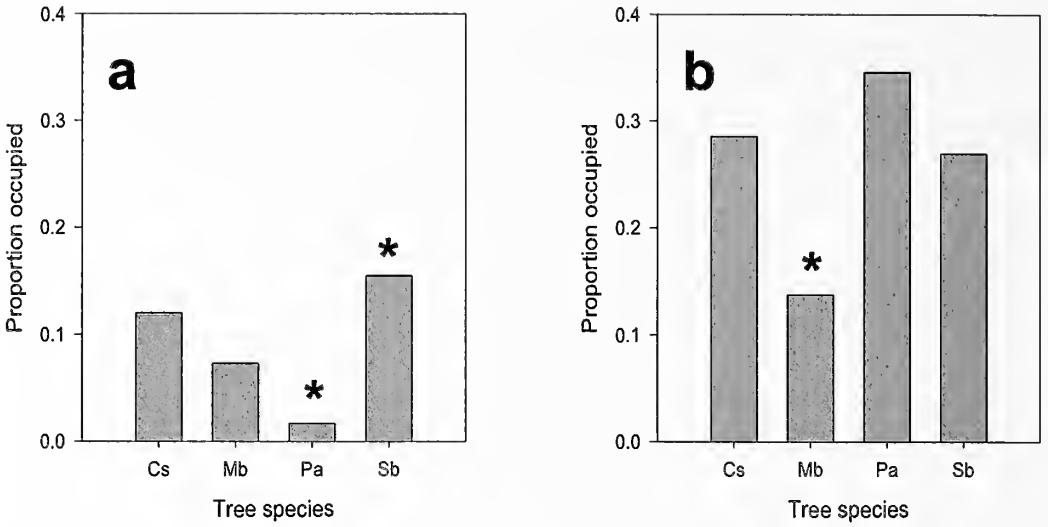

Figure 1.—Patterns of substrate use by *Phrynus longipes* in the Luquillo Experimental Forest. The proportion of individuals of eaeh tree species (Cs, *C. schreberiana*; Mb, *M. bidentata*; Pa, *Prestoea acuminata*; Sb, *Sloanea berteriana*) occupied by *P. longipes*. a. Dry season; b. Wet season. Asterisks above bars indicate significant departures from expected occupancy based on standardized residuals of chi-square contingency tables.

amblypygids in the LEF. We reconcile the observations of Bloch & Weiss (2002) with other studies by demonstrating changes in microhabitat use between sampling periods.

Two lines of evidence are consistent with the hypothesis that large, buttressed *S. berteriana* are preferred substrates for *P. longipes* in the LEF. First, *S. berteriana* was among the most frequently occupied tree species, regardless of season, especially if only relatively large trees are considered. In the dry season, *P. longipes* occupied 86% of *S. berteriana* that measured ≥ 20 cm and 53% that measured ≥ 10 cm, compared to only 7.2% that measured < 10 cm. This pattern was repeated in the wet season, as *P. longipes* occupied 75% of *S. berteriana* ≥ 20 cm, 58% ≥ 10 cm, but only 11% that were < 10 cm. Second, the positive correlation between TBL of *P. longipes* and DBH of *S. berteriana* suggests that larger individuals were best able to defend a territory on large, buttressed trees.

There was a considerable shift in microhabitat use between seasons, however, with individuals avoiding *P. acuminata* in the dry season but using it as frequently as *C. schreberiana* and *S. berteriana* in the wet season. Several reasonable hypotheses may explain this pattern. Abundance or quality of prey may change seasonally, making *P. acuminata* trunks better hunting sites in the wet season than in the dry season. For example, foraging activity of *P. longipes* (Pfeiffer 1996) and insect abundance (Garrison & Willig 1996) are greater during rainy than dry periods, suggesting that higher prey capture rates are possible during such times, even on trees that represent less suitable habitat during dry periods.

