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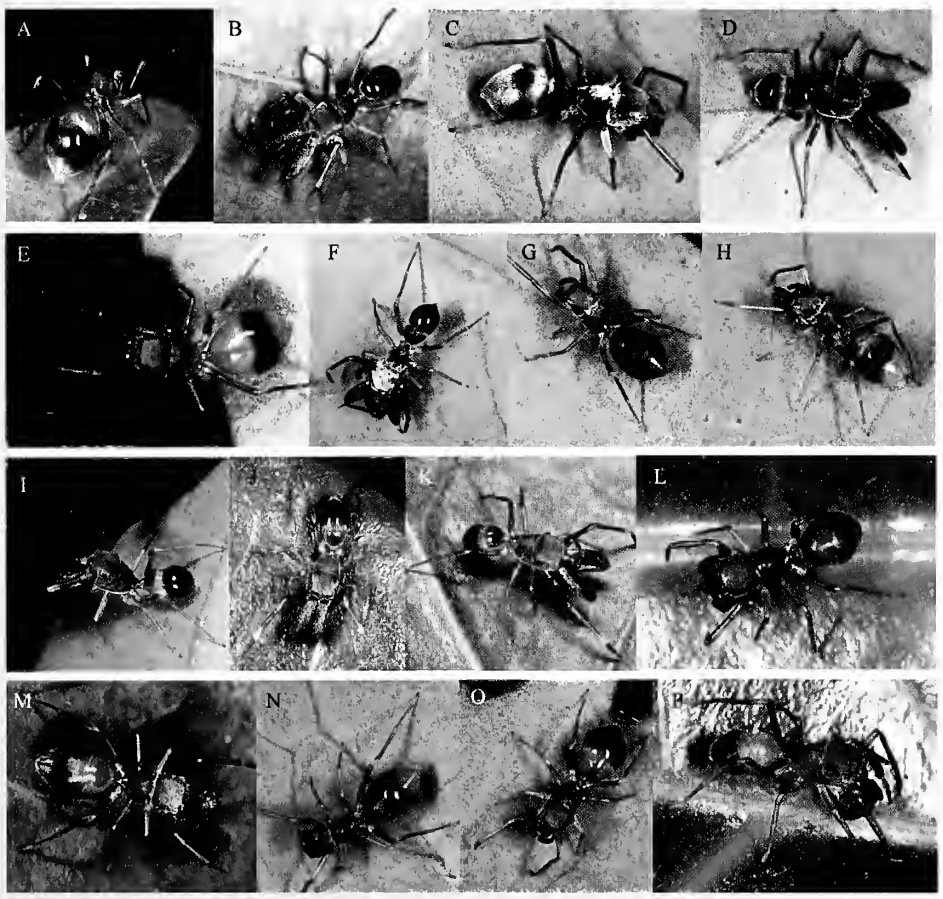

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Cover photo: Polymorphisms in *Myrmarachne bakeri* (Salticidae) at Los Baños, Philippines. Photos by R. R. Jackson.

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Analysis of sensory processing in scorpion peg sensilla

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Abstract. Primary chemosensory afferents within each peg sensillum on scorpion pectines contain a dense plexus of synaptic contacts of unknown importance to informational processing within this simple sensory structure. These connections probably contribute to the processing of chemical signals from the substrate to the encoded pattern of spike activity ascending the pectinal nerves to the CNS. A key finding of earlier studies of this system was the apparent existence of strong and long-lasting inhibitory interactions between one identifiable unit – type "B" cells – and at least two other sensory neurons – identified as "A1" and "A2" – cells within the same sensillum. Because peripheral synaptic interactions are rarely observed between primary sensory neurons, it is important to reject the alternative non-synaptic mechanism to account for the unusual spike waveform of inhibitory B units, namely, that it is derived from coincident discharge of the A1 and A2 units it is presumed to inhibit. High resolution waveform analysis of two or more units firing in close temporal proximity (within about 5 ms) showed unequivocally that type B units occur within the post excitatory period when the A units would be refractory to re-excitation. Furthermore, the number of these B/A1 or B/A2 doublets was in line with the number predicted for the observed spontaneous firing frequency of the B, A1, and A2 units in the peg. This analysis corroborates the original conclusion that B unit activity is the electrophysiological signature of an inhibitory processing event, one that strikingly transforms the information encoded and passed from each peg sensillum to the central nervous system.

Keywords: Synaptic interaction, chemosensory, electrophysiology, pectines

Synaptic coupling between peripheral sensory neurons is uncommon and especially rare among chemosensory afferents (Foelix 1975; Hayes & Barber 1982). For example, in the well-studied antennal systems of insects, the first synaptic interaction between cells appears to be in the antennal lobe of the brain (Bullock & Horridge 1965; Ernst & Boeckh 1983; Kaissling 1987). In non-scorpion arachnids, there is evidence of extensive peripheral synaptic interaction among mechanosensory neurons in spiders (Fabian-Fine et al. 2002) and whip spiders (Foelix et al. 2002; Spence & Hebets 2006), but no indication of interaction between chemosensory cells. The major chemosensory organs of scorpions, the pectines (Cloudsley-Thompson 1955; Ivanov & Balashov 1979), are organized differently. Morphological studies (Foelix & Müller-Vorholt 1983; Foelix 1985) and physiological evidence based on cross-correlation analysis of unit activity (Gaffin & Brownell 1997a; Gaffin 2001) suggest the presence of cell-to-cell synaptic interactions at the level of the first order chemosensory neurons. Further, it appears that these synapses are important in the processing of information prior to relay to the scorpion CNS (Gaffin & Brownell 1997b; Gaffin 2002).

While the morphological and physiological evidence makes a compelling case for the existence of chemical synapses in peg sensilla, the physiological evidence is correlative and indirect. For example, the pattern of inhibition in cross-correlograms could result from indirect effects of other undetected cells in the circuit (Perkel et al. 1975). Alternatively, an electrical coupling of two cells (Hestrin & Galarreta 2005) could generate a novel waveform in extracellular electrophysiological recordings. This novel waveform, when analyzed relative to the two contributing cells, would produce the semblance of an inhibitory effect in cross-correlograms. Could this be the case in scorpion peg sensilla? A further curiosity in peg recordings is that the putative inhibitory cell with the type B waveform has a peculiar inflection or notch in its otherwise highly repetitive waveform, suggesting that it could result from the combination of two coincident and subordinate events (Fig. 1).

To check the validity of previous assumptions, I looked closely at high-resolution recordings from peg sensilla of two species of scorpions where all three units (A1, A2, B) were clearly resolved. I mathematically combined idealized A1 and A2 waveforms, offset at various time intervals, to see if the B waveform is derived by simple summation of the former. I also looked for evidence of A1 and A2 unit discharges in close temporal proximity of B cell firing. If the B waveform is the expression of coupled A1 and A2 activity, then the refractory period of A1 or A2 should preclude their appearance within the B waveform. I calculated an expected number of contaminations of B by A1 and/or A2 based on spiking frequencies of the three cells and compared this to empirical observation. Based on these observations, I conclude that the B waveform does not result from a coupling of A1 and A2. This is consistent with the idea that B is a separate entity from A1 and A2 and that B exerts an inhibitory synaptic influence over A1 and A2 events in scorpion peg sensilla.

METHODS

A high quality, archived recording from a peg sensillum of *Smeringurus mesaensis* Stahnke 1957 (Scorpiones: Vaejovidae; formerly *Paruroctonus mesaensis*) and a new recording from a peg sensillum of *Paruroctonus utahensis* Williams 1968 (Scorpiones: Vaejovidae), collected near Kermit, Texas, USA (31°57′46.44″N, 102°58′53.59″W) formed the data set for this paper. A voucher specimen of *P. utahensis* has been deposited at the Sam Noble Oklahoma Museum of Natural History. The specific methods of the recording techniques are in Gaffin & Brownell (1997a, 1997b). The archive recording was relayed from an audiocassette tape through digitizing hardware (1401-plus, Cambridge Electronic Design (CED), Cambridge, UK) at 20 kHz sampling rate to a computer for analysis. I relayed the new *P. utahensis* recording directly from the preparation

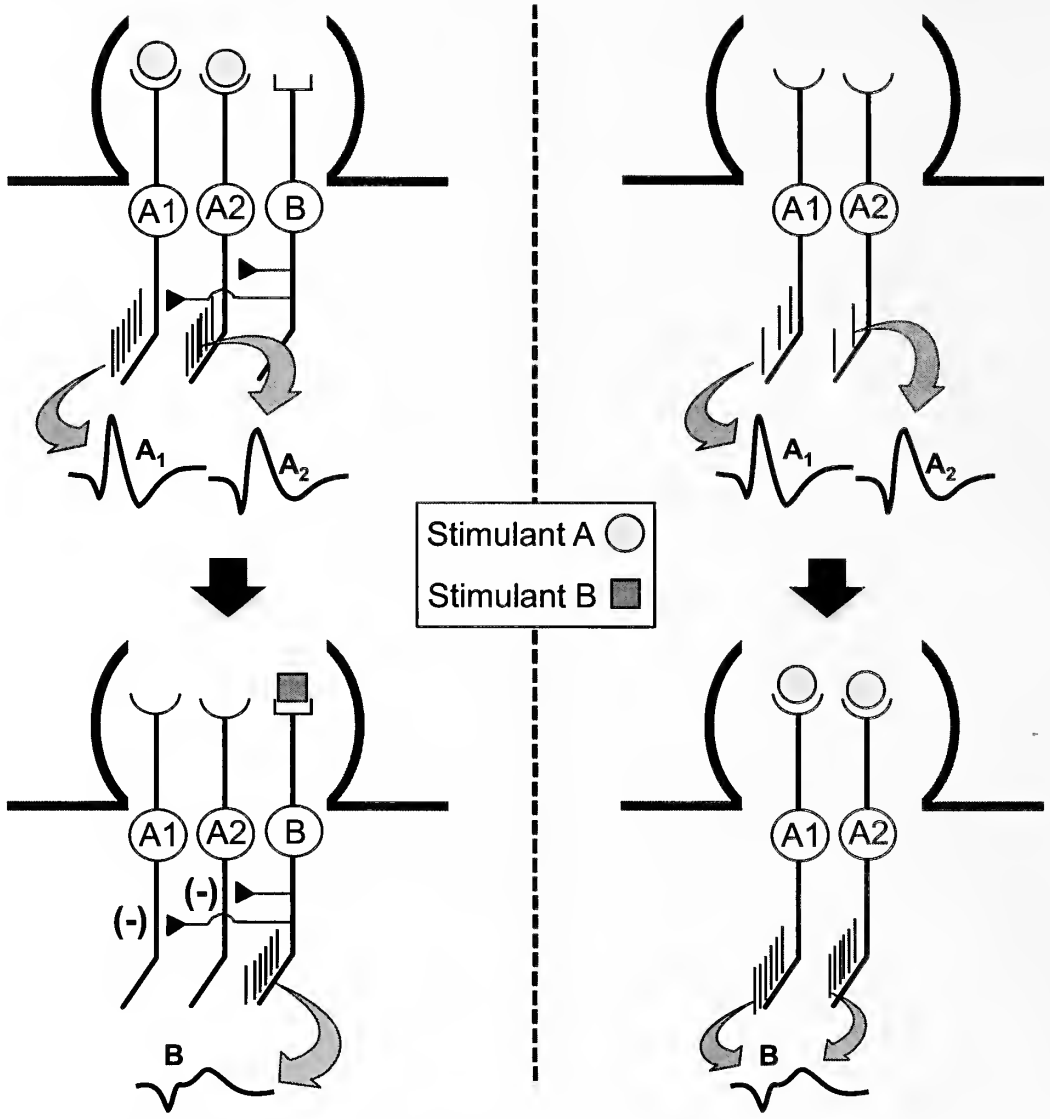
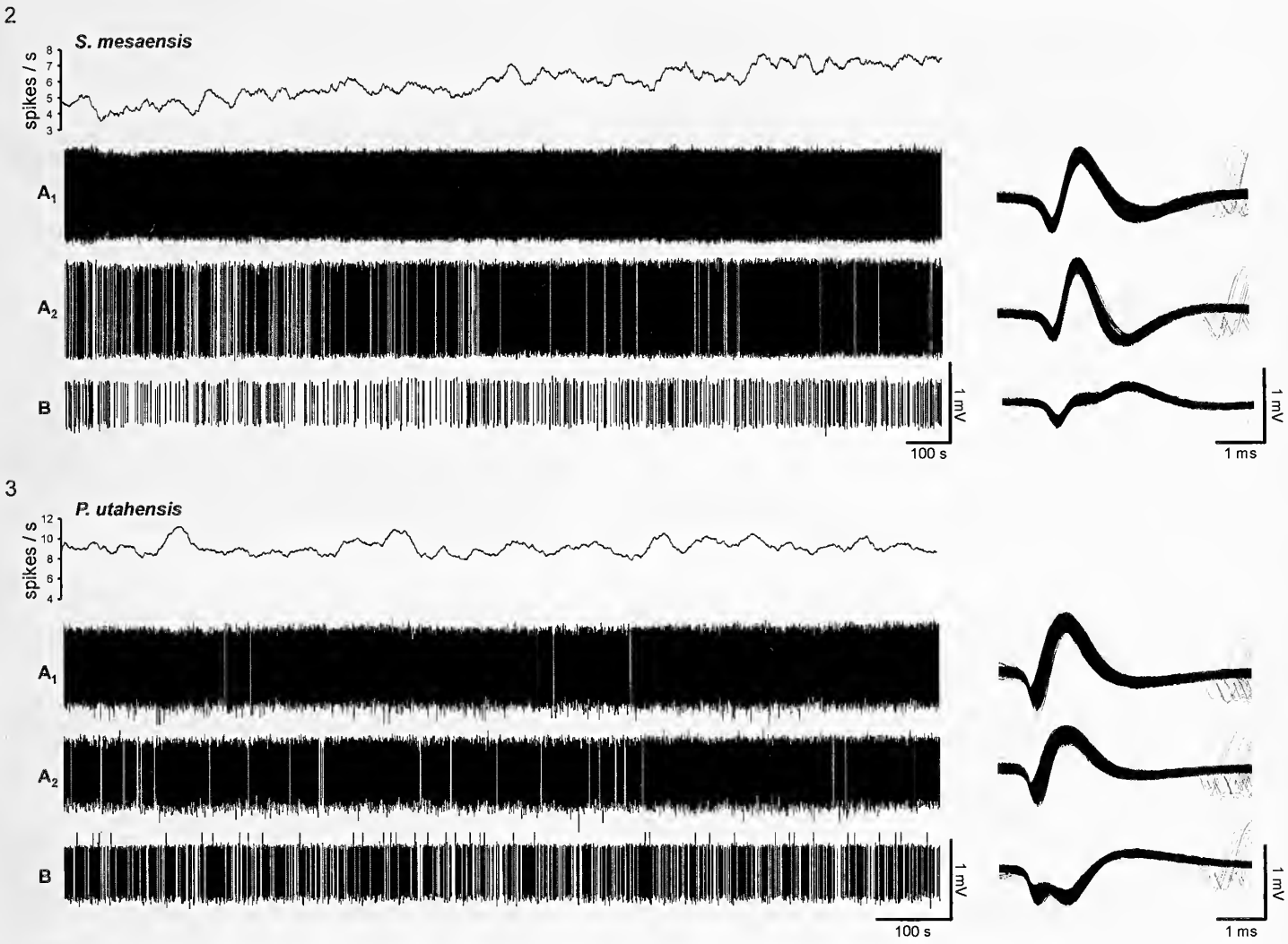


Figure 1.—Alternative interpretations of electrophysiological recordings from peg neurons. At left is a situation with three cell types A1, A2, and B where B has an inhibitory influence on both A1 and A2; each of the three neurons produce their own distinct waveforms. When the B cell is stimulated, it inhibits A1 and A2 and produces its own triphasic type B waveform. At right is a situation with only two active cells, A1 and A2. When A1 and A2 firings are coincident (as with simultaneous stimulation) their waveforms merge to produce the triphasic type B waveform.

through the digitizing software to the computer using the same settings. I used Spike 2 software (version 3.21, CED) to capture and analyze the spiking events in the records.

High-quality spike classification was necessary to support the findings of this study. The Spike 2 template matching parameters most effective in resolving sensillar waveforms included: 1) at least five similar spikes for a new template; 2) new template width 20% of amplitude; 3) no templates rarer than 1 in 150; 4) 20% maximum amplitude change for match; 5) minimum of 75% of points in template; and 6) linear waveform interpolation method. I reclassified unresolved waveforms (type 0 in Spike 2) by restricting the waveform comparison window to the first half of the triggered spike, and visually comparing and assigning each spike to the best-matched wave class. I produced auto-correlograms (Eggermont 1990), which captured same-waveform activity in the 0.5 s before and after each event, for each wave class to check the purity of spike assignments.

Once assured of accurate event classifications, I further analyzed the parsed records. I ran cross-correlograms (Eggermont 1990) to cross-reference activity between spike classes and detect activity-dependent interactions between waveforms. I averaged all classified spikes (minus those initially classified as type 0) to determine the average 75-point waveforms for the three classified spike types: A1, A2, and B. These values were then copied to an Excel spreadsheet for summation analysis. I added the 75 points forming the A1 and A2 waveforms point by point to derive a resultant waveform for comparison to the B waveform. Then, I offset the A1 and A2 waveforms by a point relative to each other and recalculated the resulting waveform. I repeated this process for 30 points positive and negative displacement of A1 and A2 relative to each other. Each one-point offset represented 50 μs of time displacement because of the 20 kHz sampling frequency (1/20,000 = 0.00005 s or 50 μs). The family of summed waveforms spanned ± 0.75 ms or 1.5 ms overall displacement relative to each other.



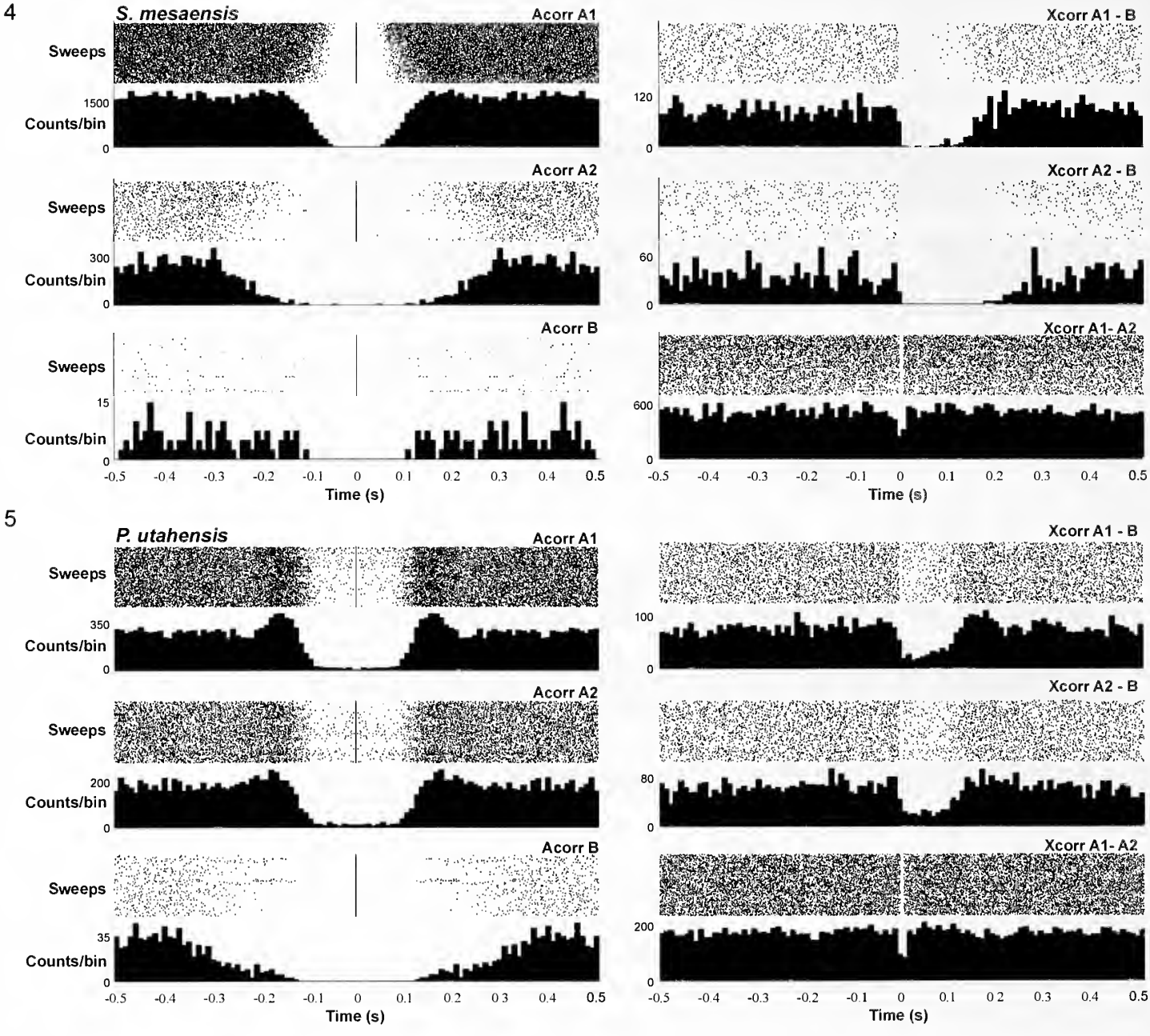
Figures 2, 3.—Electrophysiological recordings from scorpion peg sensilla. Baseline recordings from *Smeringurus mesaensis* (2) and *Paruroctonus utahensis* (3) are parsed by spike waveform analysis to separate traces classified as putative cell types A1, A2, and B; superimposed time-expanded waveforms are shown at right. The top plots show the activity of all three cells smoothed by a 30 s running average.

Finally, I compared the number of expected co-firings of putative cells to the observed number of doublets in the record. The expected number of A1/A2 doublets (i.e., the number of times that A1 and A2 fired within the same 75 point spike capture window) was calculated by first determining the average spiking frequency of each spike class (after adding in the reclassified type 0 spikes; B/A doublets were assigned to A1 or A2 based on the relative frequency of firing of A1 and A2 in the record). I multiplied the 0.004 s spike capture window (75 * 50 μs = 0.004 s) by the average spiking frequency of each class (in Hz) to determine the total time per second accounted for by each spike class. The expected number of doublets per second was then determined by adding the products of the average spiking frequency of one spike class by the time per second accounted for by the second spike class. I multiplied this number by the duration of the record to determine the expected number of A1/A2 doublets in the entire record. In a similar manner, I calculated the expected number of B/A1 or A2 doublets in the record. Finally, I compared these expected values with the actual number of reclassified doublets of each class.

RESULTS

The recordings of spike activity analyzed here had signal-to-noise ratios of about 6 to 1, well above the level required to clearly discriminate sensory spike events above background noise. The spike recognition algorithm of Spike 2 identified three distinct waveforms, which is typical for recordings from peg sensilla of *S. mesaensis* and *P. uthahensis*. The firing of these three cells (A1, A2, and B) is displayed for each of the species on separate tracings (Figs. 2, 3). In the *S. mesaensis* record, the combined spiking frequency of the three units averaged 5.8 Hz and steadily increased from the beginning to the end of the 2100 s recording (top trace of Fig. 2). In the *P. utahensis* record, the combined spiking frequency averaged 9.2 Hz and remained relatively steady from the beginning to the end of the 1200 s recording (top trace of Fig. 3).

Figures 2, 3 also show the superimposed waveforms of the three spike classes recognized by the template recognition software. In both species, the A1 and A2 waveforms were clearly distinguishable across the breadth of their patterns, and the B waveform had a characteristic inflection midway in its pattern.



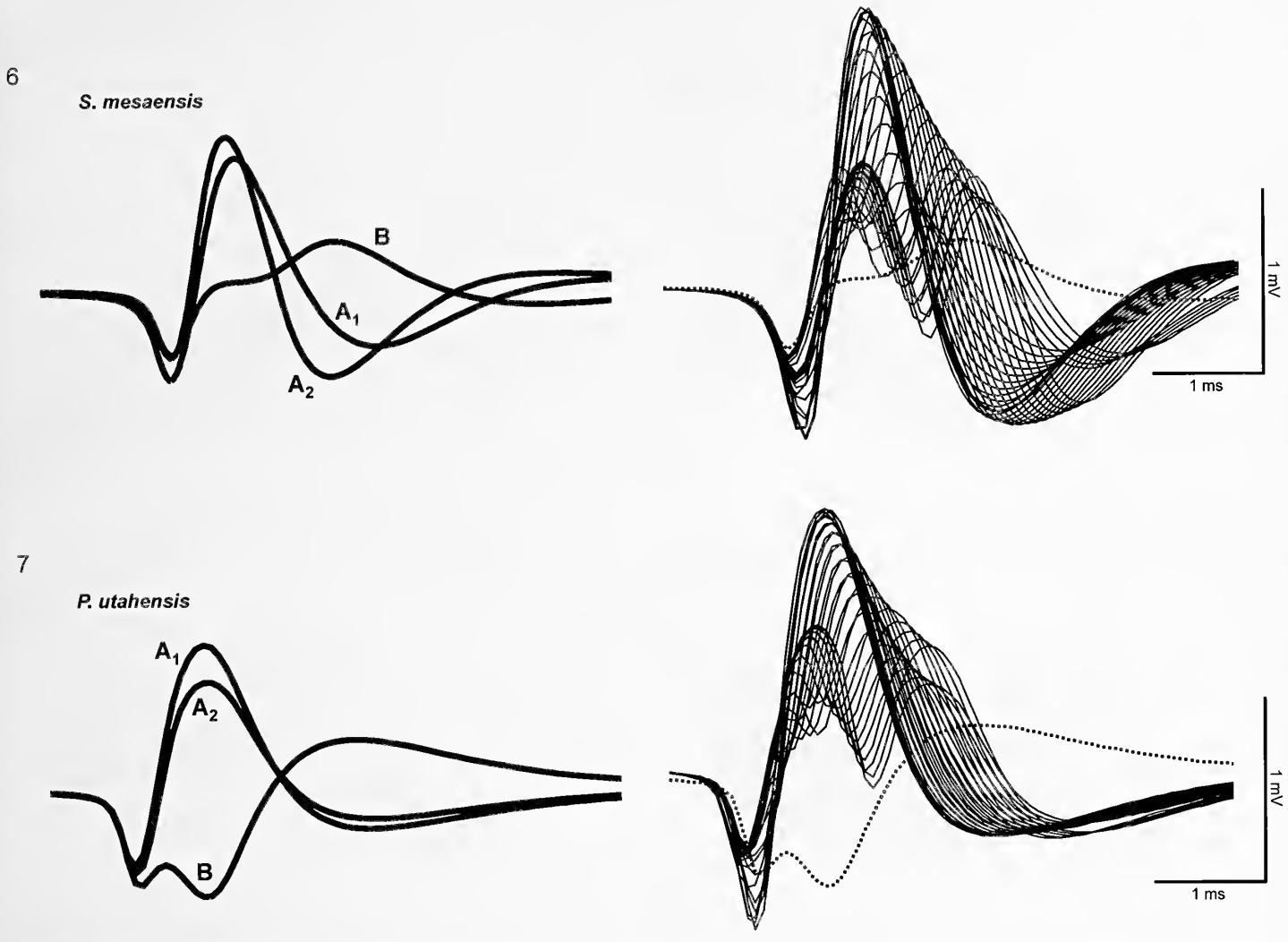
Figures 4, 5.—Correlation analysis of peg sensilla events. Auto-correlograms (right) and pair-wise cross-correlograms (left) of A1, A2, and B activities in *Smeringurus mesaensis* (4) and *Paruroctonus utahensis* (5). The raster plots at the top of each panel show the auto-activity 0.5 s before and after cell firing; the bars at bottom are sums of these tracings by 0.01 s bins.

Autocorrelograms of A1, A2, and B in *S. mesaensis* were free of contaminating events around the referenced event (Fig. 4, left) and nearly free in *P. utahensis* (Fig. 5, left). These clearings reflect the refractory period of each cell and indicate that the spike classifications were accurate. The steady increase in spiking frequency across the 2100 s *S. mesaensis* record is discernable in the raster plots at the top of the *S. mesaensis* autocorrelation panels – especially for A1. In contrast, the raster plots of *P. utahensis* autocorrelations do not show this increase, reflecting the relatively steady spiking frequency across the recording.

Cross-correlograms of A1 vs. B, A2 vs. B, and A1 vs. A2 show characteristic inhibition of A1 and A2 by B for both species (Figs. 4, 5, right). No apparent interaction exists between A1 and A2 of either species. Of note are the activities of A1 and A2 immediately prior to the firing of the B event in the top two cross-correlograms for both species. The activity is high and sustained right up to the firing of B and then drops abruptly. This is contrary to what would be expected if B were an electrical coupling of A1 and A2.

The average waveforms derived from Figs. 2, 3 are shown in Figs. 6, 7 (left) along with the results of summing the A1 and A2 waveforms in a series of time displacement calculations (right). The family of curves generated (representing a total of 1.5 ms displacement of A1 relative to A2) did not produce any curves similar to the average B waveform (dotted lines in Figs. 6, 7, right) for either species.

The left side of Figs. 8, 9 shows the unclassified waveforms that result from the near-temporal occurrence of A1 and A2



Figures 6, 7.—Summation of A1 and A2 waveforms. Right: Average waveforms for A1, A2, and B cells as calculated from individual waveform captures for *Smeringurus mesaensis* (6) and *Paruroctonus utahensis* (7). Left: Family of waveforms derived by adding the A1 and A2 waveforms successively displaced by 50 μs for a total displacement of +/− 0.75 ms (or 1.5 ms overall displacement) relative to each other. The dotted line is the average B waveform for reference.

waveforms for both species. The overlaid waveforms show a range of relative firings, similar to that generated in the calculations of Figs. 6, 7. The tracings at the top of the records indicate the time of occurrence of these A1/A2 doublets. Predictably, the frequency of occurrence of doublets in the *S. mesaensis* record directly correlates with the increase in spiking frequency of A1 and A2 as the record progresses (Fig. 2), while the frequency of A1/A2 doublets is relatively consistent across the *P. utahensis* record.

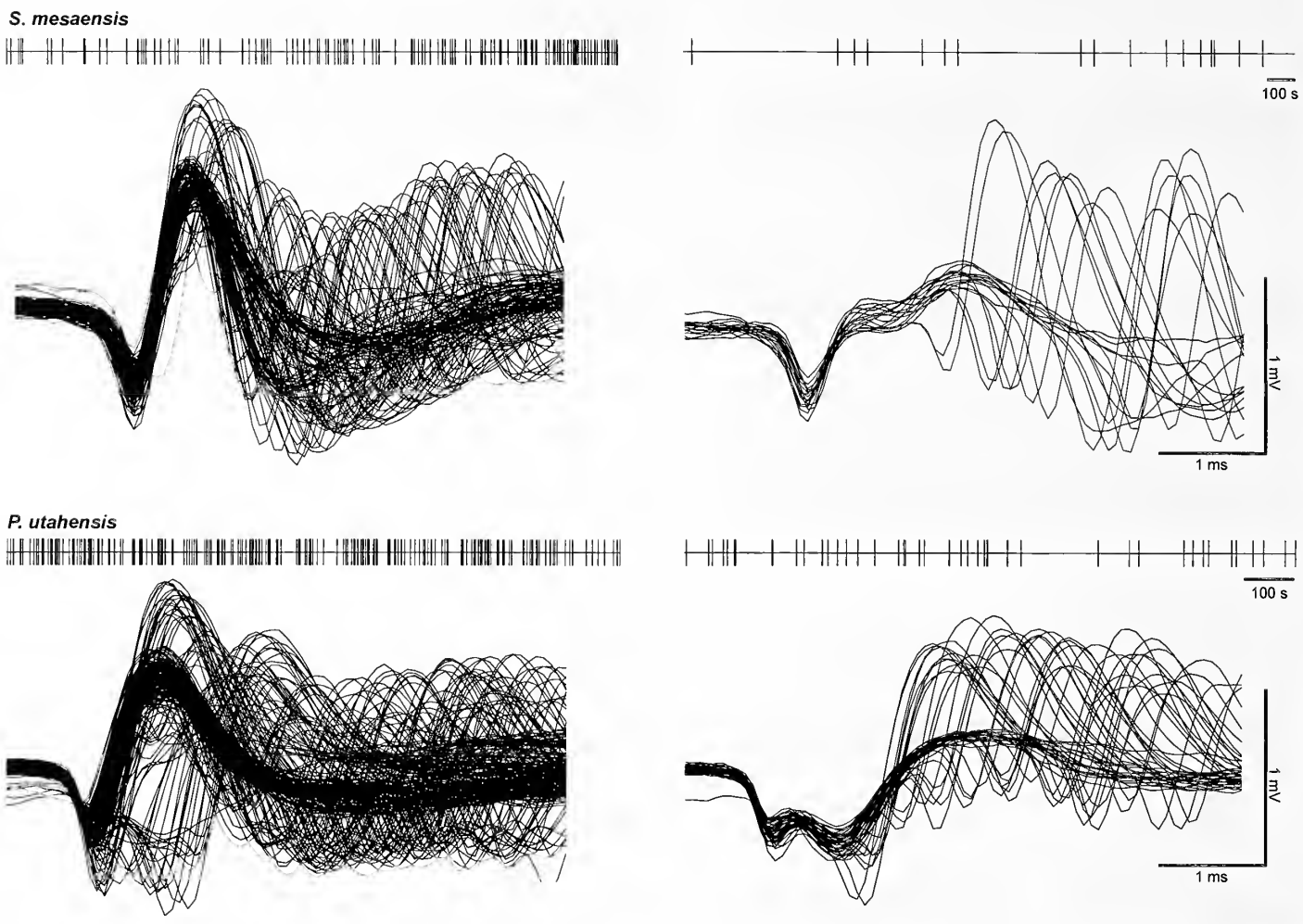
Figures 8, 9 (right side) also show the unclassified waveforms that resulted from the near-temporal occurrence of A1 or A2 waveforms after the firing of a B waveform. In all, I identified 17 of these doublets in the *S. mesaensis* record and 45 in the *P. utahensis* record. The tracing at the top of each record indicates the time of occurrence of these B/A1 or B/A2 doublets and, as before, the frequency of these doublets in the *S. mesaensis* reeord increases in direct relation to the increase in spiking frequency of the individual units.

I wanted to determine if the number of observed doublets in the records approximated the number predicted based on the unit firing frequencies and the duration given to each waveform captured by the analysis software. Since I know the average spiking frequency of each of the classified units, the time window for each spike (4 ms), and the duration of the records, I can estimate how many doublets ought to be captured by the software. Table 1 compares the number of doublets observed to the number predicted for both species. In the *S. mesaensis* record, I identified 103 A1/A2 doublets, while the predicted number based on unit firing frequency was 96. I counted 17 B/A doublets, which is similar to the expected number of 13. In the *P. utahensis* record, I identified 193 A1/A2 doublets, while the predicted number based on unit firing frequency was 150. I counted 45 B/A doublets, which is similar to the expected number of 48.

DISCUSSION

If the B cell observed in extracellular records from scorpion peg sensilla is actually a product of the electrical coupling of the A1 and A2 cells, then the following conditions should be met.

- The B waveform should be derivable from a direct addition or subtraction of the individual A1 and A2 waveforms. This was not supported.



Figures 8, 9.—Temporally close peg sensilla waveforms. 8. Superimposed images of firings of A1 and A2 waveforms for *Smeringurus mesaensis* (left) and firings of B spikes where A1 or A2 waveforms occurred within the same spike-sampling window (right). 9. Similar representations of event co-firings for *Paruroctonus utahensis*. The traces at the top of each panel show when these temporally close firings occurred in the records.

- Cross-correlograms of A1 or A2 vs. B should show restricted firing of A1 and A2 before and after the occurrence of B. They do not show activity immediately before B.
- There should be no contamination of the B waveform by other proximally occurring A1 or A2 waveforms because B results from the co-occurrence of A1 and A2, and the normal refractory period of the two waveforms would prevent them from occurring within the period of the B waveform. This is not the case. There were 17 identified co-occurrences of B and A1 or B and A2 waveforms in the *S. mesaensis* record and 45 in the *P. utahensis* record. Both of these numbers were in line with the predicted number of co-occurrences based on firing frequencies of the individual cells. The slight discrepancy is likely due to variations in the spiking frequency across the record and/or unclassifiable spikes.

Taken together, it appears clear that the B event is distinct and separate from the A1 and A2 events, and that the B event inhibits the activity of the A1 and A2 events. This finding is further supported by the ability to generate similar cross-correlogram patterns using a simulated neural network (Duffin 2000) involving two interacting units, one inhibiting the second (Gaffin 2002).

While the B event appears to be separate from A1 and A2, this does not preclude the possibility that it could result from the coupling of at least two other events within the peg sensillum. Alternatively, it could be a summation of two active conductances within the same cell. This is the first formal report of the spiking patterns in *P. utahensis*; however, the regular inflection of the B waveform has also been reported in another scorpion, *Hadrurus arizonensis* Ewing 1928 (Scorpiones: Iuridae), where it also inhibits the A1 and A2 events (Gaffin 2002). A different situation exists in the main three spiking units of *Centruroides vittatus* Say 1821 (Scorpiones: Buthidae). Again, three active cells are typical, but two of the cells have inflections in their waveforms. The waveform of the third cell is smoothly biphasic and does not appear to affect the two triphasic events. However, one of the triphasic events in *C. vittatus* excites the other (Gaffin 2001).

Previous interpretations of chemical synaptic interaction in scorpion peg sensilla are supported by these analyses. The interactions appear ubiquitous in the tens of thousands of pegs

Table 1.—Expected vs. observed number of doublets.

Species	Spike class	Number of events	A1/A2 doublets	B/A doublets	% A1 or A2	B/A doublets assigned	Total spikes	Record duration (s)	Spiking frequency (Hz)	Spike duration (s)	Time/s accounted for by spike type	Predicted doublets/s	(Expected number of doublets/s)* (record duration)	A1+A2
S. mesaensis	A1	8494	103	**17**	0.75	13	8610	2103.81	4.09	0.004	0.016	0.0228	48	96
	A2	2824	103	17	0.25	4	2931	2103.81	1.39	0.004	0.006	0.0228	48	
	B	643						2103.81	0.31	0.004	0.001	0.0067	**14**	
P. utahensis	A1	5045	193	**45**	0.55	25	5263	1200.00	4.39	0.004	0.018	0.0623	75	150
	A2	4047	193	45	0.45	20	4260	1200.00	3.55	0.004	0.014	0.0623	75	
	B	1511						1200.00	1.26	0.004	0.005	0.0400	**48**	

on the distal surfaces of pecten teeth. The utility of these extensive interactions is still under investigation. They may enhance information content in chemical identification (Gaffin & Brownell 1997a, 1997b). Alternatively, they may serve as a governor or brake on A1 and A2 and be related to the hypothesis that peg sensilla function as a parallel sampling system for rapid acquisition of ground-based chemical information (Gaffin & Walvoord 2004).

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The phylogenetic utility of the nuclear protein-coding gene EF-1α for resolving recent divergences in Opiliones, emphasizing intron evolution

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Abstract. Our focus was to design harvestmen-specific PCR primers to target both introns and exons of the nuclear protein-coding gene Elongation Factor -1 alpha (EF-1α). We tested this primer set on ten genera representing all primary lineages of Opiliones, with sets of close phylogenetic relatives (i.e., sets of several congeners) included to specifically assess utility at shallow phylogenetic levels. Our research also included the collection of parallel mitochondrial protein-coding DNA sequence datasets for the congeneric sets to compare relative rates of evolution and gene tree congruence for EF-1α versus mitochondrial data. The harvestmen primers resulted in successful amplification for nine of ten tested genera. Exon sequences for these nine genera appear orthologous to previously-reported EF-1α Opiliones sequences, which were generated using RT-PCR methods. Newly-generated exon sequences are interrupted by three separate spliceosomal introns; two introns are restricted to one or two genera, but a third intron is conserved in position across all surveyed genera. Phylogenetic analyses of EF-1α nucleotide data for congeneric sets result in gene trees that are generally congruent with mitochondrial gene trees, with EF-1α phylogenetic signal coming from both intron and exon sites, and resolving apparently recent divergences (e.g., as recent as one million years ago). Overall, the combination of gene orthology, conserved intron position, and gene tree congruence at shallow levels suggest that this gene region will prove generally useful for both phylogeographic and species-level phylogenetic analyses in Opiliones, complementing already-documented utility at higher taxonomic levels.

Keywords: Molecular systematics, phylogeography, gene tree, orthology, elongation factor -1 alpha

Both phylogenetics and taxonomy obviously have been impacted by advances in molecular biology, and most modern systematic analyses published today include some molecular component. In the arachnid order Opiliones, several molecular systematic studies have been published that consider relatively ancient phylogenetic divergences (e.g., Giribet et al. 1999, 2002; Shultz & Regier 2001). Fewer studies have considered molecular phylogenetic divergence within a species, or between closely related species, and those published have relied mostly on mitochondrial DNA sequence data. While informative, a mitochondrial-only perspective has limitations (Ballard & Whitlock 2004; Rubinoff & Holland 2005), and is quickly becoming obsolete in a molecular systematics world dominated by multigenic datasets.

A handful of nuclear genes have been used to address recent divergences within Opiliones. Those utilized offer a mix of pros and cons. Thomas & Hedin (2008) used ribosomal ITS sequences at the intraspecific level in *Fumontana deprehendor* Shear (Laniatores), and although well-behaved in this case, this gene region has been shown to be problematic in other arthropod taxa because of a lack of concerted evolution (e.g., Harris & Crandall 2000; Bower et al. 2009). Ribosomal 28S sequences have been used in various harvestmen taxa, but in our experience these data show limited variability at the shallowest levels (e.g., see Hedin & Thomas 2010). Shultz & Regier (2009) used reverse transcriptase polymerase chain reaction (RT-PCR) methods to generate exon nucleotide data for the nuclear protein-coding genes EF-1α and POL II, and applied these data to species-level relationships in the genus *Caddo*. While these data are informative at shallow levels, gathering the data requires the extra cost and expertise needed to conduct RT-PCR. Finally, Sharma & Giribet (2009) used three novel nuclear genes (H3, H4, U2 snRNA) to address relationships in sandokanid laniatoreans. Although the focus was at slightly higher taxonomic levels, several congeneric comparisons were made. Again, despite the clear utility in the multigenic perspective used in this study, these gene fragments individually are small (330, 160, 130 basepairs (bp), respectively), and our examination of these data suggests minimal variation among congeneric species.

Here we assess the phylogenetic utility of the nuclear protein-coding gene Elongation Factor-1 alpha (EF-1α) for resolving relatively shallow opilion phylogenetic divergences. In metazoans, EF-1α is a core gene involved in protein synthesis – this fact facilitates use in molecular phylogenetics (e.g., the gene is expected to be found in all taxa, have conserved function, etc). This gene is commonly used in hexapod systematics (summarized in Caterino et al. 2000), and has also seen limited use in arachnids (Shultz & Regier 2001, 2009; Hedin & Maddison 2001). Given our interest in resolving recent divergences, we expect most phylogenetic information to come from either silent substitutions at third codon exon positions (e.g., Cho et al. 1995; Reed & Sperling 1999), or from introns (e.g., Danforth et al. 1999; Hedin & Maddison 2001). Here we present the results of a commonly employed strategy: to develop PCR primers that reside in relatively conserved exons, but span variable introns that are conserved in position (see Palumbi 1996). We have discovered several EF-1α introns in Opiliones, some of which are universally conserved in position, but variable at shallow phylogenetic levels. The primers developed permit consistent and robust amplification of a fast-evolving gene region using standard PCR methods, allowing potentially rapid collection of population-level samples (e.g., many individuals from

Table 1.—PCR primers. Primers marked with an asterisk were designed after sequence collection using more general primers. The relative position (POS) of primers (5′ end) is shown based on alignment with exon sequences of Shultz & Regier (2001). Ambiguity codes are standard.

		POS
Forward Primers		
EF1-OP1	5′ -CGTGGTATYACCATYGATATCAC -3′	28
EF1-OP2	5′ -GATTTCATCAARAACATGATYAC -3′	112
EF1-OP2SCLER	5′ -GATTTCATCAAGAACATGATTAC -3′	112
*EF1-OP2ASAB	5′ -GCTGTGCTTATTGTTGCTGCYGG -3′	157
*EF1-OP2BSAB	5′ - GGTACTGGTGAGTTTGAAGCTGG-3′	178
EF1-OP3	5′ - TTTGARGAAATCCARAARGAAGT-3′	322
EF1-OP3PHAL	5′ - TTTGAAGAAATCCAAAAGGAAGT-3′	322
EF1-OP3SCLER	5′ - TTTGAGGAAATCCAGAAGGAAGT-3′	322
EF1-OP4	5′ - TACATYAAGAAGATTGGTTA-3′	352
EF1-OP4SCLER	5′ - TACATCAAGAAGATCGGTTA-3′	352
*EF1-OP5LEIO	5′ - GGAGATAACATGTTGGAACAAAG-3′	415
EF1-OP5PHAL	5′ - AACATGTTGGAACAAAGTACCCA-3′	421
EF1-OP5LAN	5′ - AACATGYTGGAAGCTTCCTCC-3′	421
EF1-OP5ISCH	5′ - AACATGTTGGARGCCAGYGC-3′	421
EF1-OP6PHAL	5′ - CATCACCACTGAAGTTAAATCTG-3′	672
Reverse Primers		
*EF1-OP5LEIORC	5′ - CTTTGTTCCAACATGTTATCTCC-3′	437
EF1-OPRC1PHAL	5′ - CAGATTTAACTTCAGTGGTGATG -3′	692
EF1-OPRC1SCLER	5′ - CGGACTTGACCTCAGTGGTGATG -3′	694
EF1-OPRC2	5′ - GANACGTTCTTNACRTTGAA -3′	767
*EF1-OPRC2LEIO	5′ - GAAACGTTCTTAACATTGAA -3′	767
EF1-OPRC2PHAL	5′ - ACGGAAACGTTCTTAACGTTGAA -3′	770
EF1-OPRC3PHAL	5′ - ATGACCTGGGCRGTGAATTCTTC -3′	854
*EF1-OPRC3LEIO	5′ - ATAACCTGGGCAGTAAATTCTTC -3′	854
EF1-OPRC3SCLER	5′ - ATGACCTGAGCCGTGAACTCTTC -3′	854
EF1-OPRC4	5′ - GAACTTGCANGCAATGTGAGC -3′	935
*EF1-OPRC4LEIO	5′ - GAACTTGCAAGCAATGTGAGC -3′	935
EF1-OPRC5PHAL	5′ - GGTTGTCTTCCAATTTCTTGCC -3′	992

multiple populations from many species). We present this primer set as a resource to be explored, modified, and utilized by other opilion systematists.

METHODS

Primer design.—Shultz & Regier (2001) generated nearly full-length EF-1α exon nucleotide sequences using RT-PCR methods for a comprehensive sample of Opiliones. These sequences were downloaded from GenBank, then compiled and translated in MacClade 4.08 (Maddison & Maddison 2003). We designed primers in conserved exon regions that might also amplify introns, using known intron positions from spiders and hexapods as a guide (Table 1, Figure 1). Within conserved regions, we manually (i.e., without software) designed 20–25 bp oligonucleotides in regions with approximately equal base composition, and GC-rich 3′ nucleotides corresponding to strictly conserved second codon positions. Most primers were designed to work for all Opiliones, but in some cases we also designed clade-specific primers (e.g., EF1-OP3PHAL is a primer that best matches the phalangioid sequences from Shultz & Regier 2001, etc.). We designed additional internal, clade-specific primers after initial sequencing with our more general primers.

Samples.—We tested primer utility on ten genera (Table 2) that represent all primary higher-level clades within Opiliones (Fig. 2). Specimens were either preserved directly in 100% EtOH in the field, kept cold, and later transferred to a −80° C freezer (or −20° C freezer in the case of *Caddo*), or preserved directly in 100% EtOH in the lab and stored in a −80° C freezer. All specimens are currently housed in the Arthropods Genomics Collection at San Diego State University. We extracted genomic DNA from leg tissues using the Qiagen DNeasy Kit; these extracts were either suspended in 200 microliters (μl) of Qiagen AE buffer, or dried down using a Speedvac and resuspended in a smaller volume of AE buffer.

Testing primer utility.—For some samples (*Siro, Caddo, Ortholasma, Dendrolasma* and *Bishopella*), we assessed primer utility in a preliminary manner. Here we conducted PCR experiments to test whether our primers resulted in amplicons of expected size, and if so, we directly sequenced these amplicons. These sequences inform us about primer success, intron position, and relative intron size, but do not address variability of the nucleotide data within a taxon (see below). We conducted PCR experiments using various forward/reverse primer combinations (Table 2). All PCR reactions included 0.8 μl of genomic DNA with 0.08 μl *Ex Taq* polymerase (Takara Bio Inc.), and 2.5 μl each of Takara dNTP mix, Takara buffer (with Mg^{2+}) and primers (at 2–4 μM concentrations). We used tissue culture water (Sigma-Aldrich Co.) to bring reaction volumes to 25 μl. Experiments were conducted on two different PCR machines, including a MJ Research PTC-100 (95° C, 2 min; 35× (95° C, 30 s; 53° C, 45 s; 72° C, 45 s); 72° C, 10 min) and an Eppendorf Mastercycler Personal (94° C, 3 min; 35× (94° C, 45 s; 45° C, 45 s; 72° C, 1 min 15 s); 72° C, 5 min). Amplification products were visualized on agarose gels, purified via polyethylene glycol (PEG) precipitation, and sequenced directly at the SDSU Micro-

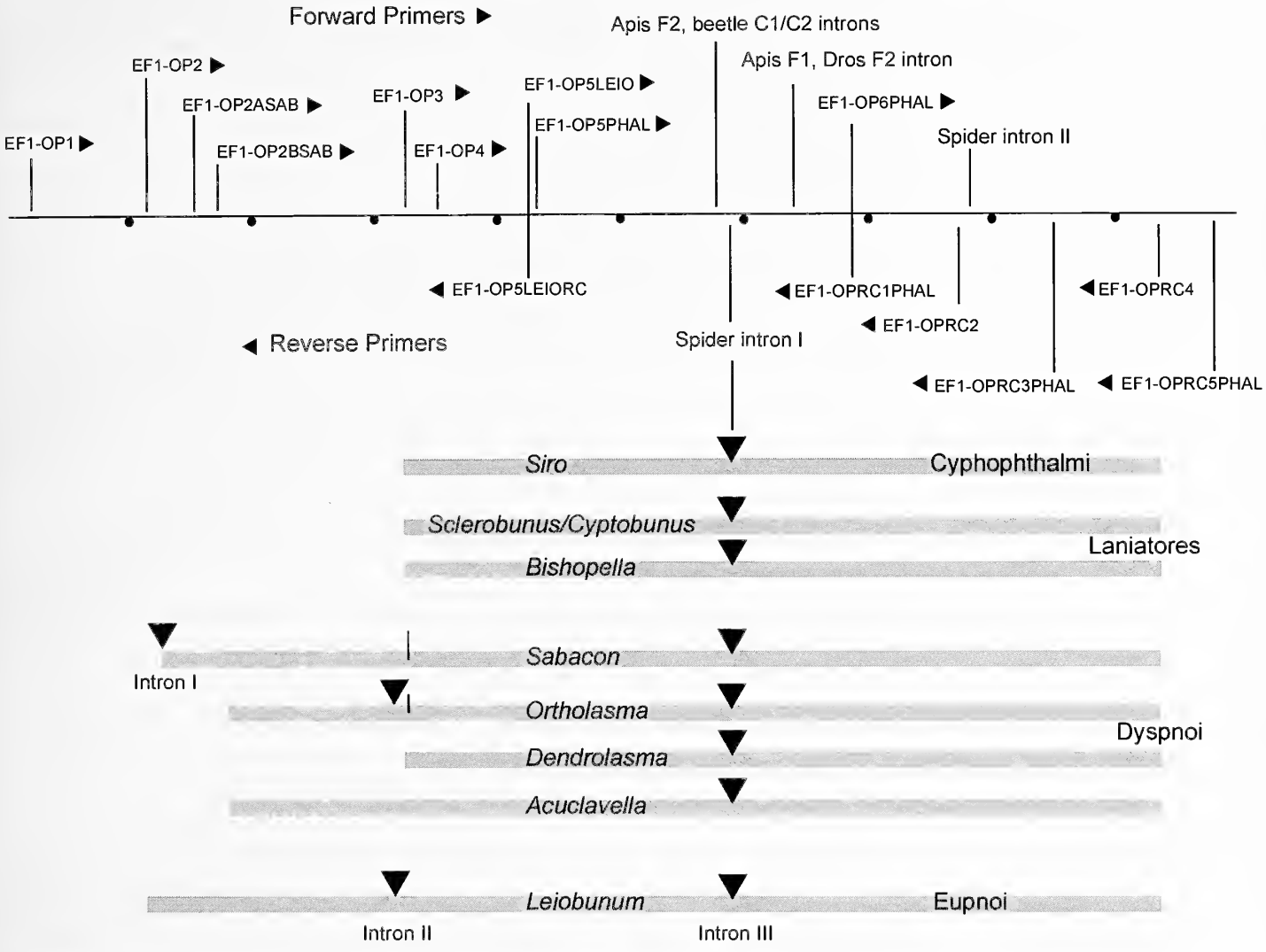

Figure 1.—Upper: Relative primer positions (see also Table 1). Spider intron positions from Hedin & Maddison (2001), hexapod intron positions from Brady & Danforth (2004). Small circles designate 100-bp intervals. Lower: Amplicon sizes and relative intron positions for opilion sample. Small hashes above amplicon boxes for *Ortholasma* and *Sabacon* indicate alternative 5′ primers used for some templates (see also Table 2).

chemical Core Facility (http://www.sci.sdsu.edu/dnacore/sdsu_dnacore.html) on an ABI Prism 3100 capillary machine.

Both strands were determined for most templates using PCR primers in sequencing reactions. For some *Leiobunum* templates, we used additional internal primers for sequencing. We assembled and edited sequence contigs using Sequencher version 4.5. For some taxa (see Results), AT-rich regions (and possible allelic length heterozygosity) within introns caused premature stops in some sequencing reads; sequences for these templates were thus determined using single reads from opposite sides of the intron. Also, for *Acuclavella* we were able to generate high-quality 780-bp sequence reads for the entire exon plus intron amplicon, and thus used only a single sequence read for several of these templates. Sites with two peaks of equal intensity on chromatograms were interpreted as representing nucleotide heterozygosity, and scored as such in data matrices using standard ambiguity codes.

To assess phylogenetic utility at shallow levels more rigorously, we collected EF-1α sequences for sets of close phylogenetic relatives (both within and between species) for *Leiobunum*, *Sabacon*, *Sclerobunus/Cyptobunus,* and *Acuclavella.* For these same taxon sets we also collected parallel mitochondrial sequence datasets, allowing us to gauge the relative variability of nuclear versus mitochondrial gene data, and ask if independent phylogenetic analyses resulted in generally congruent results. For *Sabacon*, *Sclerobunus/Cyptobunus,* and *Acuclavella,* we collected partial cytochrome oxidase I (CO1) mitochondrial sequences, following standard techniques as in Thomas & Hedin (2008). Forward primers included C1-N-1510 or C1-N-1718S, combined with C1-J-2568 or C1-J-2776S (see Hedin & Thomas 2010). For *Leiobunum*, we collected nearly full-length mitochondrial ND1 sequences using the custom primers LR-N-12945LEI (5′- TGACCTCGATGTTGAATTAA -3′) and CB-J-11638LEI (5′ - CCTWATAAACTAATCATTTAGC - 3′).

We conducted phylogenetic analyses at two different hierarchical levels. First, we conducted an exon-only analysis that included the Shultz & Regier (2001) exon data, plus newly-generated exon-only data (i.e., introns removed). This exon matrix was aligned manually, and subject to a heuristic

Table 2.—Taxon sample, including voucher and collection location information, mitochondrial sample, and successful EF-1α primers.

Taxon	Voucher no.	Collection location (N lat., W long.)	mtDNA	EF-1α primers
Sironidae				
Siro cf. kamiakensis	OP 2350	Idaho: Idaho Co., FS 311, S Route 14 (45.6853, −115.5427)	no	OP3, OPRC4LEI
Caddoidea: Caddidae				
Caddo agilis	OP2565	New Hampshire: Cheshire Co., Pisgah State Park (42.862, −72.428)	no	failed
Phalangioidea: Sclerosomatidae				
Leiobunum aldrichi	OP 829	Mississippi: Tishomingo Co., Tishomingo State Park (34.6054, −88.1927)	yes	OP2, OPRC4LEI
L. aldrichi	OP 1069	Missouri: Calhoun Co., Marshall (42.3012, −84.9674)	yes	OP2, OP5LEIORC, OPRC4LEI
L. vittatum	OP 835	Tennessee: Cumberland Co., Cumberland Mtn SP (35.9013, −84.9958)	yes	OP2, OP4, OP5LEIORC, OPRC4LEI
L. speciosum	OP 1405	Tennessee: Davidson Co., west Nashville (36.1227, −86.9061)	yes	OP2, OP4, OP5LEIORC, OPRC4LEI
L. calcar	OP 1394	North Carolina: Clay Co., Big Tuni Creek (35.1025, −83.7007)	yes	OP2, OP4, OP5LEIORC, OPRC4LEI
L. calcar	OP 814	Tennessee: Cocke Co., road to Cosby CG (35.7633, −83.2115)	yes	OP2, OPRC4LEI
L. undescribed species	OP 1383	Virginia: Grayson Co., Grayson Highlands SP (36.6247, −81.5013)	yes	OP2, OPRC4LEI
L. serratipalpe	OP 1080	Maryland: Montgomery Co., Unity, Tusculum Farm (39.2427, −77.0872)	yes	OP2, OPRC4LEI
Troguloidea: Nemastomatidae				
Ortholasma rugosum	OP 807	California: San Benito Co., NE of Pinacate Peak, off Hwy 101 (36.8602, −121.6126)	no	OP2BSAB, OP3, OPRC4LEI
Ortholasma rugosum	OP 808	California: San Mateo Co., San Bruno Mtn SP (37.6943, −122.4529)	no	OP3, OPRC4LEI
Dendrolasma mirabile	OP 1000	Oregon: Curry Co., Hwy 33, E of Gold Beach (42.5465, −124.1273)	no	OP3, OPRC4LEI
Ischryopsalidoidea: Ceratolasmatidae				
Acuclavella cf. *quattuor*	OP2233	Idaho: Idaho Co., Eagle Mt Trailhead, S side of Lochsa River (46.4292, −115.1335)	yes	OPRC4 only
A. merickeli	OP2251	Idaho: Idaho Co., along headwaters of Red River, Red River Rd (45.7853, −115.2026)	yes	OPRC4 only
A. quattuor	OP2255	Idaho: Idaho Co., Tributary of Crooked Creek, FS Rd 222 (45.5791, −115.4431)	yes	OPRC4 only
A. cosmetoides	OP2278	Idaho: Idaho Co., Canyon Creek Trailhead, US 12 (46.2101, −115.5442)	yes	OPRC4 only
A. cf. *merickeli*	OP2345	Washington: Jefferson Co., Cedar Creek, E of US (101 47.7105, −124.4095)	yes	OP2BSAB, OPRC4
A. cf. *merickeli*	OP2347	Washington: Lewis Co., tributary of Iron Creek, FS Rd 25 (46.4033, −121.9902)	yes	OP2BSAB, OPRC4
Ischryopsalidoidea: Sabaconidae				
Sabacon simoni	OP2522	Italy: Piemont, Prov. Cuneo, near Monesi di Triora (44.07381, 7.75028)	yes	OP2BSAB, OPRC4
S. cavicolens	OP721	Tennessee: Anderson Co., near Norris Dam Cave (36.221, −84.09)	yes	OP2BSAB, OPRC4
S. cavicolens	OP657	Virginia: Lee Co., Cave Spring Rec. Area (36.803, −82.92)	yes	OP2BSAB, OPRC4
S. cf. cavicolens	OP1283	North Carolina: Macon Co., Bullpen Bridge (35.015, 83.126)	yes	OP2BSAB, OPRC4
S. cf. cavicolens	OP691	North Carolina, Haywood Co., below Hebo Mtn. (35.686, −82.906)	yes	OP2BSAB, OPRC4
S. cf. cavicolens	OP1290	North Carolina: Transylvania Co., Looking Glass Creek (35.297, −82.767)	yes	OP2BSAB, OPRC4
S. cf. cavicolens	OP660	Virginia: Scott Co., Hwy 23/58, near Weber City (36.6341, −82.5604)	no	OP2, OPRC4

Table 2.—Continued.