Alternatively, decreased use of *P. acuminata* in the dry season may reflect that it is unsuitable for mating, or that an ontogenetic shift in substrate selection occurred. The courtship display of many species of whip spider requires a sheltered, level area (Weygoldt 2000), such as the root surface or the caverns that form under the roots of the most frequently selected trees. The roots of *P. acuminata* generally lack such spaces. The small crevices found between *P. acuminata* roots are, however, useful as retreat sites, especially for small individuals, and may therefore provide higher quality habitat in the non-breeding season and for juveniles.

The population density of *P. longipes* was greater during the wet season than the dry season, as indicated by much higher proportional occupancy of all four tree species (Figs. 1a,b). The increase in density is probably driven by the emergence of juveniles that hatched late in the dry season or early in the wet season. As a result, competition for optimal substrates was considerably lower in the dry season than in the wet season. In the wet season, two adults were observed occupying the same tree once (on *S. berteriana*), and an adult was observed on the same tree as a juvenile in two cases (once each on *P. acuminata* and *S. berteriana*), and multiple juveniles were observed on a single tree for all species except *M. bidentata*. This suggests that food was abundant enough throughout the wet season that it did not influence habitat selection.

To maximize fitness, individuals must respond to geographic and temporal changes in resource availability either by selecting a territory that provides them with high-quality habitat in all seasons or by moving to a different, higher-quality site when resources decline. Here, we provide evidence of a shift in substrate occupation from the wet season to the dry season in a sub-tropical whip spider, *P. longipes*. Given the limitations of this study, further research is needed to determine whether our observations are consistent with a long-term pattern or if the years we sampled represent an anomaly. Nevertheless, our findings are partially consistent with other research on habitat selection by amblypygids, particularly regarding tree selection in the dry season. However, the wet-season shift to occupying what we assume are lower-quality trees indicates that although habitat characteristics such as tree size are likely to determine whether or not a tree is suitable habitat, biotic interactions such as those that occur among *P. longipes* in the wet season are likely to further influence the small-scale distribution of a species.

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SHORT COMMUNICATION

Natural prey of the crab spider *Xysticus marmoratus* (Araneae: Thomisidae) on *Eryngium* plants

Elchin Fizuli oglu Huseynov: Institute of Zoology, National Academy of Sciences, block 504, passage 1128, Baku 370073, Azerbaijan. E-mail: hun-vey-bin@rambler.ru

Abstract. The natural prey of medium-sized juvenile (ca. 3 mm) crab spiders *Xysticus marmoratus* Thorell 1875 inhabiting *Eryngium biebersteinianum* plants was studied on the Absheron Peninsula, Azerbaijan. The percentage of specimens found feeding on prey was low (3.4%). *Xysticus marmoratus* is a polyphagous predator with representatives of four arthropod orders found in its diet. The primary food of *X. marmoratus* was ants (Formicidae), which accounted for 83.3% of the total number of prey. The length of prey killed by *X. marmoratus* ranged between 0.87–7.50 mm (mean 2.96 mm) and constituted from 28.5–300.0% (mean 96.9%) of the length of their captors. The most frequently captured size group of prey was 50–70% the length of the spiders.

Keywords: Flower-dwelling, diet, myrmecophagy, prey length

With over 2000 described species, Thomisidae is one of the largest families of spiders (Platnick 2013). However, despite the family members' great diversity and potential predatory significance, few studies have addressed the natural prey of thomisids. A survey of arachnological literature revealed that quantitative data on the natural diet are available for only fifteen species of crab spiders (Broekhuysen 1948; Nyffeler & Benz 1979; Tarabaev 1979; Morse 1981, 1983; Ricek 1982; Lubin 1983; Dean et al. 1987; Agnew & Smith 1989; Castanho & Oliveira 1997; Schmalhofer 2001; Romero & Vasconcellos-Neto 2003; Guseinov 2006; Huseynov 2007 a, b). Crab spiders (Thomisidae) do not use silk for prey capture; instead, they lie in ambush and wait until prey comes within reach of their long raptorial forelegs (Foelix 1996).