Taxon	Voucher no.	Collection location (N lat., W long.)	mtDNA	EF-1α primers
S. cf. cavicolens	OP1535	North Carolina: Avery Co., Henson Creek, N of Ingalls (36.0374, −82.0420)	no	OP2, OPRC4
Travunioidea: Sclerobuninae				
Sclerobunus nondimorphicus	OP1056	Oregon: Clatsop County, Ecola SP (45.9221, −123.9767)	yes	OP3, OP4SCLER
Cyptobunus cavicolens	OP2143	Montana: Jefferson County, Lewis and Clark Caverns (45.8386, −111.8668)	yes	OPRC3SCLER; OPRC4
Sclerobunus robustus robustus	OP885	New Mexico: Sandoval County, Jemez Mtns (35.8384, −106.4044)	yes	OP4SCLER, OPRC3SCLER
Sclerobunus r. robustus	OP1149	Colorado: Dolores County, along Dolores River (37.7679, −107.9871)	yes	OP4SCLER, OPRC3SCLER
Sclerobunus r. robustus	OP1122	Colorado: Gunnison County, S of Gothic (38.9397, −106.4072)	yes	OP3, OPRC4
Sclerobunus r. robustus	OP1164	Colorado: Custer County, Wet Mtns (38.1335, −1105.1791)	yes	OP4SCLER, OPRC3SCLER
Grassatores: Phalangodidae				
Bishopella laciniosa	OP320	Tennessee: Johnson Co., Backbone Rock Rec Area, S Damascus (36.5948, −81.8173)	no	OP3, OPRC4LEI
Bishopella laciniosa	OP108	North Carolina: Jackson Co., Upper Falls, Whitewater River (35.0336, −83.0141)	no	OP3, OPRC4LEI

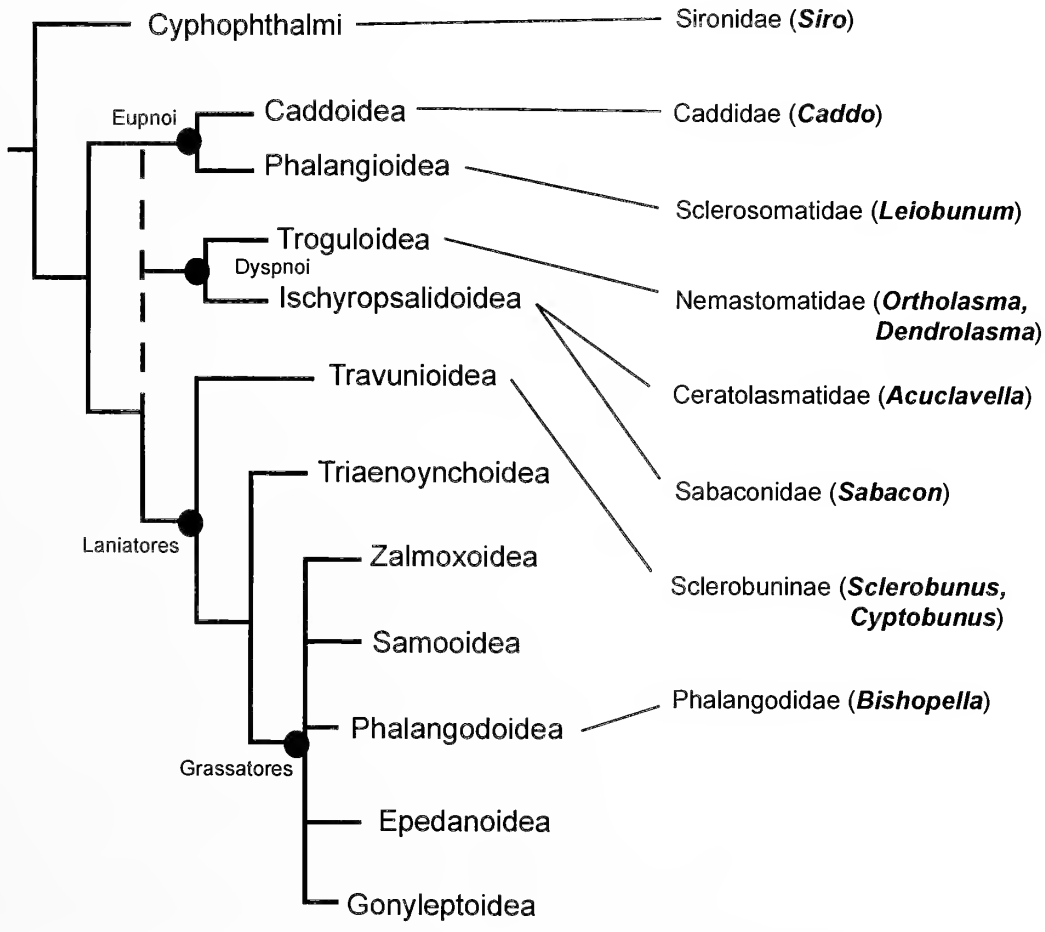

Figure 2.—Opiliones phylogeny with taxon sample. General tree structure follows Giribet & Kury (2007, fig. 3.4). The uncertain phylogenetic placement of Dyspnoi is indicated by a dashed line, representing a sister clade relationship to either Eupnoi (following Shultz & Regier 2001), or to Laniatores (e.g., Giribet et al. 1999).

parsimony search in PAUP v4.06 (Swofford 2002), using stepwise taxon addition from 1000 random replicates with tree bisection-reconnection (TBR) branch swapping. For the congeneric sets, we conducted exhaustive parsimony searches on both nuclear and mitochondrial matrices, conservatively treating EF-1α intron gaps as missing. Branch support was assessed using nonparametric bootstrap analyses (Felsenstein 1985), comprising 1000 pseudoreplicates of a branch-and-bound parsimony search for each congeneric set matrix.

RESULTS

Data availability, primer utility.—All sequences have been deposited to GenBank (accession numbers GQ870643–GQ870668; GQ872152–GQ872185). Both intron and exon alignments are available at www.treebase.org (study accession number S2469).

Using various primer combinations (Fig. 1, Table 2), we were able to amplify and generate EF-1α sequences for all surveyed genera, except for the caddoid *Caddo agilis*. We attempted eight different primer combinations for a single extraction from *Caddo*, using primers that worked well for other taxa. We suspect that the failure of this sample reflects a difference in sample preservation, as noted above. Overall, the most successful primers included the forward primers EF1-OP2, EF1-OP2BSAB, and EF1-OP3SCLER, combined with the reverse primer EF1-OPRC4LEI. The most 5′ (EF1-OP1) and 3′ (EF1-OPRC5PHAL) of our designed primers failed in all PCR experiments.

Features of the data.—Newly generated sequences were manually aligned with the exon-only nucleotide data of Shultz & Regier (2001) to identify the reading frame, intron insertions, and intron/exon boundaries. As a preliminary assessment of sequence orthology, we first removed all introns. The remaining exon data translate to expected amino acids, and with a single exception (see below) can be aligned without gaps. A strict consensus tree resulting from parsimony analysis of the exon-only data recovers expected phylogenetic placements for the newly-generated EF-1α sequences (Fig. 3), suggesting orthology of the gene copies used in this study. Ours is the first study to include the genus *Acuclavella* in a higher-level molecular analysis; we recover *Acuclavella* as sister to *Ceratolasma*, consistent with the hypothesis of Shear (1986, fig. 1).

Patterns of exon variability across divergent opilion taxa (e.g., *among* representatives of primary clades) have been addressed previously (Shultz & Regier (2001). Patterns of exon evolution *within* congeneric sets are summarized in Table 3. As expected, almost all exon variation occurs at third codon positions, with a total of 116, 14, and 7 variable sites at third, first, and second positions, respectively. Heterozygosity at exon positions is minimal, with less than 10 total sites scored as heterozygous for all newly-generated sequences.

We discovered three different introns in the harvestmen sample, here named introns I–III. The relative position of these introns, intron size, and intron/exon nucleotide boundaries are summarized in Figures 1 and 4. Intron I appeared only in two *Sabacon* samples (those amplified with EF1-OP2 primers; most *Sabacon* were amplified using downstream OP2BSAB primers). We also used the EF1-OP2 for *Leiobunum* (Fig. 1), but these sequences lack this intron. Examination of EF-Iα genomic sequences for the tick *Ixodes scapularis* (http://iscapularis.vectorbase.org/, gene ISCW020299) indicates that intron I is shared in position between *Ixodes* and *Sabacon*. Intron I is relatively small in *Sabacon* (less than 100 bp), and includes 5′ (GT) and 3′ (AG) splice site signal sequences (see Fig. 4) consistent with proposed metazoan consensus signal sequences (Senapathy et al 1990; Mount et al. 1992).

Intron II was found in all *Leiobunum* and one *Ortholasma* sample (OP 807, amplified with OP2BSAB; *Ortholasma* OP 808 was amplified using downstream primers). These taxa are not phylogenetic relatives, and differ considerably at the exon level (see Fig. 3). We did not detect this intron in *Acuclavella* and some *Sabacon*, despite using primers that spanned this region (Fig. 1). This somewhat random phylogenetic pattern of intron presence/absence is most likely the result of differential intron loss (a similar argument applies for intron I), although we cannot rule out independent intron gains (e.g., see Roy & Penny 2006). Intron II varies in size (~ 100–200 bp), and to infer canonical 5′ (GT) and 3′ (AG) splice site signal sequences in *Ortholasma* requires a two amino acid deletion (see Fig. 4). We return to this inference in the Discussion.

Finally, intron III is found in all opilion samples that we have sequeneed. This intron is shared in position with spiders (*Habronattus*, see Hedin & Maddison 2001), but not with *Ixodes*. Intron III varies considerably in size among harvestmen taxa, ranging from 60 to > 500 basepairs in length; all include canonical 5′ (GT) and 3′ (AG) splice site signal sequences (Fig. 4).

We generated high-quality intron I sequences for the taxa reported here, but in other *Sabacon* taxa a long (6-bp) poly-T region proved difficult to sequence through (data not reported). This provided our motivation to design *Sabacon*-specific primers (OP2BSAB) downstream of intron I. Intron II includes at least two long poly-T regions, but we had little difficultly in sequencing through this intron for several *Leiobunum* and a single *Ortholasma*. Our sample size is largest for intron III, where there is eonsiderable variation in intron size (see above), nucleotide composition, and sequencing difficulty. The short (Fig. 4) *Acuclavella* introns were not difficult to sequence through, although these possess both poly-T and poly-A regions. Also, despite the long *Siro* intron III, and several simple-sequence regions, sequence reads were clean. Other taxa presented mixed success, even within congeneric sets, where some samples resulted in clean bi-directional reads, whereas other reactions resulted in clean reads that failed abruptly at poly-A/T regions. The sequenee contigs for these latter samples comprise single strand reads from opposite directions, with minimal terminal overlap. Regions of terminal overlap that proved ambiguous to score were excluded from the phylogenetic analyses reported below.

Comparative phylogenetic utility.—The protein-coding mitochondrial data (CO1, ND1) translate to amino acids without stop eodons, and can be aligned without gap insertions. We compared these mitochondrial data to intron plus exon EF-1α data for the four sets of close phylogenetic relatives. Introns II (*Leiobunum* only) and III (all taxa) were aligned manually, but for the six-taxon *Sclerobunus/Cyptobunus* set, introns from two specimens (OP1056, OP2143) were too divergent to align reliably. Here we conducted phylogenetic analyses on exons-only for the six-taxon set, and analyses of introns-only for four of six taxa.

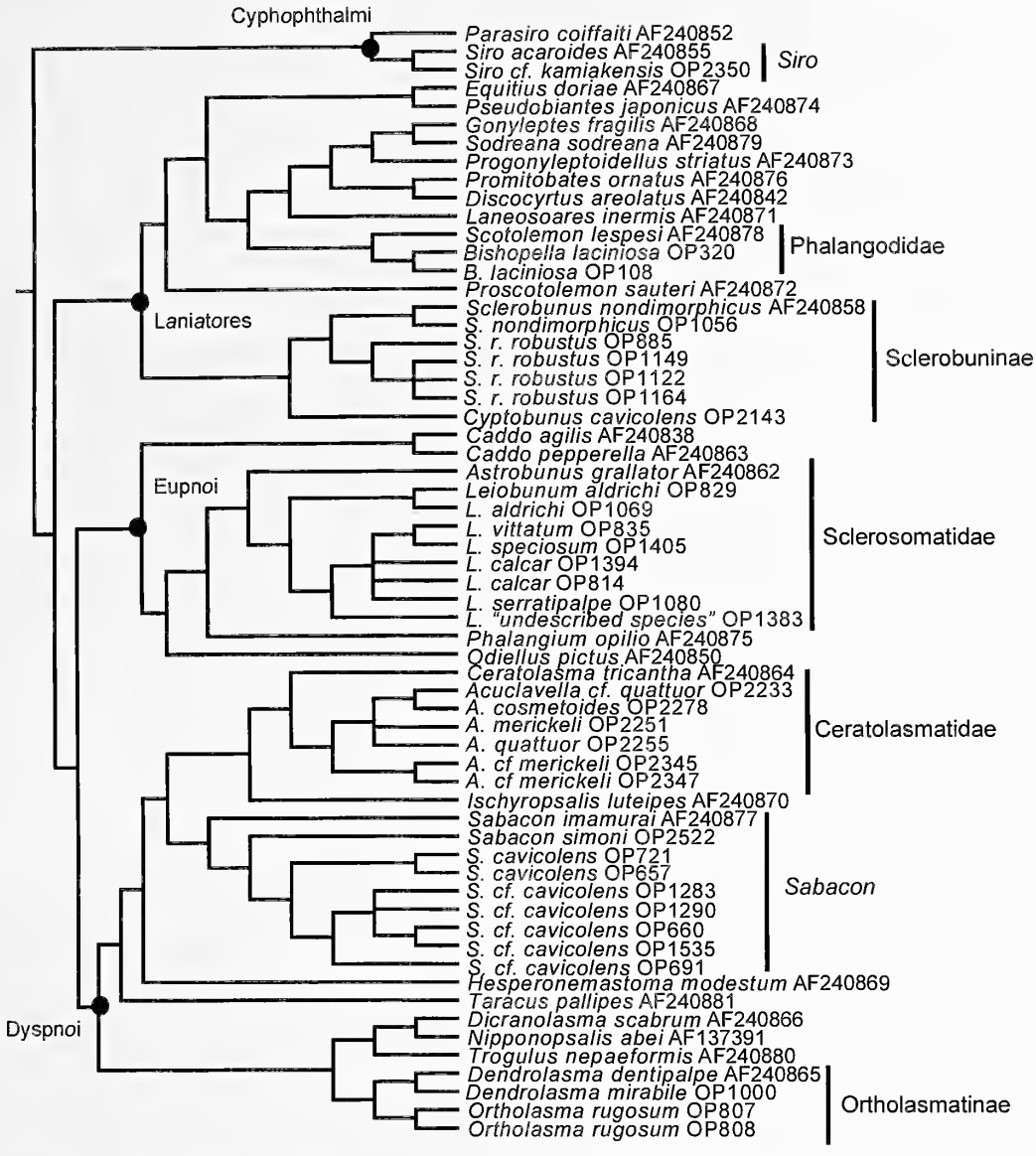

Figure 3.—Exon-only majority-rule consensus parsimony tree (N = 920, L = 2760). Higher level clade names are indicated, following taxonomy of Pinto-da-Rocha & Giribet (2007). Sequences generated in this study are shown with OP numbers; those from Shultz & Regier (2001) are shown with associated GenBank numbers. The *Sclerobunus* sequence (AF240858) generated by Shultz & Regier (2001) was originally misidentified as *S. robustus* - this sequence actually corresponds to *S. nondimorphicus*.

Results of the nine separate exhaustive parsimony searches (three for *Sclerobunus*/*Cyptobunus*, two for all other congeneric sets) are summarized in Fig. 5. These comparative analyses reveal several patterns of interest. First, all trees are well-resolved, with all but one of the searches resulting in a single most-parsimonious tree. Second, there is considerably more divergence depth in mitochondrial trees as compared to EF-1α trees, as indicated by differences in total tree length and individual branch length estimates. This reflects the well-documented higher rates of molecular evolution in animal mitochondrial genomes as compared to "average" nuclear genes (e.g., Moriyama & Powell 1997; Lin & Danforth 2004). Third, phylogenetic signal in the EF-1α data is coming from both intron and exon sites. This is most-clearly illustrated in the *Sclerobunus*/*Cyptobunus* taxon set where we conducted individual analyses on exons versus introns, but is also seen when comparing intron versus exon substitutions for specific "shallow" pairwise comparisons (see Table 3, Fig. 5). Finally, analyses of the independently-evolving mitochondrial versus EF-1α data result in generally similar gene tree estimates. Visual inspection indicates an overall pattern of topological and branch length similarity across data partitions. Moreover, we used the qualitative framework of Wiens (1998) to assess topological congruence. Among all trees, thirteen taxon bipartitions are strongly supported (bootstrap proportion values > 70; see Hillis & Bull 1993) by the mitochondrial data. Of these 13 bipartitions, 10 are also strongly supported by the EF-1α data (Fig. 5). Importantly, there is no evidence for "strong incongruence" (following Wiens 1998), where conflicting taxon bipartitions are strongly supported by the parallel datasets.

DISCUSSION

A general lack of genomic resources for Arachnida has hampered development of the type of multigenic molecular

Table 3.—Patterns of EF-1α sequence evolution. Variable intron sites were only counted at unambiguously aligned positions without indels. The following samples were not included in intron variation counts: *Sabacon* (OP660, OP1535, OP2522), *Sclerobunus/Cyptobunus* (OP1056, OP2143). Values for specific "shallow" pairwise comparisons (involving samples with mitochondrial divergence values between 2–3.2%) are shown in parentheses (see also Fig. 5).

	Variable Intron sites		Variable Exon sites		
Taxon	Intron II	Intron III	Pos 1	Pos 2	Pos 3
Sclerobunus/Cyptobunus	–	50 (10)	4	2	44 (1)
Acuclavella	–	16 (1)	2	2	16 (0)
Leiobunum	19 (1)	11 (3)	3	2	20 (1)
Sabacon	–	9 (1)	5	1	36 (4)

systematics perspective that is now seen in many other organismal groups (e.g., see Brito & Edwards 2008). In Opiliones, a core set of genes have been used at higher taxonomic levels (e.g., various mtDNA genes, 18S, 28S, histone 3, EF-1α), but nuclear gene choices at lower levels are extremely limited. We need to develop additional rapidly-evolving, informative nuclear genes for harvestmen. These will allow more accurate reconstructions of population and species' history, providing the necessary framework for addressing "shallow" systematic questions that abound in this diverse group (e.g., fine-scale historical biogeography, cryptic speciation, rapid character evolution, etc.).

The primer set and data reported here provide a starting point for other researchers to explore, modify, and utilize. The exon matrix now available (www.treebase.org, study accession number S2469) includes many conserved regions for primer design, and because the matrix includes taxonomic coverage for all higher-level harvestmen groups, it is easy to modify primers to match a group of interest. Furthermore, we have discovered an intron (intron III) that is most-parsimoniously reconstructed as being ubiquitous in Opiliones, with obviously conserved exon regions flanking this eonserved position intron. With minor experimentation, it should be easy for researchers with access to standard PCR tools to generate both intron and exon EF-1a data for Opiliones.

The most conspicuous problem that we have identified is the presence of short simple sequence regions within introns, which because of apparent length heterozygosity, sporadically interrupt direct sequence reads. Here we would first recommend designing custom primers that span only a single intron

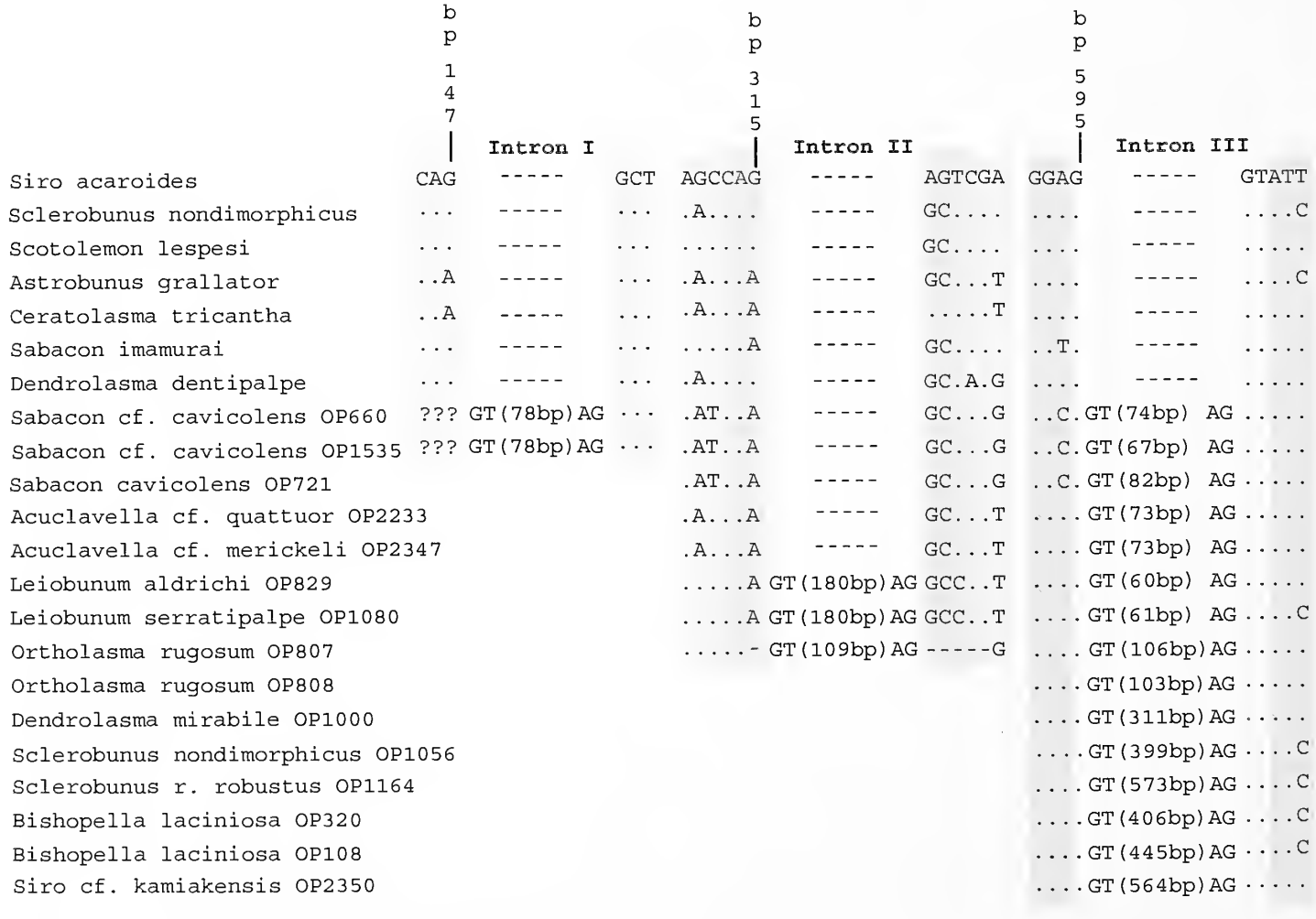

Figure 4.—Intron/Exon boundaries. Sequences generated in this study are shown with OP numbers, all those without are from Shultz & Regier (2001). Exon nucleotides are shaded in grey. Base positions are shown relative to alignment with exon sequences of Shultz & Regier (2001).

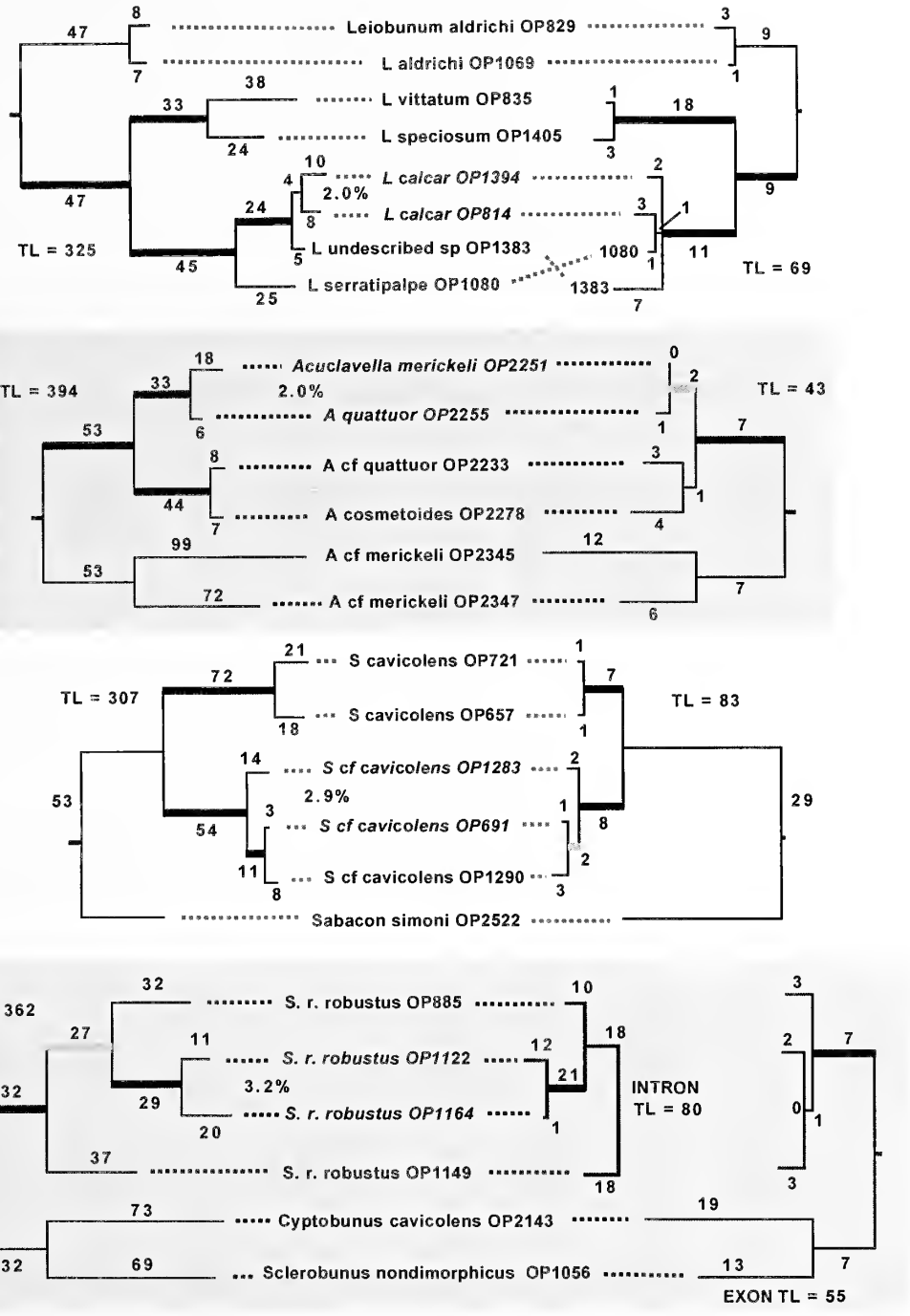

Figure 5.—Comparative mitochondrial (left) and EF-1α (right) parsimony trees. Number of parsimony inferred changes shown adjacent to branches. TL = length of most-parsimonious tree. Bootstrap proportion values above 90 are indicated by black thick branches; proportion values between 70–90 indicated by grey thick branches. All searches resulted in a single most-parsimonious tree, except for the *Leiobunum* EF-1α matrix, which resulted in 3 most-parsimonious trees (we illustrate only one of these). For mitochondrial trees, pairwise comparisons with divergence values between 2–3.2% (Kimura 2-parameter distances; Kimura 1980) are highlighted with bold, italicized text.

(after preliminary exploration of intron structure for the taxon of interest). Depending upon the severity of length heterozygosity, it may be necessary to clone PCR products, which is obviously more expensive and labor-intensive, but is still a widely used lab method. Other alternatives include allele-specific PCR, or bioinformatics approaches, such as the use of software programs that allow phase determination of length variable alleles resulting from direct sequencing (see Flot et al 2006; Flot 2007).

The data reported here reveal general congruence between nuclear versus mitochondrial gene trees (Fig. 5). Gene tree congruence, or lack thereof, is a reflection of several interacting variables, including sampling density, the depth and divergence history of the group of interest, and patterns of gene evolution (see Maddison 1997). For example, older, well-spaced speciation events are expected to result in more gene tree congruence than more-recent, "compressed-in-time" radiations. Gene flow across species boundaries in recently

separated species can cause incongruence among gene trees, as can comparisons of paralogous gene copies in multi-gene families.

Given our sample of taxa and exemplars within these taxa, we observed minimal incongruence, but again, this is an empirical issue that is expected to vary from group to group. In this context, one relevant question is how old are the harvestmen taxa within congeneric sets? We consider two indirect lines of evidence for estimating the ages of these groups. First, we have highlighted on Fig. 5 pairwise sample comparisons that differ by 2–3 percent for mitochondrial protein-coding genes. Applying a "standard" arthropod mitochondrial molecular clock (e.g., 2.3% pairwise per million years; Brower 1994; Pons et al. 2006) across trees for these individual groups would indicate divergences ranging from 1–8 million years ago (MYA). These are, of course, very rough estimates, with many assumptions which may or may not hold (a standard clock applies to Opiliones, no among-lineage rate variation, etc). Second, there is strong evidence for ancient vicariance within *Acuclavella*, leading to the primary phylogenetic separation of Olympic and Cascade mountain populations (OP2345, OP2347) from all other populations in the Idaho Rockies. In many other taxa from this region (e.g., Carstens et al. 2004; Steele et al. 2005), this west/east vicariance results from the Cascadian orogeny at approximately 5 MYA, which suggests a divergence time window generally consistent with the above molecular clock estimates.

The above divergence time estimates suggest that EF-1α data might be used to resolve divergences that are quite recent in absolute time. Although not extensive, all pairwise sample comparisons at the "one million year horizon" also show at least some divergence in EF-1α (see Table 3). Furthermore, we note that our phylogenetic treatment of insertion and deletion (indel) variation in introns was very conservative, as several indel sites appeared phylogenetically informative. More sophisticated treatment of this class of variation is expected to further increase the phylogenetic informativeness of this gene region for recent divergences (e.g., Simmons & Ochoterena 2000; Kawakita et al. 2003; Benavides et al. 2007).

In terms of gene tree congruence, one variable that seems not to be influencing our data is the presence of multiple gene copies, and potential mixing of paralogous copies. The paralogy/orthology issue is one of the most important problems negatively impacting the phylogenetic utility of nuclear protein-coding genes (reviewed in Sanderson & Shaffer 2002). Multiple gene copies are in fact known for EF-1α in other arthropods (see Danforth et al. 1999), including spiders (Hedin & Maddison 2001). "Deep paralogy," or the inadvertent comparison of *divergent* gene copies (e.g., gene copies resulting from gene duplication before the divergence of Opiliones), is apparently not an issue in our data, as mixing such paralogs would not result in the expected systematic relationships that we see in the exon-only analysis (Fig. 3). An alternative is that harvestmen species are carrying multiple copies of the EF-1α gene that are kept similar by concerted evolution (as hypothesized for salticid spiders, Hedin & Maddison 2001); distinguishing such closely-related paralogs would be more difficult in a phylogenetic analysis.

The only hint of such "cryptic" paralogy is the *Ortholasma* OP807 sequence, which requires a 2 amino-acid exon gap to align intron II splice site signal sequences (see Fig. 4). Such a gap is never present in other exon sequences, even from close phylogenetic relatives (e.g., *Dendrolasma*). This sequence may represent a paralog, but the exon appears functional (no stop codons), and is placed as expected phylogenetically. A perhaps more plausible explanation is that we have misaligned the intron boundary, but this requires non-canonical splice sites for this intron. More data is needed to distinguish between these alternative hypotheses. In general, there is little to no evidence for multiple copies of EF-1α in harvestmen. Researchers in prior studies (Shultz & Regier 2001, 2009) have not suggested this problem, relationships inferred from exons provide expected phylogenetic results, and gene trees for congeneric sets are largely congruent with independent data. However, the possibility of such paralogy should not be dismissed or overlooked in future studies.

Various technologies are now available for the generation of comparative nuclear sequence data. These include expressed sequence tag (EST) libraries, next-generation sequencing (Mardis 2008), development of anonymous nuclear loci (e.g., Noonan & Yoder 2009), etc. Ultimately, these technologies may supercede studies that focus on a single "candidate gene," for example, if we can ultimately generate whole genomic data for all samples of interest. However, these technologies are still too expensive for the average systematist, particularly in geographic areas where resources for systematics research are limited. Until then, we argue that studies of the phylogenetic behavior of "candidate genes," such as the research reported here, still have considerable utility in the arachnological and broader systematics communities.

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The web of the acacia orb-spider *Eustala illicita* (Araneae: Araneidae) with notes on its natural history

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Abstract. A great number of spiders build orb-webs and although the overall structure is the result of fixed behavioral patterns, much small-scale inter- and intraspecific variation is nonetheless evident. Thus in order to fully understand the orb-web and web-building behavior in these spiders, we need to study substantial samples of many different species of orb-weavers. However, to date only a few species have been rigorously studied both in the field and in the laboratory. Here, we investigate the ecology, behavior and orb-web of the neotropical spider *Eustala illicita* (O. Pickard-Cambridge 1889) and suggest it as suitable for further studies based on 1) the ease at which it can be located in abundant numbers in the field, 2) its willingness to build webs in the laboratory, 3) the plasticity of its behavior, and 4) its interesting ecology in the form of interactions with the swollen-thorn acacias and their ant mutualists. Here, we introduce its natural history and then provide a detailed description of orb-webs built in the field and in the laboratory, which we compare to other orb-spiders.

Keywords: Orb-web, tropical spider, swollen-thorn acacias, web parameters

Spider webs provide accurate information on the evolution, plasticity and development of behavior, since they are in effect physical remains of behavior that is 'frozen in time' (Vollrath & Selden 2007). Although the web serves other functions such as a substrate for communication via web-borne vibrations (Landolfa & Barth 1996; Watanabe 2000), and as protection from predators (Chou et al. 2005), the main function of the spider web is to intercept and retain prey (Eberhard 1986; Blackledge & Eliason 2007). Thus web-building behavior is in effect a foraging behavior that is performed hours or days before actual prey capture (Higgins & Buskirk 1992).

Orb-webs are particularly good models for the study of behavior, since they are complex, while simultaneously being highly ordered with a two-dimensional structure that makes them readily quantifiable and thus suitable for both field and laboratory studies (Zschokke & Herberstein 2005). The orb-web evolved more than 120 million years ago together with the spider's predominant prey, holometabolous insects (Peñalver et al. 2006). It is still a highly successful foraging strategy, which can be found in more than 4,000 species from 7 families (Platnick 2009). Orb-spiders modify their behavior, expressed in their web design, in response to a wide range of internal and external factors, including spider size and age (Mayer 1952), nutritional state (Crews & Opell 2006), silk supply (Eberhard 1988), recent prey experiences (Venner et al. 2000), climatic factors (Vollrath et al. 1997) and the spatial layout of the web-building site (Vollrath et al. 1997; Harmer & Herberstein 2009).

The stereotyped web-building behavior is expressed even when the spiders are missing one or several legs (Vollrath 1987), exposed to drugs and pesticides (Samu & Vollrath 1992; Hesselberg & Vollrath, 2004), or in the absence of gravity (Witt et al. 1977). It is therefore not surprising that many aspects of behavior, ecology, structural and material properties of the orb-web have been and still are the focus of numerous studies. However, it is surprising that only a relatively limited number of species has been the subject of both field and laboratory studies (Zschokke & Herberstein 2005). Some of the most studied spiders include the palearctic *Uloborus walckenaerius* (Latreille 1806), the large *Nephila clavipes* (Linnaeus 1767) from tropical and subtropical America, the neotropical *Argiope argentata* (Fabricius 1775), the cosmopolitan *Argiope trifasciata* (Forsskål 1775), the Australian *Argiope keyserlingi* (Karsch 1878), the holarctic *Zygiella x-notata* (Clerck 1757) and finally the most studied orb-weaver of them all, the holarctic *Araneus diadematus* (Clerck 1757) (Eberhard 1982; Zschokke & Vollrath 1995; Kuntner et al. 2008).

The limited number of species used can partly be ascribed to the fact that many orb-weavers do not build webs reliably in the laboratory (Zschokke & Herberstein 2005; T. Hesselberg unpubl. observ.), but is probably also the consequence of some conservatism in species choice among arachnologists. In order to fully understand web-building in orb-spiders, it is, however, important to analyse the diversity that we observe in nature more closely in the laboratory. Here, we suggest the araneid *Eustala illicita* (O. Pickard-Cambridge 1889) as a suitable neotropical spider for studies on orb-webs and web-building behavior based on the ease with which it can be located in abundant numbers in the dry tropical forest, its interesting ecology and its willingness to build webs in the laboratory. In this paper we give a general overview of its natural history and a detailed description of its web.

METHODS

Natural history.—*E. illicita* is a relatively large orb-weaver (Fig. 1). The adult female is 6–9 mm in length (7.7 ± 0.6 mm, mean ± SD, $n = 24$) and weighs 25–70 mg (36.4 ± 12.3 mg, mean ± SD $n = 24$). The abdomen is slightly elongated with a yellow/green or gray striped pattern on the ventral side and a brighter yellow and black pattern on the dorsal side. The male is slightly smaller with a shorter and narrower abdomen (Chickering 1955). It is found from Mexico to Panama (Platnick 2009). All observations on the natural history presented in this paper occurred in Parque Natural Metropolitano (9°N, 80°W), a lowland tropical dry forest at the Pacific

Figure 1.—Adult female *Eustala illicita* sitting on thorn of *Acacia collinsii*. Aggressive *Pseudomyrmex spinocola* ants can be seen patrolling close to the spider.

coast of Panama. We found that most *Acacia collinsii* trees contained numerous *E. illicita* individuals, whereas other trees seemed devoid of this species. On two mornings in June 2009, we counted, after spraying the trees with water from a plant mister, the number of *E. illicita* webs present in 18 randomly selected acacia trees and in another 18 similar sized and structured trees in the same area (we surveyed a different part of the forest on the second day, in order not to include the same trees twice). We furthermore compared the density of individual spiders per acacia tree between the dry season (December–April) and the rainy season (May–November) from seven visits to the forest in March and April 2009 and from a further seven visits in May and June 2009. Finally, we observed that spiders in the field resting away from the web seemed to show a high degree of behavioral plasticity as to what leg they used to maintain contact with the hub of the web via the signal thread. However, we decided to quantify the degree of behavioral plasticity in the laboratory, since it proved difficult to obtain reliable field data on this, as small disturbances to trees and branches caused the spiders to move and shift leg position. Spiders were treated similarly to the laboratory study (methods given below), and on days when they had built a web and were positioned away from the hub, we recorded the leg used to contact the signal thread. Since individual spiders showed a high variability from day to day in which leg they used, each spider was recorded between one and five times on consecutive days.

Webs in the field.—All measured webs were built by adult females on *A. collinsii* with resident *Pseudomyrmex spinicola* ants along trails. All sampled webs were below 2 m from the ground (141 ± 52 cm, mean ± SD, $n = 18$). For each web we measured the following parameters (Fig. 2A): 1) vertical diameter of the capture spiral (d_v), 2) horizontal diameter of the capture spiral (d_h), 3) location of the hub center from the top of the web (upper vertical radius, r_u) and 4) the vertical diameter of the hub and the free zone (H). Finally, we measured the total length of all the anchor threads that connected the web with the vegetation for a subset of the adult webs and for some early and late juvenile webs.

Webs in the laboratory.—Adult female spiders were collected throughout the year and maintained in the laboratory (23–26 ° C, 45–60% relative humidity, natural 12:12 h day/night cycle) following Zschokke and Herberstein (2005). We waited at least 5 days for the spiders to acclimate to the frames, since our prior observations indicated that some *E. illicita* spiders delay building in the laboratory (E. Triana & T. Hesselberg unpublished). We allowed each spider to build webs in a 29 × 29 × 5 cm frame made of clear Perspex. The frames were stacked like books on a shelf with thin Vaseline smeared sheets between them. They were sprayed with water from a plant mister every day. Each day a spider built a web, it was given one to two fruit flies before the web was cut using a cordless soldering iron leaving an intact radius in the north and south quadrant, thereby collapsing the web into a single vertical thread, which was left in the frame for the spider to ingest.

When a spider had built webs on two consecutive nights and at least three in total, the last web was placed in a custom-made black box with 8W fluorescent light from the side and 14W from below and photographed with a digital D60 SLR Nikon camera. The following parameters were extracted from the digital photographs using ImageJ (v1.41, National Institutes of Health, USA) (Fig. 2A): 1) Vertical diameter of the capture spiral (d_v), 2) horizontal diameter of the capture spiral (d_h), 3) location of the hub center from the top of the web (upper vertical radius, r_u), 4) the vertical diameter of the hub and the free zone (H), 5) area and 6) number of spiral turns of the hub (*E. illicita* removes the center of the hub, so the number of spirals counted here is only what are left outside of the removed center), 7) number of radii near the periphery of the capture spiral and 8) number of turns of the capture spiral (N) counted along a north, south, west and eastern radius (Nr, Sr, Wr and Er), where east is defined as the side with the signal thread (Fig. 2A) or arbitrarily in webs without a free sector.

Data analysis.—The following parameters were calculated from the measured values of the webs: 1) Capture spiral area was calculated from the Ellipse – Hub equation, $A_{cap} = (d_v/2)(d_h/2)\pi - (H/2)^2\pi$ (Herberstein & Tso 2000). The area of the free sector was not removed from the capture spiral area, but measurements from the laboratory webs show that this area constitutes less than 3% of the total capture spiral area (2.7% ± 1.8%, mean ± SD, $n = 24$). 2) The area of the hub and the free zone had a circular shape and was calculated from $A_{h+fz} = (H/2)^2\pi$. 3) Asymmetry in the web between the upper and lower part was calculated from $(r_u - r_l)/(r_u + r_l)$, where r_l is the lower radius found from $r_l = d_v - r_u$. The equation gives a value from −1 to 1, where 0 indicates a perfectly symmetric web. 4) The shape of the web was calculated from $(d_h - d_v)/(d_h + d_v)$, which again gives a value from −1 to 1, where 0 indicates a perfectly round web. 5) Average mesh size was calculated from the mesh size (distance from the inner to the outer spiral turn/N−1) along the north, south, east and western radii (Fig 2A). Comparisons between field and laboratory webs were made with the two-tailed Welsch's T-test for independent samples with unequal variances, while we compared the ratio of anchor thread lengths to web diameter with a non-parametric Friedman ANOVA. The significance level was set at $P = 0.05$.

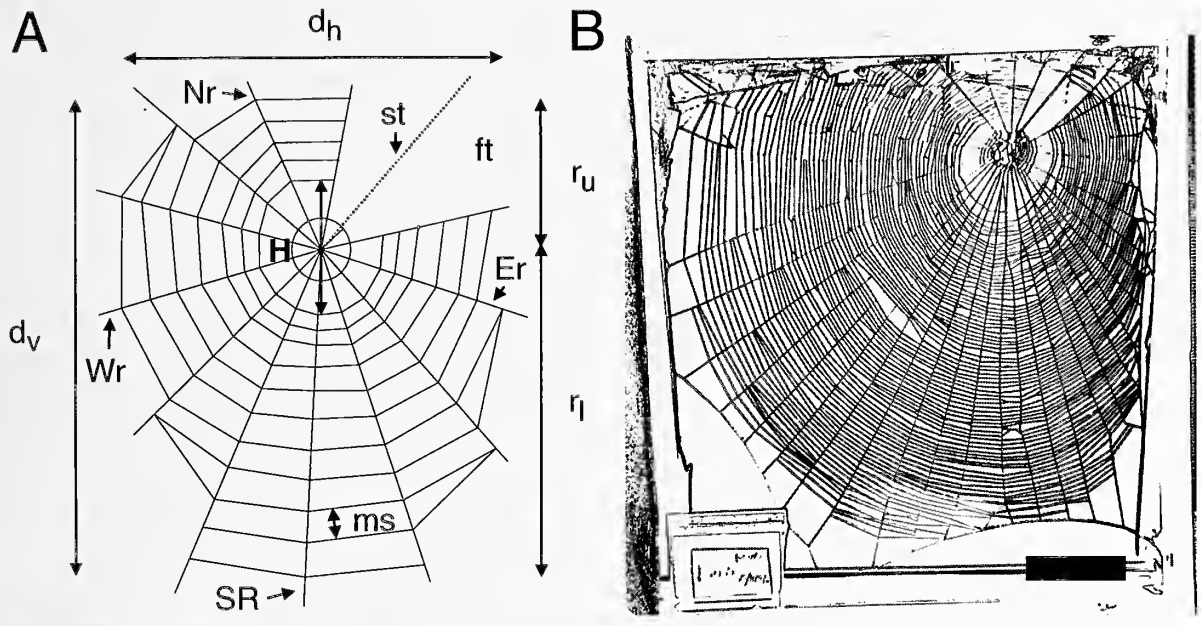

Figure 2.—Orb-web of *Eustala illicita*. A. Schematic drawing of a typical web showing the free sector (ft); signal thread (st); diameter of the hub and free zone (H); north (Nr), west (Wr), south (Sr) and east (Er) radius; mesh size (ms); upper (r_u) and lower (r_l) vertical capture spiral radius and vertical (d_v) and horizontal (d_h) capture spiral diameter. B. Photograph of an orb-web built in 29 × 29 × 5 cm frame. The photograph was inverted, sharpened and given higher contrast before the edge detect function was applied. All modifications were carried out with Corel Photo-Paint (V. 12, Corel Corporation 2003). Black bar in the lower right corner = 5 cm.

RESULTS

Natural history.—We found a close association between the spider and the swollen-thorn acacia, *Acacia collinsii*, in Parque Natural Metropolitano. We counted a mean of 6.2 ± 3.9 (mean ± SD) spiders per acacia, whereas only 0.2 ± 0.5 (mean ± SD) spiders were found per neighboring non-acacia tree (Mann-Whitney test: $U = 383.5$, $P < 0.001$). All of the four spiders in non-acacia trees were juveniles and were found on trees within a few meters of an acacia tree. However, during other visits some adults were observed on dead vegetation along a road in a different part of the forest. During the day, *E. illicita* often sat close to a bull-horn-shaped thorn of the acacia, where ants nested (Fig. 1). Resident *Pseudomyrmex* ants are extremely aggressive and usually attack all intruders, except for a few insects that have evolved mechanisms to avoid getting attacked (Janzen 1966). At present we do not know how the spider avoids attack by the ants (J.D. Styrsky, pers. comm.).

The spiders appeared to be active primarily at night, when they could be found in the hub of the webs in both the field and in the laboratory. Web-building in the laboratory usually occurred either just after dusk or shortly before dawn (T. Hesselberg, pers. observ.). During the rainy season most adult spiders also kept their webs up during the day (95% of adults observed, $n = 21$, vs. 31% of adults observed, $n = 13$, with webs during the dry season). Spiders with webs rested either on acacia branches and stems (28%, $n = 32$) or more usually on the thorns of the acacia (72%, $n = 32$) and maintained contact with a signal thread connected to the hub of the web. Spiders in the laboratory varied widely in terms of the leg used to grab the signal thread, except for the small third pair of legs, which was never used (Fig. 3). However, the other three pairs of legs were not used equally frequently. The spiders predominantly used the second pair of legs (48%, $n = 60$) to maintain contact with the web, while the front pair of legs was used less frequently (18%, $n = 60$) than the last pair (22%, $n = 60$) (Fig. 3). The signal thread sometimes ended in a V-shape and two legs held the signal line (12%, $n = 60$). In general, when one leg was used, the right leg was used significantly more often than the left (64% vs. 36%, $n = 60$, chi-square test: $\chi^2 = 4.25$, $P = 0.039$). However, this could be an artifact caused by the direction of light on the frames, which we did not control.

The web.—A total of 34 spiders was placed in frames, and 24 were photographed. The other spiders either died early or did not build webs regularly. The recorded spiders had an average web-building frequency of 80.7% ± 21.7% ($n = 24$) during the 5–10 days they were kept in the frames, which compares favorably with daily laboratory web-building frequencies of 80–90% for *A. diadematus* (Hesselberg & Vollrath 2004), 50–70% for *A. keyserlingi* (Herberstein et al. 2000; Walter et al. 2008) and 30–50% for *Z. x-notata* (Pasquet et al. 1994).

The webs of *E. illicita* showed three major deviations from the standard orb-web: 1) the upper part of the web was much reduced resulting in highly asymmetrical webs, 2) the webs had short anchor threads with no distinct frame towards the substrate, and 3) the web had a free sector (Fig. 2B). The short anchor threads were not only an artifact from laboratory frames, but were also observed in juvenile and adult webs in the field, where the ratio of the entire length of all anchor threads to the average diameter (vertical and horizontal) of the capture spiral was 1.9 ± 0.8 ($n = 32$). This is significantly lower than the ratio in juvenile and adult webs of two other sympatric neotropical orb-spiders; *Cyclosa caroli* (4.7 ± 1.5, $n = 16$) and *Nephila clavipes* (5.5 ± 1.8, $n = 30$, Friedman ANOVA $F_{(16,2)} = 24.1$, $P < 0.001$) (T. Hesselberg unpublished). The free sector was most often, but not always, found in an upper corner. Some adult females built webs without a free sector (4 out of 18 field webs, 4 out of 24 laboratory webs), and instead

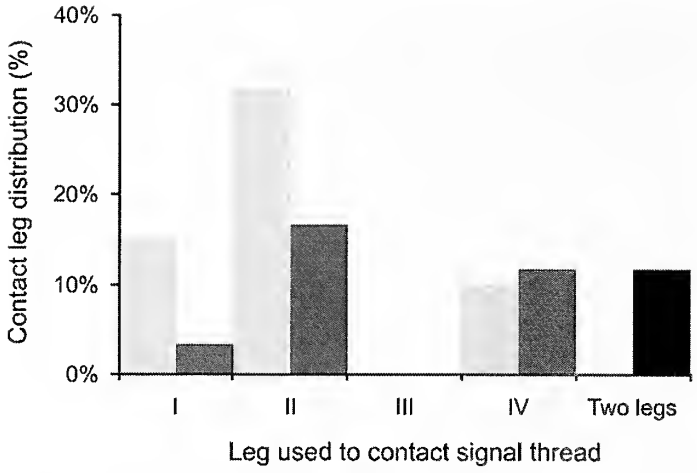

Figure 3.—Frequency distribution of leg used to contact signal thread in adult females of *Eustala illicita*. Spiders were scored when resting away from the web in laboratory frames (see method section in the text). Since individual spiders showed a high variability from day to day in which leg they used, each spider was recorded between one and five times on consecutive days. Data come from 60 observations of 24 individuals. Legs numbered from anterior to posterior end of the animal with front pair of legs labelled I. Light gray columns show right legs and darker gray columns show left legs. Signal thread sometimes ended in V-shape resulting in two different legs being in contact with it (black column).

they usually had either a signal thread that ran out of the web plane (in the field) or they had no signal thread and were found in the hub of the web (in the laboratory). The incorporation of a free sector in most webs allows comparisons to the spider, *Z. x-notata*, which also includes a free sector in the majority of its web (Venner et al. 2000).

Webs built in the laboratory were similar to webs built in nature. Natural webs and laboratory webs were almost equal in size, both in capture area and in the area of the hub and the free zone (Table 1). Laboratory webs, however, were significantly rounder and less symmetrical than natural webs (Table 1). This was probably due to adaptation to the frames provided to the spiders in the laboratory. The number of radii and the mesh size in laboratory webs of *E. illicita* were comparable to similar sized laboratory webs of *A. diadematus* (webs with a mean capture area of 236 cm^2 have 33.4 radii and a mesh size of 0.24 cm: Vollrath et al. 1997). Finally, *E. illicita* built distinct hubs with more than four closely spaced spiral turns visible, even though the spiders removed the center of the hub after completion of the web (Table 1).

Table 1.—Characteristics of adult female webs of *Eustala illicita* from the field and laboratory. All measurements given as means ± SD. Pairwise comparisons evaluated by applying two-tailed Welch's t-test for independent samples with unequal variance.

	Field webs	Laboratory webs	*t*	*p*
Sample size	18	24		
Number of radii	-	32.0 ± 7.0		
Capture area (cm^2)	300 ± 184	262 ± 69	0.95	0.348
Area of hub+free zone (cm^2)	58 ± 30	58 ± 25	0.08	0.940
Area of hub (cm^2)	-	4.7 ± 1.2		
Hub spiral turns	-	4.5 ± 1.2		
Asymmetry	−0.40 ± 0.18	−0.51 ± 0.11	2.16	0.041
Shape	−0.13 ± 0.10	−0.06 ± 0.07	2.75	0.028
Mesh size (cm)	-	0.27 ± 0.06		

DISCUSSION

In this paper, we described the observed close association between the spider, *E. illicita*, and the swollen-thorn acacia, *A. collinsii*, in Parque Natural Metropolitano on the Pacific coast of Panama. Styrsky and co-workers recently found a similar close association between *Eustala oblonga* (Chickering 1955) and the swollen-thorn acacia, *Acacia melanoceras*, in forests of the Atlantic and central regions of Panama (J.D. Styrsky pers. comm.). Furthermore, *E. illicita* and another *Eustala* with an oblong abdomen [most likely *Eustala fuscovittata* (Keyserling 1864)] have been found on *A. collinsii* in Parque Nacional Chagres in central Panama (J. Styrsky & T. Hesselberg unpubl. observ.).

The apparent association between *E. illicita* and *E. oblonga* and swollen-thorn acacias in Panama is the first reported case of such a high degree of plant specificity in an orb-weaving spider, but a few similar spider-plant interactions are known from other spider families, including Ctenidae (Barth et al. 1988), Desidae (Whitney 2004), Oxyopidae (Romero et al. 2008), Salticidae (Romero 2006), Theraphosidae (Santos et al. 2002) and Theridiidae (Gastreich 1999). However, what makes the interactions between the swollen-thorn acacias and *Eustala* spiders particularly interesting is that the trees are obligate mutualists with *Pseudomyrmex* ants (e. g. *A. melanoceras* with *P. satanicus* and *A. collinsii* with *P. spinicola*). The ants defend the plant against herbivores and encroaching plants in return for sugar, Beltian bodies and nest-space (Janzen 1966). The *Eustala* spiders might, therefore, be found in association with the two acacia species because of the protection conferred by their highly aggressive ant mutualists. A similar preference occurs in some bird species that predominantly make nests in the acacia trees, because the aggressive *Pseudomyrmex* ants prevent larger vertebrates, such as snakes and lizards from entering the acacia (Janzen 1969). However, *E. illicita*, as reported in this paper, readily builds webs in the laboratory and preliminary surveys suggest that the association with acacia trees is not obligatory, since numerous adults, both *E. illicita* and *E. oblonga*, were observed in dead vegetation along roads in Parque Natural Metropolitano at the Pacific coast and in Parque Soberania in central Panama (J. Styrsky & T. Hesselberg unpubl. observ.). Thus it is possible that the spiders employ an opportunistic strategy and inhabit dead vegetation when no suitable acacia trees can be located.

E. illicita is a promising neotropical spider for future studies on orb-webs and web-building behavior for the following reasons: 1) It is locally abundant and easy to locate in nature. The swollen-thorn acacias, and thus the spiders, are generally easy to find in the rainforest since the ants remove all other vegetation in their vicinity. However, we currently have no information on *E. illicita*'s abundance or relation to swollen-thorn acacias in the rest of Central America. 2) It builds regular orb-webs in the laboratory. *E. illicita* builds webs with a daily frequency of 80% and does not seem to be overtly disturbed by the drier and colder conditions found in the

laboratory. 3) The high degree of behavioral plasticity that *E. illicita* shows with regard to contact legs and while building a web. Especially, the location and presence of a free sector and the flexibility of frame and anchor thread lengths enable it to build webs in a range of different spatial environments (T. Hesselberg unpubl. observ.). 4) Its natural history. *E. illicita* and its sister species are unique among orb-spiders in that they occur predominantly on a few closely related plant species, all of which are protected by ants.