Xysticus marmoratus Thorell 1875 has not previously been the subject of ecological or behavioral investigation. *Xysticus marmoratus* is distributed from Eastern Europe to Central Asia (Marusik & Logunov 1994). It is a small crab spider (adult body length 4–6 mm), with adult males usually slightly smaller than females. In Azerbaijan, *X. marmoratus* occurs in arid habitats, including semi-deserts and steppes. *Xysticus marmoratus* has an annual life cycle. Adult specimens are found in October and November and inhabit grass litter. In contrast, juveniles are very abundant on flowering plants throughout the summer (Huseynov unpubl. data).

This investigation was carried out on the Absheron Peninsula, Azerbaijan. The three primary study sites were located near the villages of Shagan, Gres, and Bina (40°27′30″N 50°04′08″E), where over 95% of the total observation time was spent. Additionally, two secondary study sites were located near Gala village and Ganly-Gyol Lake. The study sites were areas of ephemeral semi-desert covered with dwarf shrubs *Eryngium biebersteinianum* Nevski, *Alhagi pseudoalhagi* (MB), *Noaea mucronata* (Forsk), and several herbs and grasses. The sites near Shagan, Bina, and Ganly-Gyol were additionally characterized by planted pines, *Pinus eldaricus* Medw., while the others were treeless.

All individuals of *Xysticus* observed during the study period were immature. They were abundant only on *Eryngium biebersteinianum*; therefore, observations were concentrated exclusively on this plant. The prey of spiders was sampled during three successive years: 1997 (2 July–9 August), 1998 (14 June–25 July), and 1999 (14 June–31 July). Fifty surveys were conducted during these periods, which took about 113 hours. During the surveys, *E. biebersteinianum* plants were thoroughly searched for *Xysticus*, and the mouthparts of each individual spider found were inspected with a hand-lens of 4× magnification to avoid overlooking small prey. Spiders with prey in their chelicerae were captured with a transparent cup, placed in separate vials containing 75% ethyl alcohol and brought back to the laboratory for measurement and prey identification. Spiders without prey were left in the field. All surveys were done in the daylight hours between 11:00 and 21:00. Most of these surveys were conducted between 11:00 and 17:00. The number of prey collected from *Xysticus* increased after 18:00. Therefore, a five-day series of double surveys was undertaken in July 1999 at Bina. On each of these days, one survey was made during the first half of the day (11:00–16:00) and another during the second half of the day (18:00–21:00).

All *Xysticus* individuals observed in the field were of approximately the same size and had a similar color pattern, suggesting that they belonged to the same species. To identify the species, I collected forty living specimens from *Eryngium* in July 2006 at Bina (where 85% of all spiders were observed in 1997–1999) and reared them in the laboratory until they reached maturity. Spiders were kept separately in glass vials (50 mm long, 8 mm diameter) under a natural light-dark regime. Two types of prey were offered to these spiders. "Innocuous" prey included various specimens of Diptera, and "dangerous" prey were workers of the ant *Lasius alienus* Forster 1850. The prey insects were collected in the garden of the Institute of Zoology, Baku, and only those between 50–100% of the spiders' body length were used in the feeding experiments. Individual spiders were fed three times a week with one of the prey types in a random order. Interactions between spiders and prey were monitored for two hours, and the prey was recorded as accepted if it was captured and consumed by spider during this period. I considered the prey to be consumed by the spider if it was not released immediately and was held in the chelicerae for at least 15 minutes after capture. Thirty-five juveniles survived to adulthood in the laboratory, and all these proved to be *X. marmoratus*. Voucher specimens of *X. marmoratus* and their prey items were deposited in the Institute of Zoology of Azerbaijan Academy of Sciences.

In total, 2,495 speeimens of *X. marmoratus* were observed in the field, 84 of which (3.4%) had prey in their chelicerae. One juvenile was consuming two prey items simultaneously. Thus the actual number of feeding events was 85. Individuals of *X. marmoratus* fed significantly less frequently during the first half of the day (6 prey records of 583 spider observations) than during the second half (38 of 614) ($\chi^2_1 = 21.1$; $P < 0.001$).

One *X. marmoratus* individual dropped its prey before it could be captured, so 84 prey items were collected for dietary analysis. These were distributed among four orders of arthropods, including three from the class Insecta (Hymenoptera, Coleoptera, and Diptera) and

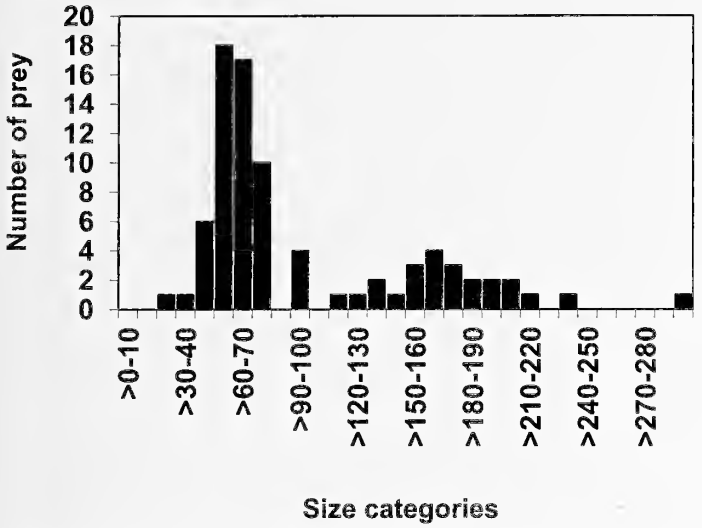

Figure 1.—Distribution of prey of *Xysticus marmoratus* in different size categories (body lengths of prey expressed as percentages of the body lengths of their captors).

one from the class Arachnida (Araneae). By far the dominant prey order was Hymenoptera, which accounted for 92.9% of total prey. Most of the Hymenoptera captured were ants (ca. 90%). They included representatives of subfamilies Formicinae [43 *Plagiolepis* sp., 8 *Cataglyphis aenescens* (Nylander 1849), 3 *Cataglyphis setipes* (Forel 1894), 1 *Stenamma* sp.)], Myrmecinae (7 *Messor denticulatus* Santschi 1927, 7 *Cardiocondyla* sp.) and Dolichoderinae (1 *Tapinoma* sp.). Except for two winged males of *M. denticulatus*, all ants were workers. Other Hymenoptera consisted of three halictid bees (*Halictus* sp., *Nomioides* sp., *Sphecodes* sp.), three stinging wasps (2 Bethylidae and 1 Thiphiidae) and two parasitic wasps (Braconidae and Scelionidae). The remaining arthropods included three adult beetles (2 Dermestidae and 1 Bruchidae), two flies (Chloropidae and Milichiidae) and a conspecific spider (juvenile *X. marmoratus*).

Of 620 ants and 442 dipterans offered to spiders in the laboratory, 542 ants (87.4%) and 397 dipterans (89.8%) were eaten by *X. marmoratus,* the rates of acceptance of these prey groups being very similar.