The observations reported here raise a number of interesting questions such as whether the spider locates the host plant by using chemical cues from the acacias or from the ants. Spiders are known to respond to visual and tactile cues from ants (see Cushing 1997 for review), but they were not believed to respond to chemical cues. However, recent studies show that spiders are able to detect air-borne chemical cues from both ants (Allen et al. 1996; Clark et al. 2000) and plants (Krell & Krämer 1998; Patt & Pfannenstiel 2008). Further questions include how host-specific the spiders are and if *E. illicita* on *A. collinsii* with *P. spinocola* ants differ in their host preference from *E. oblonga* on *A. melanoceras* with *P. satanicus*. Also, if these spiders are found in other swollen-thorn acacia – ant systems, such as for example in the *A. cornigera* – *P. ferruginea* system found from Mexico to Costa Rica (Janzen 1966). At present it is also not known whether the spiders use behavioral mechanisms or chemical mimicry to avoid attack by the aggressive ants. Some myrmecophilic spiders employ cuticular hydrocarbons to camouflage their presence (Allen et al. 2002). Answers to some of these questions are currently being pursued in our laboratory and in the working group of John Styrsky (J.D. Styrsky pers. comm.), but it is our hope that this paper will convince other arachnologists to pursue research on these fascinating spiders.

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Visual cues used by ant-like jumping spiders to distinguish conspecifics from their models

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Abstract. Despite the conceptual appeal of how morphological mimics visually distinguish between conspecifics and their models, scant attention has been given to this topic. Accurate discrimination between ants and conspecific spiders is likely to be under strong selection because approaching an ant may result in the spider's death, while approaching a different sex conspecific may result in copulation. I addressed this question by examining responses of the ant-like jumping spider *Myrmarachne bakeri* Banks 1930 (Salticidae) toward motionless, odorless lures made from dead conspecifics, ants, or lures using components of non-ant-like salticids, ant-like salticids and ants. I found that chelicerae, legs I and body, but not movement, are important cues used by *M. bakeri* to distinguish conspecifics from ants, but the relative importance of these cues differs depending on a spider's sex.

Keywords: Visual discrimination, mimicry, *Myrmarachne*, recognition

Batesian mimicry is possibly the best-documented example of a deceitful signal, and while the effects of these signals on predators have received considerable attention for over a century (Bates 1862; Wickler 1968; Ruxton et al. 2004) there is scant information on the effects of mimetic signals on conspecifics. This gap in our knowledge is not reflected in the importance of the issue at hand: Batesian mimics resemble an unpalatable or dangerous model, and mimicry has evolved due to its effect on potential predators, which consequently avoid the mimic (Edmunds 1974). However, all animals capable of processing information in the specific sensory modality of the mimetic signal may be fooled by mimics- not just predators. Consequently, if a mimic is a visually guided animal, and it looks like its model, conspecifics themselves may be fooled about its identity. This may be especially pertinent if the model is dangerous to the mimic itself.

Jumping spiders (Salticidae) have acute vision (Land & Nilsson 2002) and complex visually-mediated displays (Richman & Jackson 1992; Nelson & Jackson 2007), that are elicited by optical cues alone (Crane 1949a,b; Jackson & Pollard 1997). Salticids detect and respond appropriately toward conspecifics or prey in the absence of movement cues (Jackson & Tarsitano 1993; Jackson et al. 2005) from distances of 20 body lengths or more (Jackson & Blest 1982; Harland et al. 1999), making them ideal for investigations concerning visual identification.

Myrmarachne is a large genus of ant-like jumping spiders that resemble ants not only morphologically but also behaviorally (Cushing 1997; Ceccarelli 2008). Behavioral similarities include walking rapidly in an erratic manner on six legs and holding the first pair of legs ('legs I') in the air, simulating the ant's antennae. Morphological similarities include the shiny appearance of an ant's exoskeleton rather than the furry appearance of typical salticids, appearing to have three body parts instead of two, and having long, narrow legs instead of the short, stout legs more typical of salticids (Edmunds 1974, 1993; Cushing 1997). Here I investigate whether the ant-like salticid *M. bakeri* is able to discriminate between ants and conspecifics solely on the basis of optical cues and consider whether movement is a necessary cue for conspecific recognition. I then investigate specifically which morphological traits are necessary for identification of conspecifics.

Ants are well defended against many predators, and evidence strongly suggests that *Myrmarachne* are Batesian mimics that receive protection from predators that are averse to ants (Edmunds 1993; Nelson & Jackson 2006a,b; Nelson et al. 2006). While many species of *Myrmarachne* resemble a specific model very closely, others are less specific- they are 'poor' mimics (Edmunds 2006). *M. bakeri* appears to have no specific model (Nelson 2010) and does not resemble ants as accurately as do better known species of *Myrmarachne* (Nelson & Jackson 2006a; Nelson et al. 2004, 2005). For example, *M. bakeri* does not have a pronounced constriction in its cephalothorax, simulating the division between an ant's head and thorax. However, as with other species in this genus, *M. bakeri* has an elongated body and thin, elongated legs. Despite the relative imprecision of *M. bakeri*'s mimicry, previous studies suggest that *M. bakeri* resemble ants to other salticid species (Nelson & Jackson 2006a) and to mantids (Nelson et al. 2006).

Like all species in this genus, *M. bakeri* is sexually dimorphic, with adult males having greatly enlarged chelicerae (Pollard 1994; Nelson 2010). Although enlarged chelicerae alter the appearance of males substantially, they appear not to compromise mimicry because the chelicerae resemble an object being carried in the jaws of an ant (Nelson & Jackson 2006b).

Myrmarachne bakeri is also polymorphic (Nelson 2010). Polymorphism in *Myrmarachne* is not uncommon, but the typical pattern is for each morph to be confined to particular instars and for each morph to correspond to a distinct ant model, a phenomenon known as 'transformational mimicry' (Cushing 1997; Ceccarelli & Crozier 2007). As young juveniles, *M. bakeri* may be transformational mimics (Nelson 2010). Distinct from other species of *Myrmarachne*, *M. bakeri* adults have two color morphs: either black or similar tones or reddish/brownish tones. Many ant species sympatric with *M. bakeri* are black or reddish (X.J. Nelson: personal observation). In the present study, I used two species that are especially common, often found in the vicinity of *M. bakeri*,

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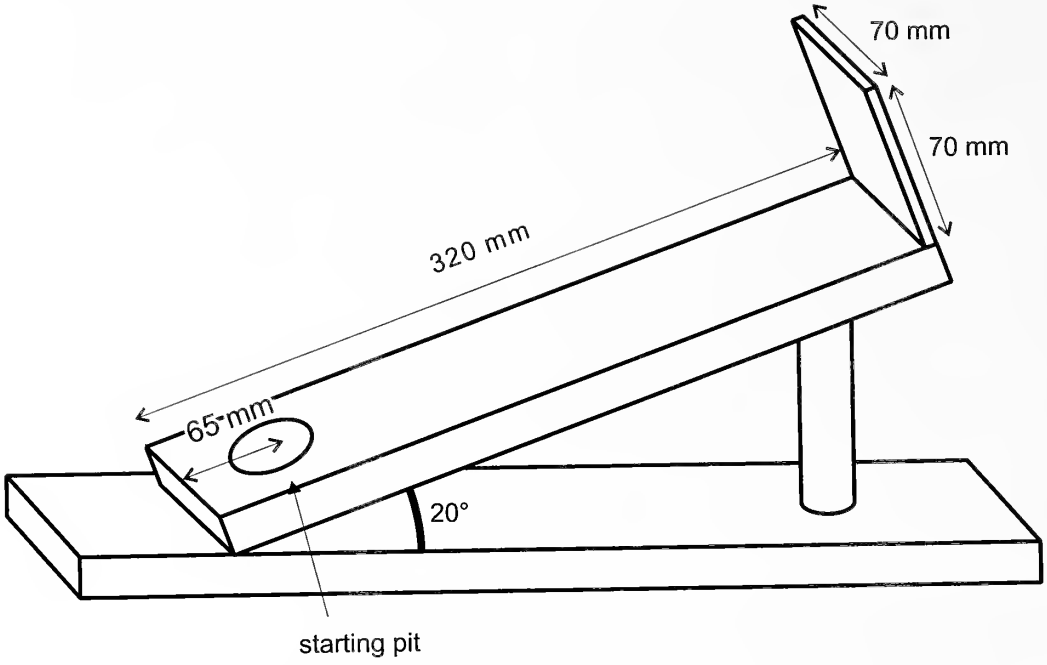

Figure 1.—Ramp used for testing *Myrmarachne bakeri* with altered and unaltered lures of conspecifics and ants.

and towards which *M. bakeri*'s responses are identical (X.J. Nelson personal observation; Nelson & Jackson 2007): *Polyrachis dives* (F. Smith 1857) and *Oecophylla smaragdina* (Fabricius 1775). *P. dives* is a black ant similar in size to *M. bakeri* and similar to the 'black' morphs of *M. bakeri*, while *O. smaragdina* is orange-brown and bears a resemblance to the 'red' morph of *M. bakeri*.

Ants are often predators of salticids and will readily attack *Myrmarachne* (Nelson et al. 2004, 2005). Consequently, *M. bakeri* is potentially at mortal risk if it does not discriminate correctly between an ant and a conspecific, yet *M. bakeri* must approach conspecifics in order to reproduce. Selection for the appropriate response to these situations, specifically to approach a conspecific of the opposite sex and to avoid a similar-looking ant, is clearly strong. In this study, I show that *M. bakeri* does discriminate correctly and elucidate some of the cues whereby this is achieved. The potential cues investigated are features that seem to be either especially conspicuous or characteristically non-ant-like attributes (e.g., presence of palps and, for males, elongated chelicerae). My approach was to make life-like lures from dead salticids and from ants that could be altered by adding or removing anatomical parts of dead arthropods.

METHODS

General.—I collected *Myrmarachne bakeri*, *Polyrachis dives* and *Oecophylla smaragdina* in the vicinity of the International Rice Research Institute (IRRI), Los Baños, Philippines (14°10′N, 121°14′E), and conducted laboratory work at IRRI and at the University of Canterbury (Christchurch, New Zealand). Spiders were tested with lures made of dead *M. bakeri*, dead ants (*O. smaragdina* and *P. dives*) and dead salticids reared in the laboratory (*Portia labiata* (Thorell 1882) and *Aelurillus cognatus* (O.Pickard-Cambirdge 1872)). Ants were collected as required for making lures (see below). Spiders were maintained in individual plastic cages, cleaned weekly, with a cotton roll through the bottom that dangled in a small cup of water, providing humidity. All spiders were fed twice a week with cultured *Drosophila* and small cultured house flies (*Musca domestica*). Testing was done between 0800 h and 1700 h using sexually mature male and female spiders. Using standard protocol for experiments on predatory behavior, spiders were fasted between 3 to 5 days prior to testing. No individual spider was tested more than once with a given lure.

Experimental methods.—A wooden ramp (see Fig. 1 for dimensions) raised at a 20° angle and supported by a wooden pole glued to a wooden base was used for testing. A thin piece of wood glued to the top end of the ramp served as a background against which the salticid saw the lure. The lure was placed 40 mm from the top end of the ramp, equidistant from both edges. The entire apparatus was painted with two coats of polyurethane.

A 200 W incandescent lamp, positioned ca 600 mm overhead lit the apparatus; fluorescent ceiling lamps provided additional ambient lighting. A white paper screen along three sides surrounded the apparatus, leaving one side open for observations. The ramp was positioned so that during tests the salticid moved away from the open side and the observer. Before each test, a *M. bakeri* was placed in a pit drilled halfway through the thickness of the ramp 200 mm from the lure. The pit was 32 mm in diameter and centered 65 mm from the bottom end of the ramp. The salticid was left in the pit to acclimate for 60 s before a piece of cardboard, which was placed over the pit, was removed, allowing the salticid to exit from the pit.

Tests began when the *M. bakeri* walked out of the pit and on to the ramp and ended when it either was within 1 mm of the lure (preventing the spider from touching the lure so as to avoid chemical contamination of the lure) or walked off the top of the ramp. If the salticid jumped off the ramp at a point below the lure or if it stayed in the pit for more than 30 min

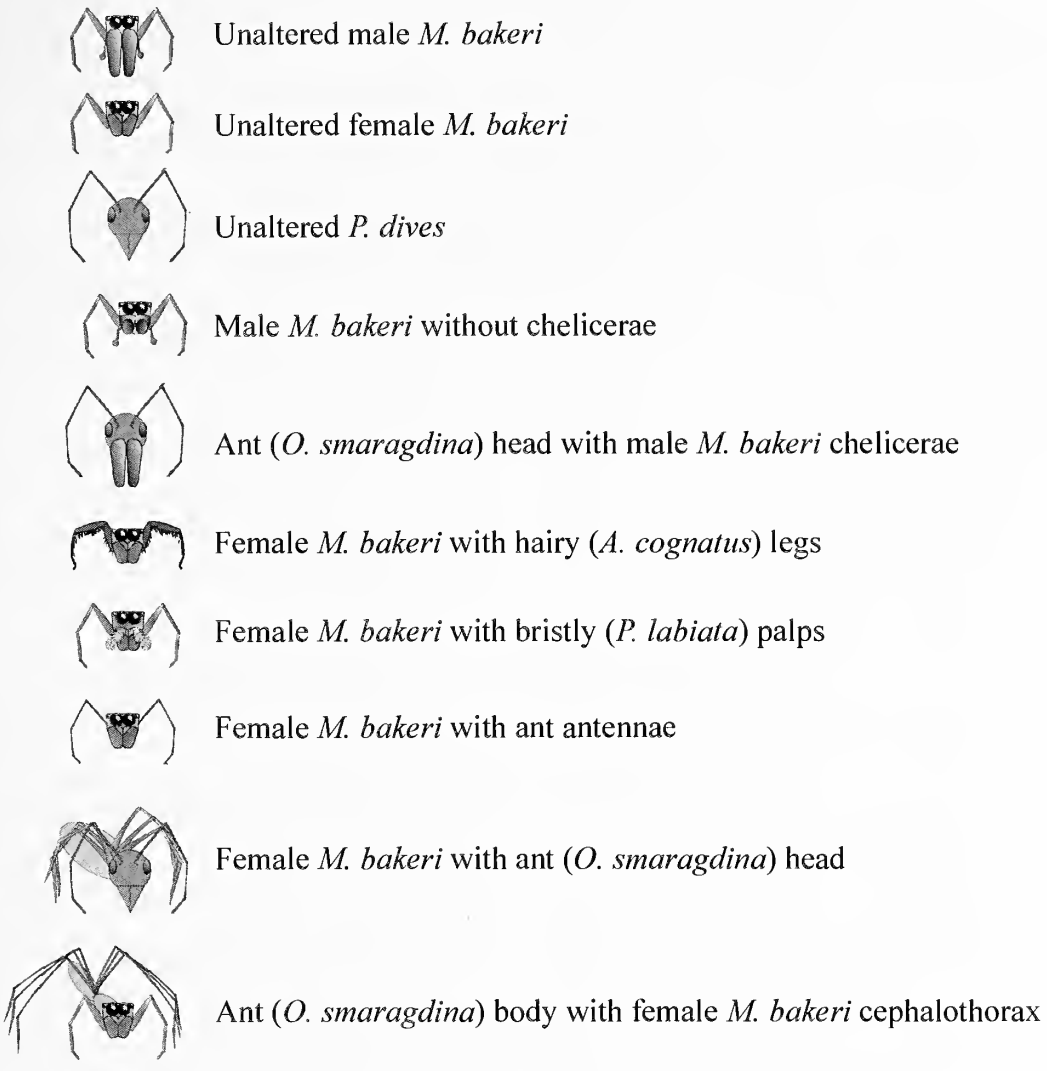

Figure 2.—Lures used for testing cues by which *Myrmarachne bakeri* distinguishes conspecifics from ants.

(no spiders walked under the ramp), tests were aborted (< 5%). Spiders that did not display were excluded from the analysis. The ramp was wiped with 80% ethanol and allowed to dry for 30 min between each test to eliminate possible chemical traces from the spiders.

Lures.—Ten lure types were made (Fig. 2), using whole arthropods ('unaltered lures') or anatomical portions ('altered lures') of three species of saltieids (*M. bakeri*, *P. labiata*, *A. cognatus*), and two species of ants (*O. smaragdina* and *P. dives*), which were combined in various ways. The questions addressed for each lure type are described below.

Lures were made by immobilizing an arthropod with CO_2 and placing it in 80% ethanol. One day later, I mounted the arthropod in a life-like posture on the centre of one side of a disc-shaped piece of cork (diameter ca 1.25 × body length of the arthropod; thickness ca 2 mm) using forceps to position it. The lure was then sprayed with a transparent aerosol plastic adhesive for preservation (see Jackson & Tarsitano 1993). I altered lures by removing body parts from a dead arthropod with a scalpel prior to mounting on the cork disc, in some instances replacing them with body parts from another dead arthropod by gluing them with adhesive spray in the relevant location (see Fig. 2). Of the ten lure types, seven were altered to test for specific cues used for conspecific recognition and for discrimination between conspecifics and ants.

In prior studies (Nelson & Jackson 2007, summarized in Table 1), Jackson and I described the characteristics of typical responses by *M. bakeri* to live conspecific males and females or live ants. I here use these prior observations to assess the responses of *M. bakeri* toward altered and unaltered lures. I posed the question such that comparisons were made between displays to a certain stimulus and 'other' displays (all other displays). I analyzed the data using Fisher exact tests. For example, to address the question whether test spiders displayed in the same way as to a male *Myrmarachne* I compared the number of spiders that used typical display behavior exhibited toward males (as described in Nelson & Jackson 2007) versus the number of spiders that exhibited other displays. Results are reported with Bonferroni adjustments for multiple comparisons. Distances at which displays were initiated and display duration were analyzed using ANOVA in Stat View Version 5 (SAS Institute Inc.).

1) *Is movement a necessary cue for recognition of ants and conspecifics?* Lures made from unaltered males and females of *M. bakeri* and an unaltered ant (*Polyrachis*

Table 1.—Outline of behavioral characteristics of the displays of male and female *Myrmarachne bakeri* toward conspecific males and females and toward ants (based on Nelson & Jackson 2007).

	Male	Female	Ant
Male	Abdomen raised and twitching	Abdomen lowered and twitching	Abdomen raised but not twitching
	Body sometimes held high	Body held low	Body sometimes held high
	Palps stationary	Palps moving	Palps stationary
	Legs tight in on the body	Legs spread wide away from body	Legs in normal posture
Female	Abdomen raised and twitching	Abdomen raised but not twitching	Abdomen raised but not twitching
	Body held 'normal' or low	Body sometimes held high	Body held high or low
	Palps stationary	Palps stationary	Palps sometimes moving
	Legs tight in on the body	Legs tight in on the body	Legs in normal posture

dives) were used. Lures faced 45° away from the starting pit on the ramp and were tested with both male and female *M. bakeri* ('standard methods'). This ensured that potential cues from both the abdomen and cephalothorax were visible to the test spider. Responses toward these stationary lures were assessed based on responses toward live animals of the same sex and species (see Table 1).

2) *Are palps an important optical cue by which* M. bakeri *recognizes conspecific females?* The hairless palps from a dead *M. bakeri* female were removed and replaced with the bristly palps of a non-ant-like salticid, *Portia labiata*. Standard methods were used for testing.

3) *Are chelicerae an important optical cue by which* M. bakeri *recognizes conspecific males and distinguish them from ants?* Two lure types were made, one by cutting the chelicerae off a *M. bakeri* male and the other by gluing the chelicerae of a *M. bakeri* onto the 'face' (anterior part of the ant's head or spider's cephalothorax) of an ant (*Oecophylla smaragdina*). To human observers the former lure resembled a female *M. bakeri*. The second lure, to human observers, resembled a male *M. bakeri*. Standard methods were used for testing.

4) *Are legs I an important optical cue by which* M. bakeri *recognizes conspecific females and distinguish them from ants?* Two lure types were made. In the first type, the hairless legs I of a female *M. bakeri* were exchanged with the antennae of an ant. The second type was made by exchanging a *M. bakeri* female's legs I for the hairy and robust legs I of a non-ant-like salticid, *Aelurillus cognatus*. Standard methods were used for testing.

5) *Relative importance of the body and of the face in male recognition of females and ants.* The head of an ant (*Oecophylla smaragdina*) was exchanged for the cephalothorax of a female *M. bakeri*. This provided two lure types, one with the 'body' (thorax and abdomen) of an ant and the cephalothorax of *M. bakeri* and the other with the abdomen of *M. bakeri* and head of *O. smaragdina*. These lures were tested only with male *M. bakeri*. Lures were placed so they faced directly toward the starting pit (0°), thereby providing cues from the 'face' only.

Voucher specimens of all species have been deposited in the IRRI Taxonomy Laboratory in Los Baños, the Philippines, and in the Florida State Collection of Arthropods, Gainesville, Florida, USA.

RESULTS

Is movement a necessary cue for recognition of ants and conspecifics?—Movement is not a necessary cue for eliciting *M. bakeri*'s typical displays toward conspecifics and ants. *M. bakeri* responded to dead, odorless, unaltered lures from conspecific males and females and from ants in the same way as they responded to living conspecific females and males and living ants (Table 1) between 79 and 100% of the time (Tables 2 & 3).

Are palps an important optical cue by which *M. bakeri* recognizes conspecific females?—Females ($P = 0.10$, $df = 1$, $n = 17$; Table 2, comparison 8 vs 2) and especially males ($P = 1.00$, $df = 1$; $n = 13$; Table 3, comparison 8 vs 2) displayed toward altered lures of conspecific females with bristly palps (from *Portia labiata*) in much the same way as toward lures made from unaltered females (Fisher exact tests).

Are chelicerae an important optical cue by which *M. bakeri* recognizes conspecific males and distinguishes them from ants?—Both females ($P < 0.01$, $df = 1$, $n = 25$; Table 2, comparison 5 vs 3) and males ($P < 0.001$, $df = 1$, $n = 22$; Table 3, comparison 5 vs 3) displayed toward the altered lure of an ant with *M. bakeri* male chelicerae differently from how they displayed to a lure made from an unaltered ant (Fisher exact tests). Instead, ants with chelicerae were treated as conspecific males by both males ($P = 1.00$, $df = 1$, $n = 24$; Table 3, comparison 5 vs 1) and females (Fisher exact test, $P = 0.199$, $df = 1$, $n = 24$; Table 2, comparison 5 vs 1) (Fisher exact tests). Females ($P < 0.001$, $df = 1$, $n = 28$; Table 2, comparison 4 vs 1) and males ($P < 0.001$, $df = 1$, $n = 34$; Table 3, comparison 4 vs 1) responded differently toward lures made from an unaltered male and from a male without chelicerae (Fisher exact tests). Neither males ($P = 0.26$, $df = 1$, $n = 25$; Table 3, comparison 4 vs 2) nor females ($P = 0.011$, $df = 1$, $n = 25$; Table 2, comparison 4 vs 2) displayed toward males without chelicerae similarly as toward a conspecific female (Fisher exact tests). However, females ($P = 0.427$, $df = 1$, $n = 29$; Table 2, comparison 4 vs 3), but not males ($P < 0.001$, $df = 1$, $n = 32$; Table 3, comparison 4 vs 3), displayed

Table 2.—Response toward lures used to determine the cues used by female *Myrmarachne bakeri* to distinguish ants (*Polyrachis dives* and *Oecophylla smaragdina*) from conspecifics. Missing percentages due to inability to interpret displays. * Displays were not typical of female-female displays.

Lure number	Lure	*n* tested	*n* displayed	Percent displayed as to a conspecific male (*n*)	Percent displayed as to a conspecific female (*n*)	Percent displayed as to an ant (*n*)
1	Unaltered male *M. bakeri*	19	13	100 (13)		
2	Unaltered female *M. bakeri*	19	10		90 (9)	
3	Unaltered *P. dives*	20	14			78.6 (11)
4	Male *M. bakeri* without chelicerae	20	15	13.3 (2)	26.7 (4)	60 (9)
5	*O. smaragdina* with male *M. bakeri* chelicerae	19	11	81.8 (9)		18.2 (2)
6	Female *M. bakeri* with hairy (*Aelurillus cognatus*) legs I	17	3*		0	
7	Female *M. bakeri* with *O. smaragdina* antennae as legs I	17	7		100 (7)	
8	Female *M. bakeri* with bristly (*Portia labiata*) palps	20	7		42.9 (3)	

toward the male lure without chelicerae in much the same way as toward an ant (Fisher exact tests).

Are legs I an important optical cue by which *M. bakeri* recognizes conspecific females and distinguish them from ants?—Hairless legs are a necessary cue for females ($P = 0.014$, $df = 1$, $n = 13$; Table 2, comparison 6 vs 2) to identify conspecific females, but not necessary for males to identify conspecific females ($P = 1.00$, $df = 1$, $n = 13$; Table 3, comparison 6 vs 2) (Fisher exact tests), as males displayed toward altered lures of females with hairy legs I (*Aelurillus cognatus*) in much the same way as toward lures made from unaltered females.

Ant antennae alone do not elicit the display behavior typical of *M. bakeri* males ($P < 0.001$, $df = 1$, $n = 22$; Table 3, comparison 7 vs 3) and females ($P = 0.002$, $df = 1$, $n = 21$; Table 2, comparison 7 vs 3) to ants (Fisher exact tests). Instead, both males ($P = 1.00$, $df = 1$, $n = 15$; Table 3, comparison 7 vs 2) and females ($P = 1.00$, $df = 1$, $n = 17$; Table 2, comparison 7 vs 2) displayed toward the altered lure of a conspecific female with ant antennae in the same way as they did toward lures made from an unaltered conspecific female (Fisher exact tests).

Relative importance of the body and of the face in male recognition of females and ants.—Males displayed toward the altered lure made from a conspecific female with an ant's head in much the same way as to a lure made from an unaltered conspecific female ($P = 0.262$, $df = 1$, $n = 16$; Table 3, comparison 9 vs 2), and significantly differently to typical responses in interactions with ants ($P = 0.034$, $df = 1$, $n = 23$; Table 3, comparison 9 vs 3) (Fisher exact tests).

Males responded toward the altered lure of an ant with the cephalothorax of a *M. bakeri* female significantly differently from their response toward an unaltered ant ($P = 0.008$, $df = 1$, $n = 21$; Table 3 comparison 10 vs 3) (Fisher exact test). Instead, males generally responded initially toward the altered lure of an ant with the cephalothorax of a *M. bakeri* female in the same way as they did when courting conspecific females

Table 3.—Response toward lures used to determine the cues used by male *Myrmarachne bakeri* to distinguish ants (*Polyrachis dives* and *Oecophylla smaragdina*) from conspecifics. Missing percentages due to inability to interpret displays. * Lure facing pit (0°). ** All changed display (as toward ants) when they circled the female as part of the courtship dance and then saw the ant's body.

Lure number	Lure	*n* tested	*n* displayed	Percent displayed as to a conspecific male (*n*)	Percent displayed as to a conspecific female (*n*)	Percent displayed as to an ant (*n*)
1	Unaltered male *M. bakeri*	21	18	100 (18)		
2	Unaltered female *M. bakeri*	17	9		88.9 (8)	
3	Unaltered *P. dives*	18	16			93.8 (15)
4	Male *M. bakeri* without chelicerae	20	16	31.2 (5)	50 (8)	18.8 (3)
5	*O. smaragdina* with male *M. bakeri* chelicerae	10	6	100 (6)		
6	Female *M. bakeri* with hairy (*Aelurillus cognatus*) legs I	10	4		75 (3)	
7	Female *M. bakeri* with *O. smaragdina* antennae as legs I	9	6		100 (6)	
8	Female *M. bakeri* with bristly (*Portia labiata*) palps	15	4		100 (4)	
9	Female *M. bakeri* with *O. smaragdina* head*	8	7		57.1 (4)	42.9 (3)
10	*O. smaragdina* with female *M. bakeri* cephalothorax*	8	5		80 (4)**	20 (1)

(their initial view of the lure was face on) ($P = 1.00$, $df = 1$, $n = 14$; Table 3, comparison 10 vs 2) (Fisher exact test). However, during typical courtship with a living conspecific female, males perform dances involving side-to-side stepping (Nelson & Jackson 2007). When test males danced in front of the lure, they got into a position from which the lure was visible from the side, instead of face-on. At this point, the ant's body was visible and in all cases the males immediately switched behavior and briefly displayed as to an ant before fleeing (Table 3).

Display distance and duration.—Sex had no main effect on the distance ($F_{(1,167)} = 0.806$, $P = 0.371$) at which displays were initiated toward altered and unaltered lures, nor on their duration ($F_{(1,167)} = 0.773$, $P = 0.381$). However, *M. bakeri* displayed toward unaltered lures from further away than toward altered lures ($F_{(1,167)} = 8.325$, $P = 0.004$), although display duration did not differ ($F_{(1,167)} = 1.887$, $P = 0.171$). There was no interaction effect of distance ($F_{(1,167)} = 1.659$, $P = 0.5839$) or duration ($F_{(1,167)} = 0.091$, $P = 0.763$).

Female display duration was not affected by lure type ($F_{(7,72)} = 0.801$, $P = 0.589$, Fig. 3a). However, lure type did have a significant effect on the distance from which females initiated displays. ($F_{(7,72)} = 3.134$, $P = 0.006$, Fig. 3b). Fisher's PLSD post-hoc tests showed that females displayed toward *Polyrachis dives* from further away than toward conspecific females ($P = 0.002$), females with ant antennae ($P = 0.001$), females with hairy legs ($P = 0.034$), females with bristly palps ($P = 0.001$), males without chelicerae ($P = 0.007$), and males ($P = 0.025$). In other words, females displayed from further away toward ants than to anything that resembled a conspecific, except *Oecophylla smaragdina* with male chelicerae.