Eighty-one natural prey items were measured. Their lengths varied from 0.87–7.50 mm (mean ± SD: 2.96 ± 1.77 mm) and constituted from 28.5–300.0% (96.9 ± 57.5%) of the length of their captors, which ranged from 2.25–3.80 mm (3.05 ± 0.30 mm). The size distribution of the prey in relation to the sizes of their captors is shown in Fig. 1. Most of the prey did not exceed the length of their captors (70.4%, n = 57). These included *Plagiolepis*, *Cardiocondyla*, *Tapinoma* and *Stenamma* ants, bethylid and scelionid wasps, as well as beetles, flies, and a conspecific spider. The most frequently captured (60.5%, n = 49) were medium-sized arthropods from 50–100% spider body length, while small prey, not exceeding half the length of the spiders, were represented by only eight items (9.9%). About one third of the prey of *X. marmoratus* consisted of large arthropods exceeding the length of their captors. These prey consisted of *Cataglyphis* and *Messor* ants, thiphiid and braconid wasps, and halictid bees. Many of the large prey (23.5%, n = 19) exceeded 150% of the body length of their captors.

The percentage of *X. marmoratus* individuals found while feeding was low (< 10%), as is typical of cursorial spiders (Nyffeler & Breene 1990) and crab spiders in particular (Nyffeler & Benz 1979; Dean et al., 1987; Romero & Vasconcellos-Neto 2003; Guseinov 2006; Huseynov 2007a). The difference in prey capture rate of *X. marmoratus* at different times of the day is likely related to the fluctuation of ant activity on *Eryngium* throughout the day. In the summer, on the Absheron Peninsula, most ants are inactive during the first half of day, apparently because of high surface temperature, and start to forage only after 18:00, when the temperature decreases. In the evening large numbers of ants, especially *Plagiolepis* sp., appeared on *Eryngium*, which might result in increased prey capture by spiders.

This investigation has shown that *X. marmoratus* is a polyphagic predator feeding on a wide range of arthropods. The heavy prevalence of worker ants in its diet is unusual, since these insects possess effective defensive equipment, such as strong mandibles, a hard cuticle, poisonous stings or formic-acid spray (Blum 1981), making them unpalatable to most cursorial spiders (Nentwig 1986). Although some tropical thomisid species have been reported to feed exclusively on ants (Lubin 1983; Castanho & Oliveira 1997), such a high rate of ant capture (83.3%) has never been recorded for crab spiders from temperate regions. However, worker ants were found in lower proportions in the diets of *Xysticus cristatus* (Clerck 1757) and *Xysticus loeffleri* Roewer 1955 (Nyffeler & Benz 1979; Guseinov 2006). These data suggest that myrmecophagy is a widespread phenomenon within the genus *Xysticus*.

Does *X. marmoratus* prefer ants to other prey? Such a conclusion cannot be derived from dietary data alone. The prevalence of ants in the diet of *X. marmoratus* could be related to their abundance in its habitat. Indeed, at least in the second half of the day, ants were by far the most abundant visitors to *Eryngium*. In any case, the present field and laboratory findings unambiguously indicate that *X. marmoratus* is a quite competent ant-feeder.

Experimental studies of prey size preference in spiders have shown that while most cursorial spiders prefer prey not exceeding their own size, the crab spider *Xysticus cristatus* readily accepted insects two times larger than itself (Nentwig & Wissel 1986). Although *X. marmoratus* sometimes captured very large prey, most of its prey consisted of arthropods smaller than itself. This is in contrast with observations of two other thomisid species, *Thomisus onustus* Walckenaer 1805 and *Runcinia grammica* (C.L. Koch 1837) that also inhabit *Eryngium* plants in the same localities. Over 90% of the prey of these spiders consisted of large insects exceeding the size of their captors (Huseynov 2007 a, b). However, both of these thomisids fed primarily on non-ant prey and did not accept ants in the laboratory as readily as did *X. marmoratus* (Huseynov unpubl. data). Thus the bias toward smaller prey in the diet of *X. marmoratus* is apparently due to the prevalence of small ants in its microhabitat.

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Ubick, D.
Uetz, G.
Valdez-Mondragón, A.
Vetter, R.
Viera, C.
Vink, C.
Weiss, M.
Wilder, S.
Yip, E.
Zaragoza, J.

CONTENTS

Journal of Arachnology

Volume 42 Number 1

Invited Review

Featured Articles

Short Communications