Male display duration was not also affected by lure type ($F_{10,80} = 1.189$, $P = 0.311$, Fig. 3a). Lure type did have a significant effect on the distance from which males initiated displays ($F_{9,81} = 4.214$, $P < 0.001$, Fig. 3b), which followed similar patterns to those of females (see Fig. 3b), with ants being displayed at from the greatest distance, followed by conspecific males, both with and without chelicerae.

DISCUSSION

Myrmarachne bakeri distinguishes conspecifics from ants based on the elongated chelicerae of conspecific males, legs I, as well as body and other facial cues, such as the size and position of the eyes. Taken in combination, results from these display distance and display type data suggest that the enlarged chelicerae of males are fundamental for male recognition - even lures of ants with male chelicerae were displayed at as if they were males. This is intriguing, as non-ant-like salticids respond to male *Myrmarachne* as if they were ants carrying something in their mandibles (Nelson & Jackson 2006b). However, absence of chelicerae is not the sole cue used to distinguish males from females, as neither sex responded to lures of males without chelicerae as if they were females. This is an interesting finding because to the human observer a *M. bakeri* male without chelicerae looks very similar to a *M. bakeri* female. However, both males and females displayed from further away in the presence of ant lures than conspecific lures, implying that they are able to distinguish ants from conspecifics before approaching so close that it may be dangerous (Nelson et al. 2004).

Males did not appear to attend strongly to cues from the palps or legs of females, generally displaying toward these altered lures in the same way as toward conspecific females, and from similar distances. Furthermore, males displayed to lures of females with the head of an ant as if they were females, suggesting that cues from the body are important in recognition of females. Nevertheless, cues from the female's cephalothorax are used, as they also responded to lures made from an ant with the head of a spider in a manner typical of that used toward females - that is, until they saw the ant's body, whereupon they quickly displayed as to an ant and fled. However, females did appear to attend to cues from the legs of females. Unlike males, they responded differently toward unaltered lures of conspecific females than toward lures of females with the legs of other salticids.

A control lure in which body parts were cut and reassembled might have been useful to account for the effects of cutting and gluing. However, responses toward "combination lures" of females and ants, in which males responded to the "face" as to a female, but upon circling the lure and encountering the abdomen of the ant, changed tactics rapidly, suggest that glued 'intact' controls were unnecessary. The actual part that was being responded to in each case was unaltered, but the displays were very clear (one of courtship, the other escape after a brief 'aggressive' display) despite these lures being glued together.

To the human eye, *M. bakeri* legs I and the ants' antennae appear very similar and it seems that they also appear that way to *M. bakeri*. Although neither males nor females were able to distinguish ants on the basis of antennae alone, females appear to be more sensitive than males to the finer distinctions between ants and conspecifics, generally displaying toward lures containing ant parts from further away than toward lures of conspecifics or conspecifics with salticid parts. These findings suggest that *M. bakeri* uses general templates for conspecific recognition. If, on the whole, the cues fit the template, a 'decision' is made regarding the identity of the individual that is the source of the cues. Yet males and females seem to differ in the cues they use for recognizing conspecifics. For example, although males did not discriminate between the combination lure of a conspecific female with hairy legs I and the lure of the unaltered conspecific female, females did discriminate, while the display distance of males, but not females, toward males without chelicerae was more similar to that of males toward males than females. An especially striking example of template matching in jumping spiders occurs with *Maevia inclemens* (Walckenaer 1837). Males of this species are dimorphic, both in morphology and in courtship behavior. Despite these differences females recognize males and will mate with both morphs (Clark & Uetz 1992); however, if the behavior of one morph is superimposed (through the use of computer animation) on the body of the other morph, female receptivity is significantly lowered, suggesting that females match the behavior and morphology of each morph to an existing template (Clark & Uetz 1993).

Predator-prey interactions necessitate the recognition of the subject as either one or the other. In many cases this may often be achieved simply through size: if it is the bigger one, it is a potential predator, and if it is the smaller one it is a potential prey (Prete 1990; Prete et al. 2002). *Myrmarachne* lives in the

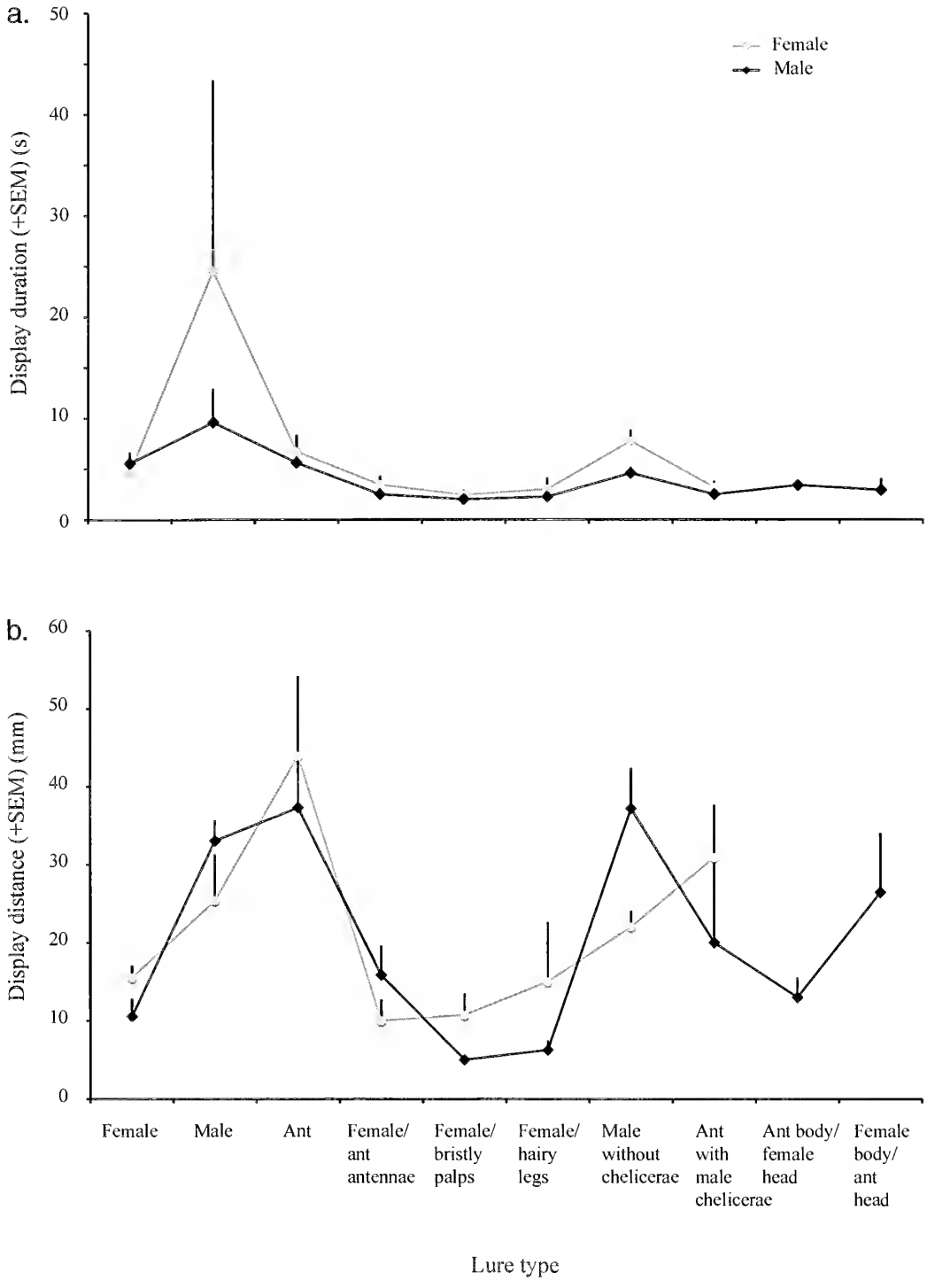

Figure 3.—Mean (+SEM) (a) duration and (b) distance of male and female displays toward lures.

vicinity of ants, and as both model and mimic are active, cursorial predators they often come near each other. In a twist to traditional examples of mimicry, the model itself is a potential predator of the mimic (Nelson et al. 2004, 2005), and this makes the task of distinguishing between the model and its conspecifics critical for *Myrmarachne*.

Other studies have shown that various species of salticids have the ability to recognize prey on the basis of optical cues alone. The most detailed studies of the cues by which salticids make vision-based discriminations have come from work on prey recognition in araneophagic (spider-eating) salticids in the genus *Portia* (Jackson & Tarsitano 1993; Harland & Jackson 2001, 2002). These studies suggest that the presence of the large, forward-facing anterior medial eyes (AME) are crucial in distinguishing jumping spiders from other spiders. In this study it was not possible to make realistic lures while altering the appearance of the AME. However, these results suggest that this would be a factor well worth further investigation.

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A new species of *Mesobuthus* (Scorpiones: Buthidae) from Xinjiang, China, with notes on *Mesobuthus songi*

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Abstract. A new species, *Mesobuthus bolensis* from the Province of Xinjiang, in the Western region of China, is described. The new species can be defined by a densely granular carapace; carinae, granulation, and metasomal segment V without any dark pigmentation; carinae of carapace and pedipalp patella dispersively granular. Furthermore, restudy of the characters of *Mesobuthus songi* Lourenço, Qi & Zhu 2005, described from the southern region of Pulan, Xizang (Tibet), China led us to accommodate this species in the genus *Hottentotta* Birula, as a new combination *Hottentotta songi* (Lourenço, Qi & Zhu 2005).

Keywords: Scorpion, *Hottentotta*, new species, new combination

In comparison with scorpion faunas of adjacent regions (e.g., Vachon 1958; Tikader & Bastawade 1983; Fet 1989), the diversity of scorpions in the Province of Xinjiang appears to be rather poor. However, taking into account that this region of China remained inaceessible for several decades and considering its extensiveness, it is quite possible that this fauna has been largely underestimated. Early studies of the region of Xinjiang have been performed by foreign experts, in particular by the Russian scorpiologist Birula (1897, 1904, 1911, 1917). Subsequently, no other experts have been involved in the study of Xinjiang scorpions. Also, considering the particular climate of this area and the diversity of its environment, inventories seem far from complete. More recently, new studies have focused on this area again, and produce new interesting results, including the discovery of new species (Lourenço et al. 2009; Sun et al. 2009). As part of a global research project on the entire Chinese scorpion fauna, our research team is conducting intensive field work in Xinjiang. Among the scorpions found in this region, most specimens belong to the genus *Mesobuthus*. Two of these were collected in the northwest region of Xinjiang, and correspond to one more new species. The new species represents the sixth known species of this genus from China.

In this contribution we also restudy the characters of *Mesobuthus songi* Lourenço, Qi & Zhu 2005, recently described from the southern region of Pulan, Xizang (Tibet) in China. This new analysis led to the conclusion that most characters of this speeies most likely associate it to the genus *Hottentotta* Birula.

In previous publications (Fet & Lowe 2000), it was clearly pointed out that the relationships of *Mesobuthus* to other genera, and in particular to *Hottentotta*, remain poorly defined. This is particularly true for the species distributed in the Middle East, and also in China. Further analysis (e.g. Gantenbein et. al. 2003) is still needed for clarifying their respective positions.

METHODS

Specimens were examined and measured under a Leica M165c stereomicroscope with an ocular micrometer. Illustrations were produced using a Leica M165c stereomicroscope with a drawing tube. All measurements follow Stahnke (1970) and are given in millimeters (mm), except for the chela (Vachon 1952). Trichobothrial notations follow Vachon (1974) and morphological terminology mostly follows Hjelle (1990), except for the carinae of a pedipalp patella, which follows Soleglad & Fet (2003). The specimens used in this taxonomic work come from the Museum of Hebei University, Baoding (MHBU), and the Muséum national d'Histoire naturelle, Paris (MNHN). All illustrations and measurements of the new species were based on the male holotype (Ar.-MHBU-XJ0701) and the female paratype (Ar.-MHBU-XJ0610); illustrations and measurement of *Mesobuthus longichelus* Sun, Zhu & Lourenço 2009 were based on the female holotype (Ar.-MHBU-XJ0801); illustrations of *Hottentotta songi* were based on the female holotype (Ar.-MHBU-XZ3101).

TAXONOMY

Family Buthidae C.L. Koch 1837
Genus *Mesobuthus* Vachon 1950

Mesobuthus Vachon 1950:152–153.

Type species.—*Androctonus eupeus* C.L. Koch 1839, by original designation.

Diagnosis.—Total length 40–85 mm. Carinae of carapace granular, and central median, posterior median and lateral median carinae forming distinct shape of lyre. Dorsal trichobothria of femur arranged in β-configuration; trichobothrium *db* usually basal to *est* on the fixed finger, or on level with *est*; line joining trichobothria v_1 and v_2 of pedipalp chela perpendicular, or nearly perpendicular, to axis of movable finger articulation. Movable finger of pedipalp-chela with distinct granules divided into 10–14 rows and 4 terminal granules (not including the terminal denticle). Ventrolateral carinae of Metasoma segment V formed of disjunct and unequal granules, often enlarged posteriorly (Vachon 1950; Sissom 1990).

Distribution.—Species of *Mesobuthus* occur in Asia, Balkan Peninsula and Caucasia.

Mesobuthus bolensis new species
(Figs. 2, 3, 5–11, 14–18, 21, 22; Table 1)

Material examined.—CHINA: *Xinjiang*, Holotype male, 15 km SW of Bole City, 44°44′N, 81°59′E, 31 July 2007, D. Sun & L. Zhang (Ar.-MHBU-XJ0701). Paratype: 1 female, area close to Yining County, 44°00′N, 81°32′E, 14 August 2006, F. Zhang, H. X. Ma & S. N. Liu (Ar.-MHBU-XJ0610).

Etymology.—The specific name refers to Bole, Xinjiang, China, the type locality of the new species.

Diagnosis.—Species of moderate to large size, with respect to the genus, reaching a total length of 57 mm in male and 71 mm in female. General coloration pale brownish-yellow to yellow; all carinae, granules and metasoma segment V without any dark pigment. Anterior median, central median, and posterior median carinae of carapace granular and somewhat dispersive; dorsointernal and dorsomedian carinae of patella dispersive granular. Tarsus and basitarsus with many long setae; tarsus ventrally with two longitudinal rows of long setae. Metasoma segments and aculeus elongate; aculeus longer than vesicle. Both movable and fixed fingers with 12 oblique rows of granules. Pectinal tooth count 28–12 (right pecten injured, not complete) in male and 22–22 in female. Trichobothrial pattern of Type A-β (Vachon 1974, 1975), orthobothriotaxic. Several similarities that justify *Mesobuthus bolensis* sp. n. to be undoubtedly associated with *Mesobuthus longichelus*: 1) similar general morphology, especially the metasoma segment I–IV and telson; 2) similar basic coloration; 3) both with 12 oblique rows of granules on movable fingers and with nearly same pectinal tooth number (all in females). However, the new species can be distinguished by the following features: 1) larger, reaching total length of 57 mm in male and 71 mm in female, vs. 52 mm in female for *M. longichelus*; 2) metasoma segment V without any dark pigment, whereas ventral and lateral surfaces of segment V of *M. longichelus* with inconspicuous variegated black pigment (Figs. 21–24); 3) carapace with much denser granules (Figs. 2–4); 4) anterior median, central median, and posterior median carinae of carapace and dorsointernal and dorsomedian carinae of patella dispersive granular, whereas those carinae not like that for *M. longichelus* (Figs. 2–5, 7, 12–13); 5) ventrolateral carinae of metasoma segment V granular, with posterior granules enlarged, whereas it is strongly marked posteriorly; with 2–4 strong and extroversive lobed granules in *M. longichelus* (Figs. 21–24); 6) chela manus more robust than that for *M. longichelus* (Figs. 14–17, 19–20).

Description.—Based on male holotype (Ar.-MHBU-XJ0701).

Coloration: Basically pale brownish-yellow to yellow, prosoma: carapace pale brownish-yellow, only eyes surrounded by black pigment. Mesosoma and metasoma: pale brownish-yellow; vesicle yellow and aculeus dark reddish to blackish on its extremity. Venter: pale brownish-yellow, except for the pectines, which are pale yellow. Chelicerae: pale brownish-yellow without any variegated pigmentation; teeth dark reddish. Pedipalps: yellow; rows of granules on dentate margins of the fingers blackish-brown. Legs: pale yellow without spots.

Morphology: prosoma: anterior margin with a very weak median concavity and slightly serrate centrally. Carinae moderately strong, granular; anterior median, central median and posterior median carinae granular and somewhat dispersive, especially the central median carinae; central median carinae directly connected with posterior median carinae, and not with lateral median carinae; posterior median carinae terminating distally in a small spinoid process that extends distinctly beyond the posterior margin of the carapace. Carapace with coarse granules, especially in the anterior; intercarinal surfaces not smooth, finely to coarsely granular; furrows moderate. Median ocular tubercle slightly anterior to the center of carapace; median eyes separated by almost two ocular diameters; three pairs of lateral eyes. Mesosoma: tergite: I to VI tricarinate; all carinae moderately developed, strongly granular; each carina on I–VI terminating distally in a small spinoid process that extends very distinctly beyond the posterior margin of tergite; intercarinal surfaces moderately granular, exterior surfaces coarsely and densely granular; VII pentacarinate; two pairs of lateral carinae moderate to strong; median carinae weak, present only on proximal half; intercarinal surfaces smooth, exterior surfaces coarsely and densely granular. Sternites: III–VII smooth; lateral margins serrate; VII with four weakly marked carinae, granular. Pectines: moderately long; pectinal teeth 28–12 (right pecten injured, not complete). Metasoma: segments I to III with 10 carinae, segment IV with eight carinae; all carinae moderately strong, granular, except for the dorsal carinae, strong; median lateral carinae complete on segment I, only covered one third length of segment on II and obsolete, remaining one or two granules at distal end on III. Intercarinae surfaces on segments I to IV slightly concave and smooth, except for the surfaces between dorsal and dorsolateral carinae on segment I, which are weakly granular. Segment V pentacarinate; ventral carina moderate to strong; ventrolateral carinae granular, with posterior granules enlarged and serrate; dorsolateral carinae granular, moderately developed anteriorly, weakly to obsolete posteriorly; dorsal and lateral surfaces on V smooth; ventral smooth, except for few sparse granules. Telson smooth dorsally and weakly granular ventrolaterally; aculeus long, more than a half of telson length. Chelicerae: dentition as defined by Vachon (1963) for the family Buthidae. A weak subdistal tooth and two very small basal teeth on movable finger. Pedipalps: trichobothrial pattern: orthobothriotaxic A-β (Vachon 1974, 1975). Femur pentacarinate, moderately to strongly granular; ventrointernal carina with spinoid granules; dorsal surface not smooth, finely granular. Patella with eight carinae, very weakly to moderately granular; dorsointernal and dorsomedian carinae dispersive granular; intercarinal surface smooth. Chela smooth without carinae; both movable and fixed fingers with 12 oblique rows of granules; movable finger with a strong basal tubercle on dentate margin. Legs: Tarsus and basitarsus with many long setae; tarsus ventrally with two longitudinal rows of long setae; tibial spurs strong on legs III and IV; pedal spurs moderately developed on all legs.

Variation.—Based on female paratype (Ar.-MHBU-XJ0610).

There is some variation between the holotype and female paratype, particularly the sexual dimorphism. 1) anterior median carinae obsolete anteriorly; 2) carapace with much denser granules; 3) intercarinal surfaces weakly granular; 4) Sternite VII with four moderately marked carinae, granular; 5)

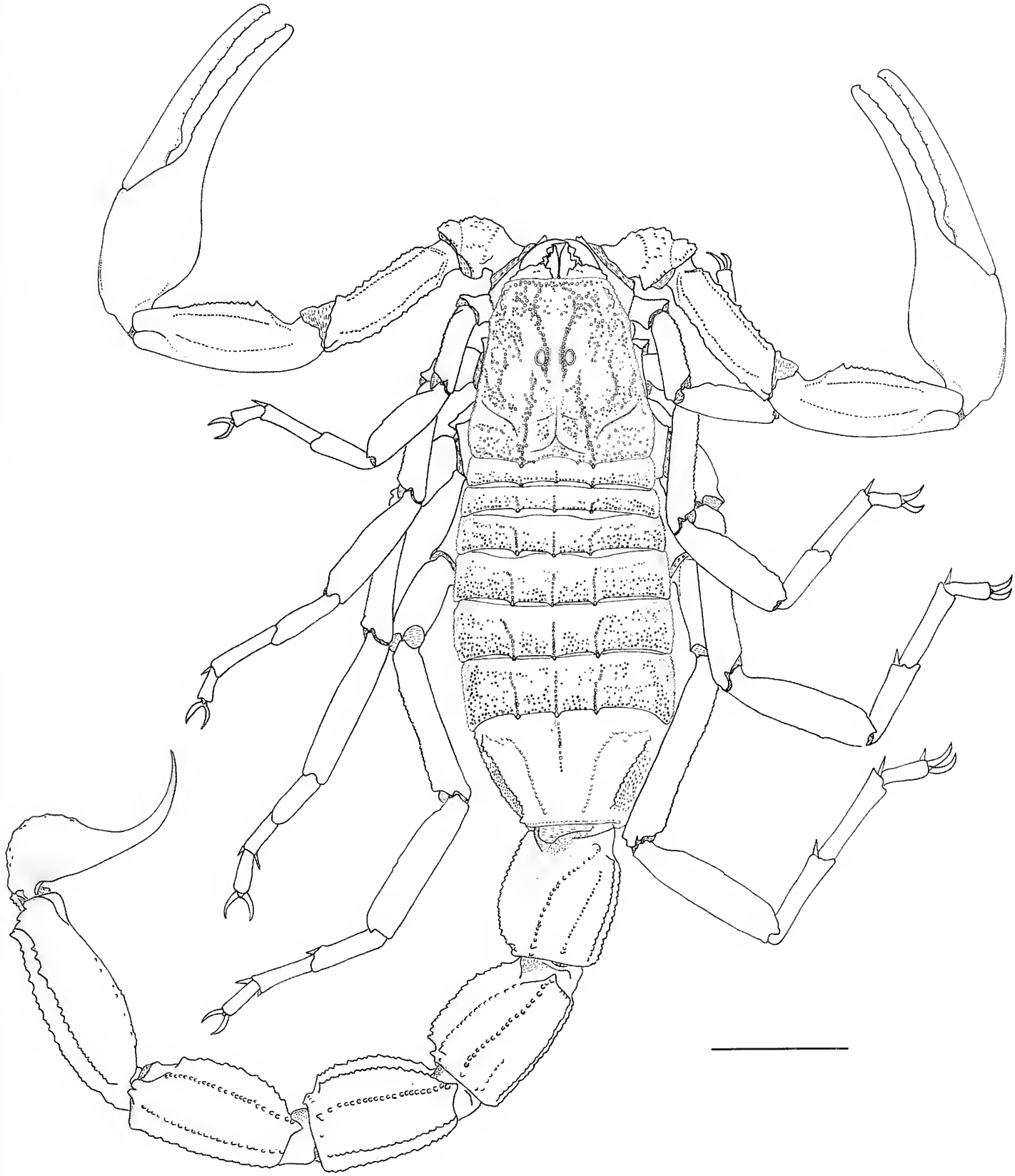
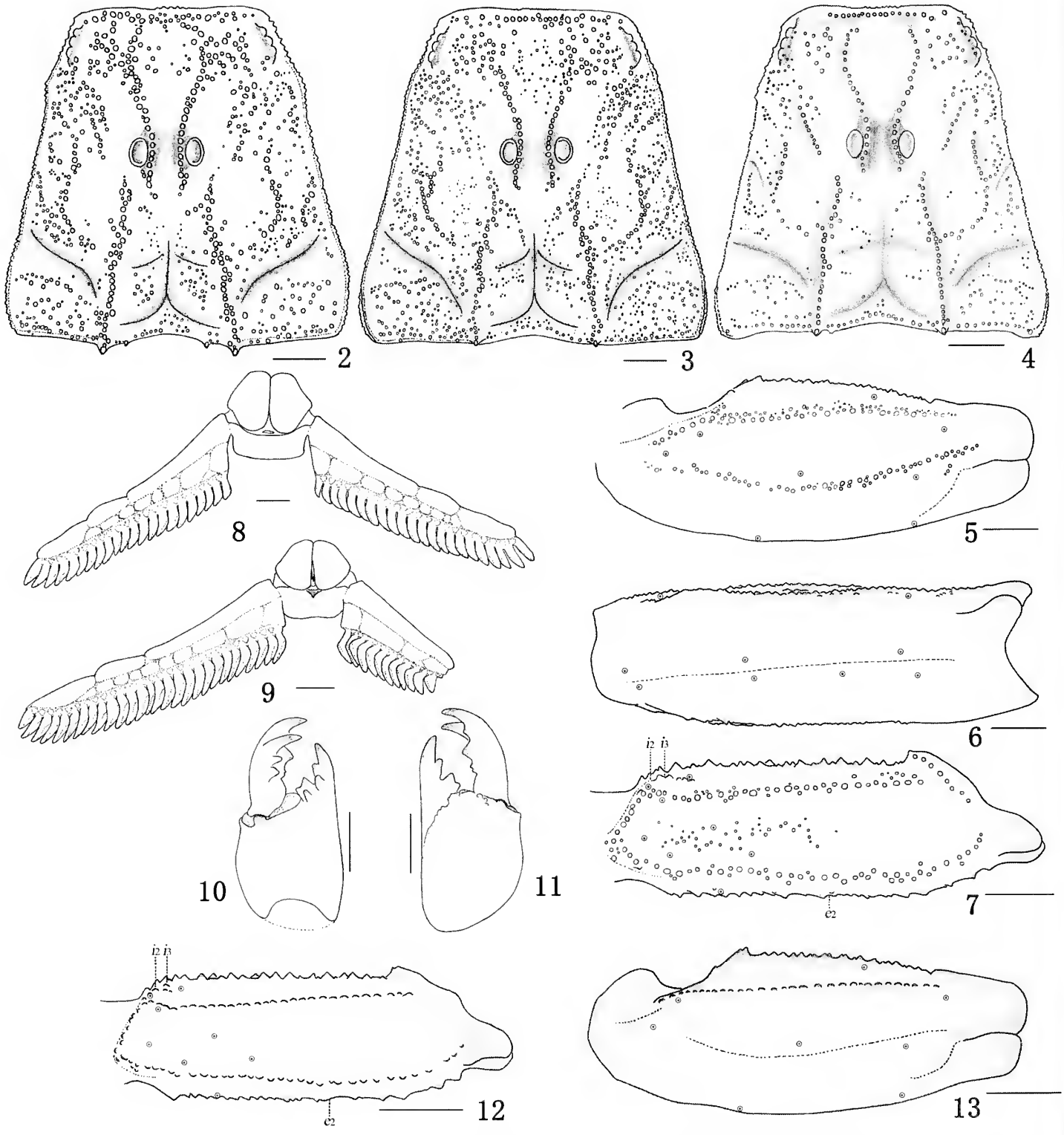
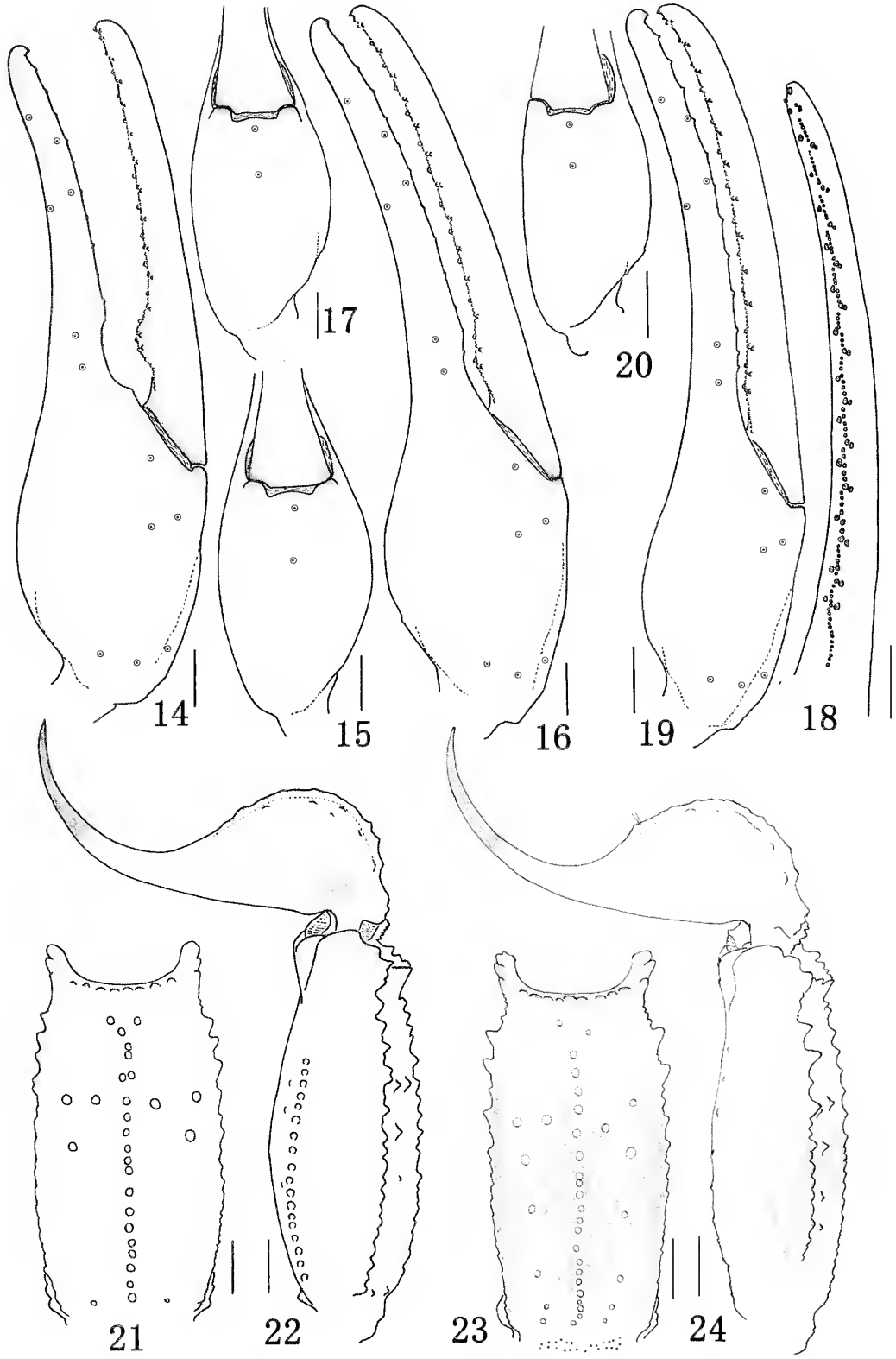


Figure 1.—*Mesobuthus bolensis* new species, male holotype, dorsal view. Scale bar = 5.0 mm.



Figures 2–13.—*Mesobutlus* species. 2, 9–11. *M. bolensis* new species, male holotype. 3, 5–8. *M. bolensis* new species, female paratype. 4, 12, 13. *Mesobutlus longichelus* Sun, Zhu & Lourenço, 2009, female holotype. 2–4. Carapace, dorsal aspect; 5, 6, 13. Palpal patella (5, 13. dorsal; 6. external); 7, 12. Palpal femur, dorsal aspect. 8, 9. Genital operculum and pectines, ventral aspect; 10, 11. Chelicera: 10. Ventral; 11. Dorsal. Scale bar = 1.0 mm.



Figures 14–24.—*Mesobuthus* species. 14, 15, 21, 22. *M. bolensis* new species, male holotype. 16–18. *M. bolensis* new species, female paratype. 19, 20, 23, 24. *M. longichelus* Sun, Zhu & Lourenço, 2009, female holotype. 14–17, 18, 19. Chela (14, 16, 19. Dorso-external; 15, 17, 20. Ventral); 18. Disposition of granulations on the dentate margins of the pedipalp chela movable finger, dorsal aspect; 21, 23. Metasomal segment V, ventral aspect; 22, 24. Metasomal segment V and telson, lateral aspect. Scale bar = 1.0 mm.

Table 1.—Morphometric values (in mm) of the holotype and paratype of *Mesobuthus bolensis* new species and female holotype of *Mesobuthus longichelus*.

	M. bolensis new species		*M. longichelus*
	♂ (holotype)	♀ (paratype)	♀ (holotype)
Total length	56.56	70.78	52.08
Carapace:			
Length	6.46	7.85	5.85
Anterior width	3.77	4.69	3.38
Posterior width	6.54	8.31	6.14
Metasomal segment:			
Length	4.69	5.46	3.81
Width	4.15	4.69	3.33
Metasomal segment:			
Length	5.23	6.00	4.71
Width	4.00	4.54	3.19
Metasomal segment:			
Length	5.54	6.46	4.95
Width	4.00	4.54	3.19
Metasomal segment:			
Length	6.00	7.00	5.48
Width	4.00	4.23	3.10
Metasomal segment:			
Length	6.69	8.77	6.14
Width	3.54	3.92	2.95
Depth	2.85	3.15	2.24
Telson:			
Length	7.08	7.85	6.23
Width	2.69	3.08	2.08
Depth	2.31	2.69	2.04
Aculeus length	4.00	3.92	3.39
Pedipalps:			
Femur length	5.69	6.54	5.05
Femur width	1.62	1.92	1.48
Patella length	6.62	7.77	6.10
Patella width	2.46	2.92	2.14
Chela length	11.62	13.92	10.62
Chela width	2.85	2.92	1.96
Chela depth	3.15	3.69	2.39
Movable finger length	8.00	9.77	7.62
Pectines:			
Tooth count (left-right)	28–12	22–22	22–23

* right pecten injured, not complete.

dorsal surface of femur of pedipalps moderately granular; 6) movable finger only with a weak basal tubercle.

Distribution.—This species is currently known only from China (Xinjiang).

Ecology.—This region in Xinjiang is comparatively rainy, which is the widest and driest province in China, with an average annual rainfall of 200–400 mm, mostly in the summer. The new species was found mainly in habitats composed of desertified grassland and low foothills, under rocks and clods. Its microhabitat is similar to *M. eupeus*, which is the dominant scorpion species in this region; in contrast, the new species is quite rare. We could not find any specimens in comparatively humid sand or sandy soil, while *M. eupeus* is abundant in this kind of microhabitat.

Genus *Hottentotta* Birula 1908

Androctonus: C.L. Koch, 1838:45.
Buthus (in part): Thorell, 1876:103.
Buthus (*Buthus*) (in part): Pocock, 1890:126.
Buthus (*Hottentotta*) Birula, 1908:141.
Hottentotta: Werner, 1934:269; Kovařík, 2007:1.
Buthotus (*Buthotus*): Vachon, 1979:236.
Mesobuthus (in part): Tikader & Bastawade, 1983:186; Lourenço, Qi & Zhu, 2005:3.
Hottentotta (*Hottentotta*): Francke, 1985:4.
Hottentotta (*Balfourianus*): Francke, 1985:4.

Type species.—*Scorpio hottentotta* Fabricius 1787, by original designation.

Diagnosis.—Total length 30–130 mm. Dorsal trichobothria of femur arranged in β-configuration; trichobothrium *db* on the fixed finger of pedipalp usually located between *est* and *et*, or may be on level with trichobothrium *est*, rarely between *est* and *esb*; line joining trichobothria v_1 and v_2 of pedipalp chela obliquely oriented relative to axis of movable finger articulation, with v_2 external to v_1. Movable finger of pedipalp-chela with distinct granules divided into 11–16 rows and 4–6 terminal granules (not including the terminal denticle). Ventrolateral carinae of fifth metasomal segment with all granules more or less equal in size and never lobate (Vachon & Stockmann, 1968; Sissom 1990; Kovařík 2007).

Distribution.—Species of *Hottentotta* occur in Africa, Middle East, parts of Asia.

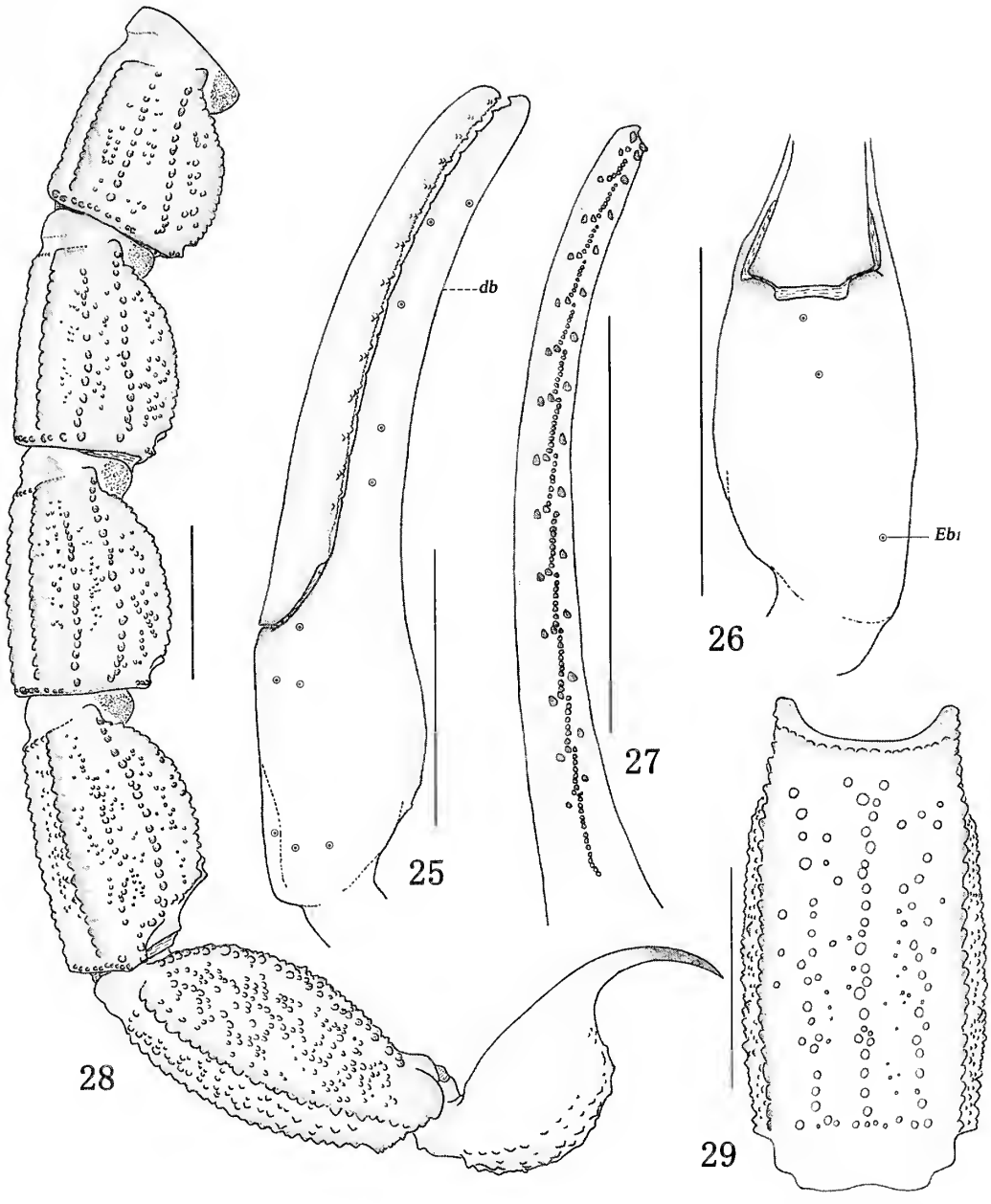
Remarks.—The study of the following characters, relative positions of trichobothria on the fixed finger and ventral surface of chela, ventrolateral carinae of metasoma segment V, led us to transfer the species *M. songi* to the genus *Hottentotta*.

Hottentotta songi (Lourenço, Qi & Zhu 2005) new combination (Figs. 25–29)

Mesobuthus songi Lourenço, Qi & Zhu 2005:3, figs. 1–17.

Material examined.—CHINA: *Xizang (Tibet)*, male holotype, southern region of Pulan, low valley of the Kongque River, near border with Nepal, 30°09′–30°15′N, 81°10′–81°18′E (estimated), July 1931, collector unknown (MNHN). Paratypes: 8 females and 7 males (MNHN), 1 female (Ar.-MHBU- XZ3101), 2 males (Ar.- MHBU- XZ3102–03), collected with holotype.

Diagnosis.—Total length reaching 69 mm (male) and 80mm (female). General coloration reddish-yellow to reddish-brown,with blackish zones on the carinae of the body. Carinae and granulations strongly marked on carapace, tergites and metasomal segments. Trichobothrial pattern: orthobothriotaxic A-β (Vachon 1974, 1975). Trichobothrium *db* on the fixed finger of pedipalp located between *est* and *et* (Fig. 25); line connecting trichobothrium v_1 with v_2 not vertical to the joint of mobile finger markedly (Fig. 26).



Figures 25–29.—*Hottentotta songi* (Lourenço, Qi & Zhu, 2005), new combination, female paratype: 25, 26. Chela: 25. Dorso-external; 26. Ventral. 27. Disposition of granulations on the dentate margins of the pedipalp chela movable finger, dorsal aspect; 28. Metasomal segments I–V and telson, lateral aspect; 29. Metasomal segment V, ventral aspect. Scale bar = 5.0 mm.

Fixed and movable fingers with 13 oblique rows of granules in males and females, and with four terminal granules (Fig. 27). Intercarinal spaces of metasoma strongly granular, denser from segment I to segment V; ventrolateral carinae of metasoma segment V moderate to strong granular, with all granules more or less equal in size, especially never lobate and larger posteriorly (Figs. 28, 29). Pectinal tooth count 31–34 in males and 27–29 in females. Very intense setation on body and pedipalps.

Description.—See Lourenço, Qi & Zhu (2005).

Distribution.—China (Xizang).

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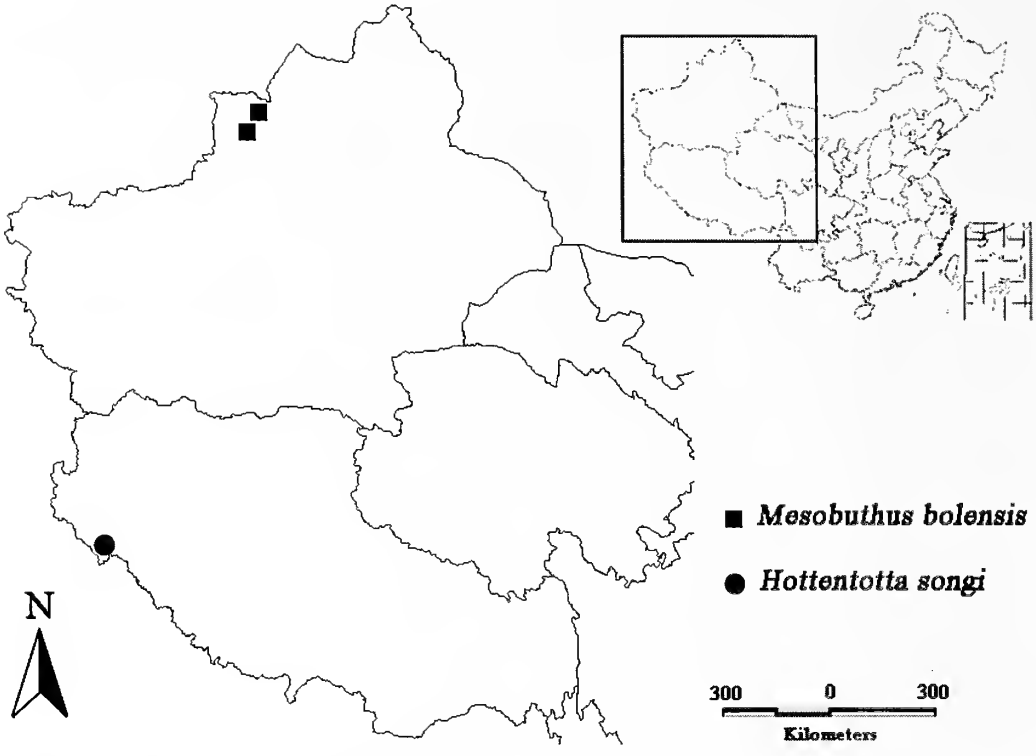

Figure 30.—Known distribution of *Mesobuthus bolensis* new species and and *Hottentotta songi* (Lourenço, Qi & Zhu, 2005).

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The enigmatic Pennsylvanian arachnids *Areomartus ovatus* and *Vratislavia silesica* (Trigonotarbida)

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Abstract. *Areomartus ovatus* Petrunkevitch 1913, from the Pennsylvanian (Kanawah Formation; Bashkirian?) of Cotton Hill, Fayette County, West Virginia, USA is redescribed. Originally placed in the family Eophrynidae of the extinct arachnid order Trigonotarbida, it lacks unequivocal eophrynid features. Nevertheless, *Areomartus* Petrunkevitch 1913 was used as the type genus of a now superfluous eophrynid subfamily Areomartinae Petrunkevitch 1955. The present revision suggests that too much emphasis was placed on the primary diagnostic character of *Areomartus*, hexagonal fields across the carapace, in a rather poorly preserved and incomplete specimen. *Areomartus ovatus* is thus removed from Eophrynidae and treated as Trigonotarbida incertae sedis. *Vratislavia silesica* (Römer 1878) from the Pennsylvanian (Langsettian?) of Kłodzko (formally Glatz) in Silesia, Poland is another problematic eophrynid. The holotype is believed lost, and thus interpretations rely on published figures. Opisthosomal morphology suggests that *V. silesica* actually belongs in a different trigonotarbid family: Anthracosironidae.

Keywords: Fossil, Areomartinae, Coal Measures, West Virginia, Silesia

Areomartus ovatus Petrunkevitch 1913 is a poorly known and enigmatic fossil arachnid from the mid-Pennsylvanian Coal Measures of Fayette County, West Virginia, USA. It was briefly described by Petrunkevitch (1913), who placed it in the extinct order Trigonotarbida (then under the older name Anthracomarti) who defined the monotypic genus based on a unique and unusual character: "Cephalothorax triangular, wider than long, its surface divided into hexagonal fields." The specimen is only known from the body (Figs. 1–3) with most of the limbs missing. The original description is rather brief. The accompanying photograph is small and yet there seem to be discrepancies between this and the interpretative drawing, for example in the degree of curvature of the opisthosomal sclerites.

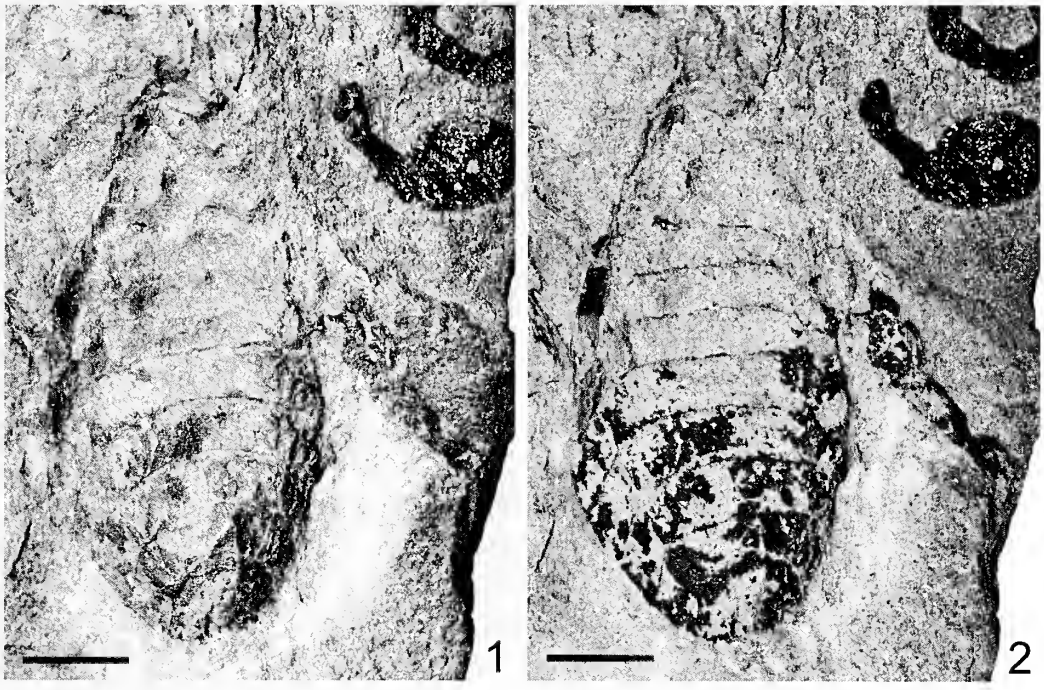
Petrunkevitch (1913) assigned *A. ovatus* to the trigonotarbid family Eophrynidae. This lineage is typically characterized (Pocock 1902; Dunlop 1995; Garwood et al. 2009) by a heavily tuberculate dorsal surface of both the carapace and opisthosoma, the latter usually with four large spines projecting from the posterior margin. At least according to current interpretations, *A. ovatus* differs markedly in having smooth tergites and no posterior spines. As noted above, an irregular ornament of hexagons on the carapace is not known from other eophrynids, or other fossil arachnids in general. Petrunkevitch (1913) commented that the carapace, from which eyes incidentally were not described, seemed unusually small compared to the opisthosoma, which implies that this body region may not be completely preserved. The species was briefly mentioned in several instances by Petrunkevitch (1949, 1953, 1955), by which time the diagnosis of the genus was amended to "Tergites smooth. Carapace triangular, with shallow, hexagonal depressions." (Petrunkevitch 1955) In summary, *A. ovatus* seems to be a rather atypical eophrynid based on the published literature.

Another problematic eophrynid is *Vratislavia silesiaca* (Römer 1878) from the coal measures of Silesia in southwestern Poland. Historically it one of the oldest records of Trigonotarbida, although Römer's original description is extremely brief and referred the fossil to the genus *Architarbus* Scudder 1868, implicitly a member of another extinct arachnid order, Phalangiotarbida. It was correctly identified as a trigonotarbid by Haase (1890), who transferred it to *Anthracomartus* Karsch 1882. Subsequently, Anton Frič raised a new genus, *Vratislavia* Frič 1904, for Römer's fossil. Frič provided the first illustrations of the holotype (Figs. 4, 5), which appears to consist primarily of the (? ventral) opisthosoma and some partial limbs. *Vratislavia* was placed in the family Anthracomartidae, although Petrunkevitch (1913, 1953, 1955) subsequently transferred it to Eophrynidae, making reference in his 1953 monograph to a series of posterior spines on the opisthosoma shown in Frič's illustrations. By this time the holotype could not be traced (see also Material). The figured spination is indeed a typical eophrynid feature, as noted above, but in the absence of a type specimen or photographic documentation their presence in *Vratislavia* relies on the accuracy of Frič's observations. In some cases, these have been found wanting (see, e.g., comments in Pocock 1910), where some trigonotarbids were interpreted by Frič (1904) as spiders because of the supposed presence of opisthosomal spinnerets, structures which could not be confirmed by later observations. Haase (1890) also examined the original specimen of *V. silesiaca*, but made no mention of any spines in his (albeit brief) description.

As part of a planned revision of eophrynids and their relatives, a redescription of *A. ovatus* and a reconsideration of *Vratislavia silesica* are proposed here to confirm whether they even belong in this family and/or preserve sufficient characters for phylogenetic analysis.

METHODS

Material.—The holotype, and only known specimen, of *Areomartus ovatus* was obtained from the United States National Museum, Smithsonian Institution (USNM, No. 60686), Washington D.C., USA. Notes accompanying the specimen imply that it was collected as part of the United States Geological Survey in 1895 by B. Phillips, although one note also states "Lacoe Coll." The repository number was incorrectly stated by Petrunkevitch (1913) as 1196. In fact, this is the locality number and presumably refers to the Cotton Hill type locality. Oddly, a further handwritten label, probably by Petrunkevitch, names the fossil as "*Architarbus ovatus*"; *Architarbus* is, as noted above, a representative of a different



Figures 1, 2.—Holotype and only known specimen of *Areomartus ovatus* Petrunkevitch, 1913 (USNM 1196) (Trigonotarbida incertae sedis) from the Pennsylvanian of West Virginia, USA. 1. Photographed dry under low angle lighting to bring out surface relief. 2. Photographed under 70% alcohol to reveal full segmentation and dark patches of carbonized cuticle. Scale bars = 2 mm.

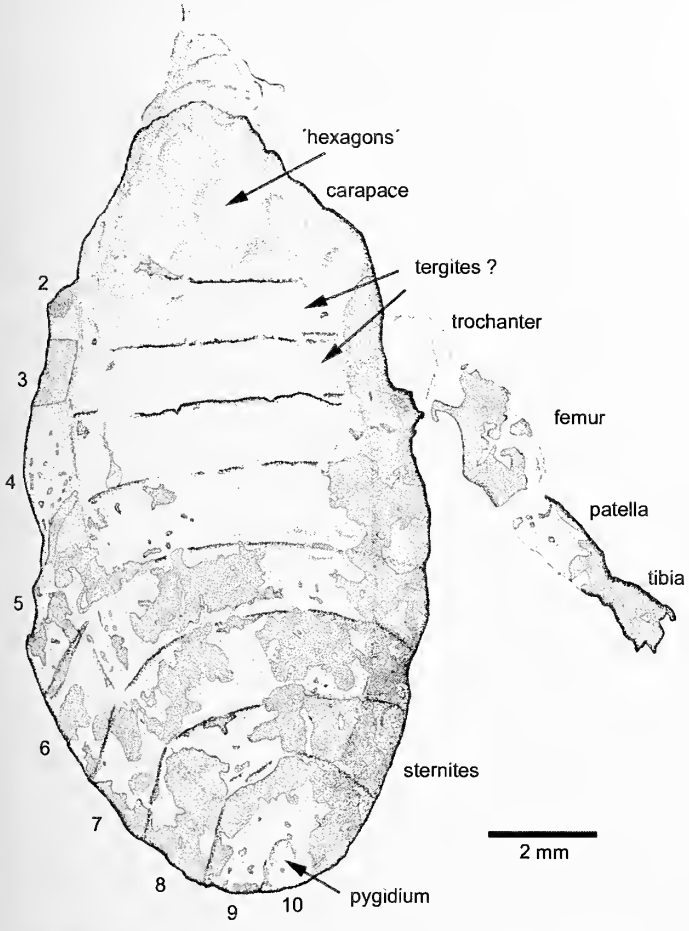
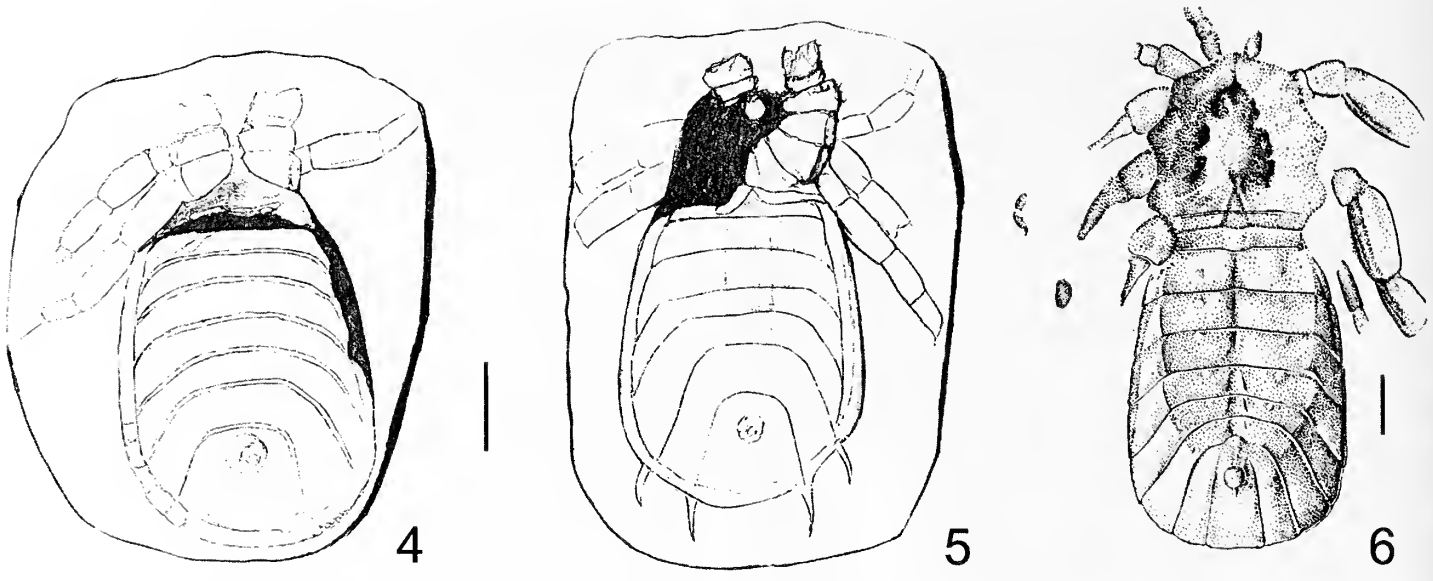


Figure 3.—Camera lucida drawing of the specimen shown in Figs. 1, 2.

extinct order, Phalangiotarbida (= Architarbida in some older literature). The holotype of *Areomartus ovatus* consists of a single specimen, without a counterpart, preserved in a small, quadratic piece of shale about 3 cm across. The reverse side preserves some plant fragments. The specimen was photographed both dry (Fig. 1) and under 70% alcohol (Fig. 2) using a Canon Eos 400 digital camera with a macro lens. It was drawn (Fig. 3) under a Leica MZ12 stereomicroscope with a camera lucida attachment. Images were assembled using Adobe Photoshop©.

The holotype of *Vratislavia silesiaca* originated from the "Ferdinandgrube", near Glatz (now Kłodzko) in the Silesia district of Poland. According to Ferdinand Römer's published notes, it was discovered by Herr Sabarth of Dortmund, a "Markschieder" or mining official responsible for delimiting claims, and passed on to the local mineralogical Museum in Breslau (= Wrocław). Römer personally loaned it from Breslau to Haase in 1890 and the holotype was explicitly cited by Schwarzbach (1935) as being present as Nr. 557 of the Geological Institute Breslau. However, it could no longer be traced by the time of Petrunkevitch's (1953) monograph, or in a more recent search of the most likely repository, the Muzeum Geologiczne (Geological Institute, Wrocław University: Cybulskiego 30, 50–205 Wrocław – A. Pacholska, pers. comm.); see also comments in Dunlop & Rössler (2002) about the fate of another Breslau trigonotarbid specimen.

Age.—Petrunkevitch (1913) gave the stratigraphic horizon of the *A. areomartus* holotype as "lower Kanawha." The Kanawah Formation belongs to the upper part of the Pottsville Formation (e.g., Cardwell et al. 1968). The Kanawah is noted as yielding much of the productive coal deposits in the West Virginia area (see Martino 1996 for a regional overview) as well as numerous plant and animal



Figures 4–6.—Anthracosironidae. 4, 5. *Vratislavia silesiaca* (Römer 1878) from the Pennsylvanian of Silesia, drawings of the (? lost) holotype reproduced from Frič (1904, p. 13, figs. 5, 6); 6. *Anthracosiro woodwardi* Pocock 1903a, dorsal and ventral opisthosomal features partially superimposed, from the more or less contemporary British Middle Coal Measures; reproduced from Pocock (1911, fig. 36). Note particularly the similar curvature of the posterior opisthosomal segments. Scale bars ca 2 mm, based on the described body lengths in the original publications.

fossils from the Coal Measures. The Kanawah Formation spans a time range of ca. 305–317 mya, and thus corresponds roughly to the late Bashkirian and early Moscovian stages in international stratigraphic terms, approximately equivalent to the Namurian and Westphalian stages of European terminology. A precise stratigraphic position for *A. ovatus* is not available either in the original description or the notes accompanying the specimen, but a Bashkirian age is tentatively adopted here.

No published details are available of the horizon yielding *V. silesica*. In his summary of the Silesian fossil arachnids, Schwarzbach (1935) wrote: "Ferdinandgrube bei Glatz (jedenfalls [in any case] Hausdorf b. Neurode). Oberkarbon". This suggests that the Ferdinandgrube is equivalent to an adjacent fossil site, Neurode (= Nowa Ruda). According to Dunlop & Rössler (2002), arachnid-yielding horizons here belong to the Langsettian (Bashkirian) substage (ca 313 mya) within the Silesian Intra-Sudeic Basin [see Bossowski et al. (1995) for a regional overview]. In the absence of any further details, I have adopted a Langsettian (= Westphalian A in European terminology) age here.

SYSTEMATIC PALAEONTOLOGY

Order Trigonotarbida Petrunkevitch 1949
Trigonotarbida incertae sedis
Areomartus Petrunkevitch 1913

Type species.—*Areomartus ovatus* Petrunkevitch 1913 by original designation. No further species known.

Areomartus ovatus Petrunkevitch 1913
Figs. 1–3

Areomartus ovatus Petrunkevitch 1913:102, pl. X, fig. 58, text-fig. 59; Petrunkevitch 1949:259–250, Fig. 123; Petrunkevitch 1953:86; Petrunkevitch 1955:109, fig. 73(2).

Material examined.—USA: *West Virginia*: Holotype, Cotton Hill, Fayette County, B. Phillips, Pennsylvanian, lower Kanawah Formation (= Bashkirian?) [USNM 6068 (part only)].

Description.—Incomplete arachnid; total preserved length 11.5 mm, maximum width 4.8 mm. Outline torpedo-shaped, apparently more pointed anteriorly, but unclear whether entire carapace region is preserved. Putative carapace region triangular, length 3.5 mm, basal width 3.8 mm. Eyes equivocal, but carapace bears ca. nine (sub)hexagonal fields up to about 0.7 mm across. Similar fields appear to continue over the next two segments. Differentiation into dorsal tergites and ventral sternites indistinct (see Remarks), thus measurements (in mm) simply given for visible segments along their midline: 2, 0.8; 3, 0.8; 4, 0.8; 5, 0.9; 6, 0.9; 7, 1.2; 8, 0.6; 9, 1.0. Segments provisionally numbered in comparison to better preserved trigonotarbids and become increasingly strongly curved posteriorly; ninth and tenth segment surrounding a small, circular pygidium, diameter ca 0.3 mm. Tuberculation or other opisthosomal ornament not apparent, but division into median and lateral plates implicit. Isolated limb, probably leg IV, preserved on right side. Demarcation of individual articles indistinct, but approximate article lengths in mm: trochanter, 1.2; femur, 2.0; patella, 1.3; tibia (probably incomplete), 1.0. More distal articles and other appendages equivocal.

Remarks.—The holotype of *Areomartus ovatus* is not especially well-preserved. Despite the torpedo-shaped body, which is often seen in Phalangiotarbida, the distribution of its segments does not correspond to a typical phalangiotarbid arrangement (c.f. figures in Pocock 1911; Petrunkevitch 1913) in which there tends to be a shortening of the anterior opisthosomal segments. The provisional assignment of the holotype to '*Architarbus*' on one of the specimen labels can thus be rejected and *Areomartus ovatus* does appear to be a bone fide trigonotarbid, with the typical round pygidium

towards the back of the opisthosoma. Petrunkevitch (1913) assumed a fossil primarily in dorsal view, but interpreting its segmentation is not easy. The strong curvature of the sclerites, at least towards the posterior of the specimen around the (ventral) pygidium, is far more consistent with sternites than tergites. Nevertheless there are hints of a division into median and lateral plates, which are typical for trigonotarbid tergites. Dorsal and ventral elements may in fact be to some degree superimposed and a subtle change in the way the sclerites overlap each other was noted in the present study between segments 3 and 4 (Fig. 3). Conceivably, the anterior third represents purely dorsal features and the posterior two-thirds primarily ventral features.

What of the triangular carapace and its putatively diagnostic hexagons? These structures are indeed present, and are best seen under low angle lighting (Fig. 1). However, Petrunkevitch (1913, fig. 59) does seem to have overemphasized both their symmetry and regularity, and his original drawing does not indicate the fact that similar depressions continue, albeit weakly, onto the succeeding sclerites (Fig. 3). Whether they are biological or taphonomic features is hard to tell, but the latter option is perhaps more likely. Overall, the torpedo-like shape and proportions of the fossil would probably allow the species to be recognized again. *Areomartus ovatus* is not a *nomen dubium*, but it preserves no convincing apomorphies of Eophrynidae, or any other trigonotarbid family. Given these uncertainties about many of its morphological details, the species is treated here as Trigonotarbida incertae sedis.

Petrunkevitch (1955) divided Eophrynidae into two subfamilies: Areomartinae, defined by smooth or granular tergites, and Eophryninae, defined by tergites with conspicuous rows of tubercles. Defining a taxon on a variable character state (i.e. a smooth or granular dorsal surface) is problematic. In any case, Rössler & Dunlop (1997) resurrected Haase's (1890) family Kreischeriidae for the more 'granular' eophrynids. Since Kreischeriidae now accommodates most of the areomartine genera sensu Petrunkevitch, and since *Areomartus* itself has been removed here from Eophrynidae, a subfamily based around this genus becomes superfluous and should be abandoned. Any remaining eophrynid taxa with 'smooth' tergites (see e.g., *Vratislavia* below) are probably misplaced at the family level.

Family Anthracosironidae Pocock 1903b

Vratislavia Frič 1904

Type species.—*Architarbus silesiacus* Römer 1878 by monotypy. No further species known.

Diagnosis.—? Anthracosironids with a pear shaped-opisthosoma, ca 1.3 times longer than wide, terminating in four prominent and slightly incurving spines.

Vratislavia silesiaca (Römer 1878)

Figs. 4, 5

Architarbus silesiacus Römer 1878:55.

Anthracomartus (*Architarbus*) *silesiacus* (Römer); Haase 1890: 650.

Vratislavia silesiaca (Römer); Frič 1904:44–45, pl. 13, figs. 5, 6, text-figs. 56A, B; Pocock 1911:7; Petrunkevitch 1913:97; Schwarzbach 1935:5, 6, fig. 5; Petrunkevitch 1953:89; Petrunkevitch 1955:109, fig. 74(3).

Material.—Poland: holotype, from the "Ferdinandgrube bei Glatz" (= Kłodzko), Lower Silesia, Sabarth, Pennsylvanian (Langsettian?), [originally in the Geological Institute, Breslau (= Wrocław), Nr. 557, now missing, presumed lost].

Description.—See Frič (1904), who provided the only relatively complete description, and mentioned a body length of 10 mm and a width of 4 mm.

Remarks.—In the absence of a type, it is tempting to treat *Vratislavia* as an incertae sedis taxon too. However, its opisthosomal proportions coupled with the reported terminal spination offer a diagnostic character combination which could potentially be recovered in future material. Whether it is an eophrynid, as assumed by Petrunkevitch, is debatable. Eophrynidae usually have a heavily ornamented body (Pocock 1902; Dunlop 1995; Garwood et al. 2009) and, like most trigonotarbids, a more rounded to oval opisthosoma, typically only marginally longer than wide.

The proportions of *V. silesiaca* are far more like another trigonotarbid family: Anthracosironidae (compare Figs. 4, 5 vs 6). Of particular note is a somewhat elongate, pear- to lozenge-shaped opisthosoma, noticeably longer than wide, with a bluntly rounded posterior end and strongly procurved opisthosomal segments around the anal operculum. The type genus, *Anthracosiro* Pocock 1903, was described in detail by Pocock (1903a, 1903b, 1911). As pointed out by Frič (1904), the original illustration of *Anthracosiro woodwardi* Pocock 1903 (see Pocock 1903a, fig. A, probably based on NHM 1551) hints at very small spines at the back of the opisthosoma in a similar position to those drawn for *V. silesiaca*. A detailed restudy of *Anthracosiro* is planned which should allow this spine character to be investigated further. It is not seen in other published illustrations. An early Devonian trigonotarbid from Wales, United Kingdom, has also been assigned to the Anthracosironidae by Dunlop & Selden (2004), but since it is mostly known in dorsal view, it offers few characters for direct comparison to the largely ventrally preserved *Vratislavia*. Overall, the habitus of *V. silesiaca* is much more consistent with an anthracosironid than an eophrynid. An unequivocal placement of incomplete and/or missing fossils will always be difficult. The presumption here is that the defensive marginal spination is adaptive, and thus prone to be a homoplastic character, and that spination (in isolation) is insufficient grounds to justify placement in Eophrynidae.

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Caves as islands: mitochondrial phylogeography of the cave-obligate spider species *Nesticus barri* (Araneae: Nesticidae)

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Abstract. Around 1000 obligate cave species have been described from the continental United States. This taxonomically diverse group of species eontains both terrestrial obligate cave species (troglobites) and aquatic obligate cave species (stygobites). The greatest diversity of troglobites in the United States occurs on the southern Cumberland Plateau in south-central Tennessee and northeastern Alabama. The troglobitic spider *Nesticus barri* Gertsch 1984 is known from nearly 60 caves in this area. We studied the mitochondrial phylogeographic structuring of this species, sampling individuals from twelve caves across the species' range. We found that *N. barri* populations within individual caves are generally not genetically diverse; that *N. barri* is divided into genetically distinct subpopulations, with mitochondrial cytochrome oxidase I genetic distances between subpopulations ranging from 0.021 to 0.045; and that female-based migration between caves is minimal or nonexistent, even over small geographic scales (< 15 km). This is the first genetic study of a troglobitic taxon from this biodiverse region. Our results contrast with those from previous studies on stygobitic crayfish from this area, which showed high levels of gene flow between caves.

Keywords: Cumberland Plateau, troglobite, gene flow, mitochondria, cytochrome oxidase I

Caves are home to a unique and diverse community of species. Species that complete their life cycles within caves and are never found outside of caves are known as 'obligate cave species', and around 1000 such species have been described from the continental United States (Culver et al. 2000). The dominant taxonomic groups are arachnids, crustaceans, and hexapods, but also known are mollusks, diplopods, fish, and salamanders (Culver et al. 2000). Terrestrial obligate cave species are known as troglobites and aquatic obligate cave species are referred to as stygobites. Cave obligate species have often evolved in a convergent manner such that most have small to absent eyes, are light colored, and have long appendages, a condition referred to as troglomorphy (reviewed in Porter 2007).

Obligate cave species are not distributed evenly across the continental United States. Fewer than one fifth of all counties have even one troglobite or stygobite (Culver et al. 2000), whereas a few areas have an exceptionally diverse cave fauna. For troglobites, the highest diversity is found on the southern Cumberland Plateau in northeastern Alabama and southern Tennessee. The Cumberland Plateau is one of the largest karst regions in the eastern United States, and is exceptionally cave-rich (Christman and Culver 2001). Culver et al. (2000) found that Jackson County in northeastern Alabama has more troglobitic species (52) than any other county in the United States, and that Marshall County, to the south of Jackson County, had the fourth most species of troglobites (32). Recent surveys in southern Tennessee found similarly high levels of troglobitic diversity, with Franklin County having 34 species of troglobites, and Marion County 24 species (Culver et al. 2000; Lewis 2005).

Cave habitats, as compared to surface habitats, are limiting in that cave species are often restricted in where they can live and their ability to move between habitats (caves). Accordingly, many troglobites have extremely small ranges, with nearly 70% of troglobitic species and subspecies limited to a single county, and many species known from a single cave (Culver et al. 2000). Troglophiles, which are able to survive outside caves, though they tend to complete their life cycle within caves, and trogloxenes (such as bats), which do not complete their life cycle in caves but often use them for shelter, usually have larger ranges and higher levels of gene flow because of greater continuity between habitats (Caccone 1985).

The only genetic studies on the troglobitic or stygobitic fauna of the southern Cumberland Plateau were conducted by Buhay and Crandall (2005) and Buhay et al. (2007) on two genera of stygobitic crayfish (*Orconectes* and *Cambarus*). They found that these crayfish have large population sizes, high genetic diversity, and extensive gene flow between caves as evidenced by haplotypes shared among multiple caves across several counties (Buhay and Crandall 2005; Buhay et al. 2007). Contrasting results have been found in studies of troglobites from other areas, including several species of troglophilic and troglobitic spiders of the Appalachians (Hedin 1997a), which showed significant population structure and little evidence for migration between caves. This difference may be due to the generally broader connections present between subterranean aquatic habitats than between subterranean terrestrial habitats (Porter 2007).

Spiders of the genus *Nesticus* are diverse in the southeastern United States, where at least 30 different species occur in the southern Appalachian Mountains and Cumberland Plateau (Gertsch 1984; Hedin 1997b; Hedin and Dellinger 2005). These medium-sized (2 to 7 mm) spiders are limited to cool, moist microhabitats in the southeastern United States. About one-third of this regional fauna includes troglophilic or troglobitic species (Hedin and Dellinger 2005).

In addition to taxonomic studies, several studies have been conducted on *Nesticus* spiders. Hedin (1997b) studied the phylogenetic history of the *Nesticus* species of the southern Appalachian Mountains and population genetics of the

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Table 1.—Summary of genetic samples – including cave sites, sample sizes, haplotype identities, and network inclusion information.

Network-cave	County	State	Number of individuals	Haplotype
A- Sewanee Blowhole	Franklin	Tennessee	3	A
B- Salt River Cave	Jackson	Alabama	2	B
C- White Cricket Cave	Marion	Tennessee	5	C1 (×4), C2 (×1)
C- Tate Spring	Marion	Tennessee	2	C3
D- Lost Cove Cave	Franklin	Tennessee	3	D
E- Lost Cove Cave	Franklin	Tennessee	5	E1
E- Keith Cove Cave	Franklin	Tennessee	5	E2
E- Grapeville Cave	Franklin	Tennessee	3	E2 (×2), E3 (×1)
F- Guess Creek Cave	Jackson	Alabama	4	F1
F- Gross Skeleton Cave	Jackson	Alabama	1	F2
F- Bishop Cave	Marshall	Alabama	3	F3
G- Tate Cave	Jackson	Alabama	6	G1 (×3), G2 (×2), G3 (×1)
G- Jess Elliot Cave	Jackson	Alabama	2	G2

Nesticus tennesseensis complex (1997a). This species complex is found in eastern Tennessee, western North Carolina, western Virginia, and southern West Virginia. He found a small number of closely-related haplotypes in each individual cave population, whereas haplotypes between populations were divergent, indicating that these populations last shared a common ancestor a relatively long time ago. As no haplotypes were shared between populations, there is evidently little to no gene flow between populations of these spiders (Hedin 1997a).

Nesticus barri Gertsch 1984 is a troglomorphic (pale, eyeless, and long-limbed) species known from around 60 caves across the hotspot of troglobite diversity in Jackson and Marshall Counties, Alabama, and Franklin and Marion Counties, Tennessee (Gertsch 1984; Hedin and Dellinger 2005; Lewis 2005). They spin webs that act as both a home and a means to catch prey. They hang upside down from their webs and do not stray far from them throughout their lives (Gertsch 1984; Hedin 1997b). Female spiders carry their egg sacs on their spinnerets until the offspring hatch (Reeves 1999). On the basis of morphology, Hedin and Dellinger (2005) synonymized *N. valentinei* Gertsch 1984, a species known from only one cave on the edge of *N. barri's* range, with *N. barri* (Gertsch 1984). Previous molecular work with *N. barri* was limited to four individuals that were sequenced for phylogenetic analysis by Hedin (1997b).

The objective of this study was to examine the mitochondrial phylogeographic structuring of *N. barri*. We gathered specimens from caves across the range of the species to determine levels of genetic diversity within individual caves and across the range of the species, and to determine how much gene flow occurs between caves. We hypothesized that there would be little to no gene flow between caves. We also gathered genetic evidence to support or reject the synonymization of *N. valentinei* with *N. barri*. Our study constitutes the first to examine genetic structuring of a troglobite from the southern Cumberland Plateau.

METHODS

Samples.—Forty-five specimens were obtained from twelve different caves (Table 1) that spanned the range of *Nesticus barri* (Figs. 1, 2). Individuals were preserved in the field in 95% ethanol and taken back to the laboratory where they were stored at −80° C. We report, for the first time, the presence of *N. barri* in Grapeville Cave and Sewanee Blowhole in Franklin County, Tennessee, USA; these are the northernmost records for this species. To protect sensitive cave habitats, cave locations are referred to only by Tennessee and Alabama Cave Survey names and by approximate locations on maps; detailed collection information can be obtained from the authors.

DNA extraction, amplification, and sequencing.—DNA was extracted using the tissue from one leg of small individuals or the femur of large individuals according the manufacturer's instructions for the DNeasy Blood and Tissue Kit (Qiagen; P/N: 69506). Initial polymerase chain reaction (PCR) amplifications for part of the mtDNA cytochrome oxidase I (COI) gene were done using the primers LCOI (5′-GGTCAACAAATCATAAAGATATTG-3′) and HCOI (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) from Folmer et al. (1994). We later developed a species-specific replacement for the LCOI primer that was more effective in *N. barri* (LCOI-barri; 5′-GGACTTTGTATTTTATTCTTGGGTC-3′). Two different polymerase enzymes were used: Amplitaq Gold PCR Master Mix (Applied Biosystems; P/N: 4318739) or Taq DNA Polymerase (Sigma; P/N: D5938). When Taq DNA Polymerase was used, PCR conditions were 1 min at 94° C, 2 min at 50° C, and 90 s at 72° C (× 35 times). When Amplitaq Gold PCR Master Mix was used, the conditions were 5 min at 95° C, followed by 35 cycles of 15 s at 95° C, 15 s at 50° C, and 1 min at 72° C. Successful PCR reactions were purified according to the manufacturer's instructions for the QIAquick PCR Purification Kit (Qiagen; P/N: 28106). Sequencing reactions on both strands were performed by the DNA Analysis Facility at Yale University and were analyzed on an Applied Biosystems 3730 sequencer. The resulting sequences were edited using Sequencher (v. 4.9; Gene Codes Corp., Ann Arbor, MI). Sequences have been submitted to GenBank (Accession #GQ421645-GQ421688).

Intraspecific analyses.—No indels were present, and sequences were aligned by eye. Numbers of variable sites, transitions, transversions, and predicted amino acid changes were determined in Mesquite (v. 2.6; http://mesquiteproject.org). The

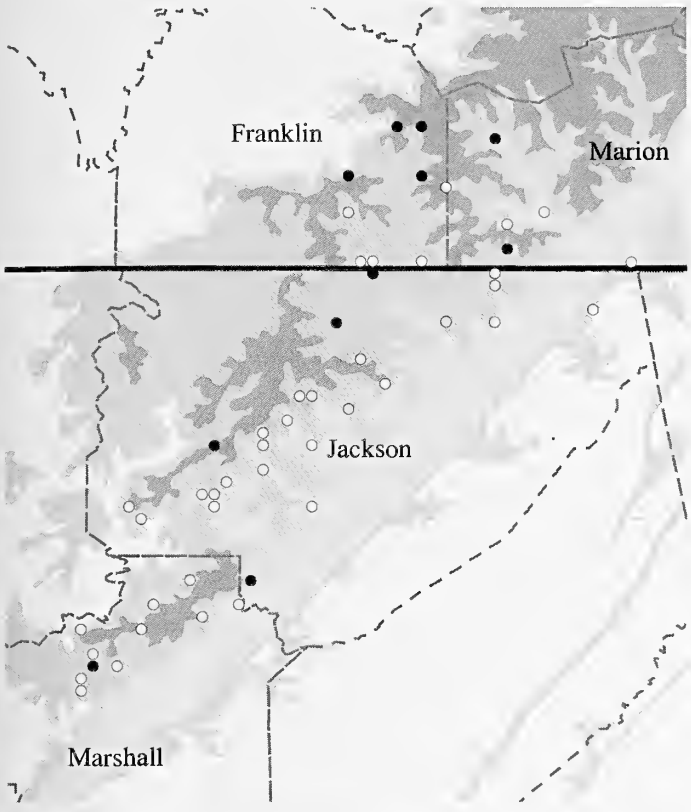

Figure 1.—Extent of the Cumberland Plateau in southern Tennessee and northeastern Alabama and distribution of *Nesticus barri*. The top of the Cumberland Plateau is indicated in dark grey, and lower elevations are indicated by lighter shades. Approximate location of caves in which *Nesticus barri* has been found (all circles) and caves which we sampled (filled circles). Tate Cave and Jess Elliot Cave, whose entrances are close together, are indicated by a single circle.

number of haplotypes present was determined by combining identical sequences, including those that differed only in length at one end of the sequence or by one or more ambiguous bases. We used TCS (v. 1.21; Clement et al. 2000) to group these haplotypes into networks. We used PAUP* (v. 4.0b10; Swofford 2001) to calculate mean uncorrected 'p' distances between haplotypes both within and between TCS networks. We also examined population structure by calculating *F*-statistics in Arlequin (v. 2.0; Schneider et al. 2000) among the caves where we sampled four or more individuals (Table 1).

Phylogenetic analyses.—To test the monophyly of *N. barri*, and to allow us to compare intraspecific diversity within *N. barri* with interspecific diversity between *N. barri* and other *Nesticus* species, we used partially overlapping COI sequences from nine other nesticids. These included sequences from seven other *Nesticus*, and two sequences from the more distantly-related *Eidmanella pallida* (Emerton 1875) (GenBank Accession #GQ421636-GQ421644). Six of the seven *Nesticus* species included here are found in the same geographic area as *N. barri* (Gertsch 1984; Hedin and Dellinger 2005); *N. silvestrii* Fage 1929 is found in the Pacific Northwest, and is an outgroup to the *Nesticus* species of the Appalachians (Hedin 1997b).

We used MrBayes (v. 3.1.2, Ronquist and Huelsenbeck 2003) to conduct Bayesian phylogenetic analyses on a matrix of all sequences (both ingroup and outgroup). We partitioned the data by codon position, and for each partition we used a General Time Reversible (GTR) model with six substitution rates, estimated nucleotide frequencies, and invariable sites. These model parameters were unlinked between partitions with the exception of substitution rates, which were linked for the 1st and 2nd codon position partitions due to the small number of changes in the 2nd codon position. We calculated clade credibility values from 4000 trees by sampling every 1000th tree from two runs of 5,000,000 trees after discarding the first 3001 sampled trees of each run. We used AWTY (Wilgenbusch et al. 2004) to confirm stationarity and convergence of the Bayesian analyses.

We also conducted a distance-based neighbor-joining bootstrap analysis (1000 replicates) in PAUP*. We used Modeltest (v. 3.7; Posada and Crandall 1998) to identify the model that best described the evolution of the sequences (as selected by the Akaike Information Criterion; Posada and Buckley 2004) and used the parameters identified in Modeltest (GTR + Γ + invariable sites) in distance analyses.

RESULTS

Molecular evolution within *N. barri*.—Amplification and sequencing were successful for 44 of 45 *N. barri* specimens. Sequences ranged in length from 598 to 633 bp, with a mean length of 628 bp. No indels were observed. Seven ambiguous nucleotides were present in 27,632 bp of sequence gathered from *N. barri*. Disregarding ambiguous nucleotides, there were 53 variable sites in the *N. barri* dataset. Forty-six of these sites varied by a transition substitution, five by a transversion substitution, and two sites exhibited both transition and transversion substitutions. Based on translations of the nucleotide sequences, nine of 211 amino acids were predicted to be variable. No stop codons were observed within any translated amino acid sequence.

Population structure.—Among the 44 sampled individuals we identified fifteen haplotypes (Table 1). Thirteen of these haplotypes were found in a single cave, and two haplotypes were shared between geographically-adjacent caves. Individual cave samples were generally not genetically diverse; eight caves were fixed for a single haplotype, three caves had two haplotypes, and one cave had three haplotypes. In three of the cases where a single cave had multiple haplotypes, those haplotypes differed by one or two nucleotides. In one exceptional case (Lost Cove Cave) two haplotypes were present and these haplotypes differed by 14 nucleotides.

The fifteen *N. barri* haplotypes fell into seven unconnected haplotype networks based on the 95% parsimony probability (Templeton et al. 1992), which separated networks that differed by more than ten nucleotides (Fig. 3). Several 'networks' contained only a single haplotype (A, B, D), and no network contained more than 3 haplotypes (Fig. 3). Most haplotypes within a network differed by a single nucleotide, with one network (F) containing three haplotypes that differed by as many as three nucleotides, and another (C) containing three haplotypes that differed by as many as seven nucleotides (Figure 3). No cave contained haplotypes from more than one network with the exception of Lost Cove Cave, with one haplotype from each of network D and E (Table 1). Mean pairwise genetic distances between haplotypes from unconnected networks ranged from 0.021 to 0.045 (uncorrected 'p' distance; Table 2). Mean pairwise distances between haplotypes

Figure 2.—*Nesticus barri* female in web, Marlow Holes, Franklin County, Tennessee. Photo by Alan Cressler.

within a network ranged from zero (for those networks containing a single haplotype) to 0.007 (Table 2).

The seven genetic networks are also largely geographically continuous. Caves with spiders from a single network are in the same area (Fig. 4). The greatest geographic distance between caves containing spiders with haplotypes from the same network was 37 km in network F (Fig. 4). The closest that we found spiders from two different networks were the two found in Lost Cove Cave (Fig. 4, Table 1). This is exceptional, as all other caves had haplotypes from a single network. The two cases where haplotypes were shared between caves involve caves that are geographically proximate: haplotype G2 in Tate Cave and Jess Elliot Cave (entrances less than 0.5 km apart), and haplotype E2 in Keith Cove Cave and Grapeville Caves (entrances ~11 km apart).

We tested for population structure among all caves where we sampled four or more individuals (Table 1), using F statistics. For the nine comparisons involving caves whose spiders were from different haplotype networks, F_{ST} values ranged from 0.72 to 1.00 and were significant ($P < 0.01$). For the comparison between Keith Cove Cave (with spiders with haplotype E2, Table 1) and Lost Cove Cave (haplotypes D and E1, Table 1), the F_{ST} value was 0.24 ($P = 0.037$).

Phylogeny.—Despite the relatively short length of the sequences we gathered, the phylogenetic tree constructed from the *N. barri* haplotypes, representative sequences from seven *Nesticus* species, and two *Eidmanella* individuals, was largely congruent with the phylogeny for the Appalachian *Nesticus* species previously reported by Hedin (1997b) (Fig. 5). We identified *N. silvestrii* as the sister group to the Appalachian *Nesticus* species, *N. archeri* Gertsch 1984, *N. pecki* Hedin & Dellinger 2005, and *N. stygius* Gertsch 1984 as early-diverging, and *N. barri* most-closely related to *N. furtivus* Gertsch 1984, *N. georgia* Gertsch 1984, and *N. jonesi* Gertsch 1984. We found support (Bayesian clade credibility value of 91%; Fig. 5) for the monophyly of *N. barri*. Within *N. barri*, we identified seven primary lineages (A–G) that correspond directly to the haplotype networks (Fig. 3). When these lineages contained more than one haplotype (lineages C, E, F, and G; Fig. 5) we found strong support for their monophyly. It is notable that relationships among the seven primary lineages of *N. barri* are poorly resolved (Fig. 5), with no sister-lineage relationships being strongly supported in either Bayesian or neighbor-joining analyses.

DISCUSSION

The caves of a four-county area spanning the Tennessee/Alabama state line are inhabited by more species of troglobites than any other known comparable area in the United States. Despite this great species diversity, no detailed genetic studies have been conducted on any troglobite from this area. *Nesticus barri* is a troglobitic spider that is known from caves across this area. The objectives of this study were to examine mitochondrial genetic diversity and population structure in *N. barri*, to support or reject the recent synonymization of *N. valentinei* with *N. barri*, and to compare the results from *N. barri* to previous studies on stygobites from this area. This study also represents an additional step towards building a comparative molecular phylogeographic perspective for the diverse cave-obligate fauna of the region.

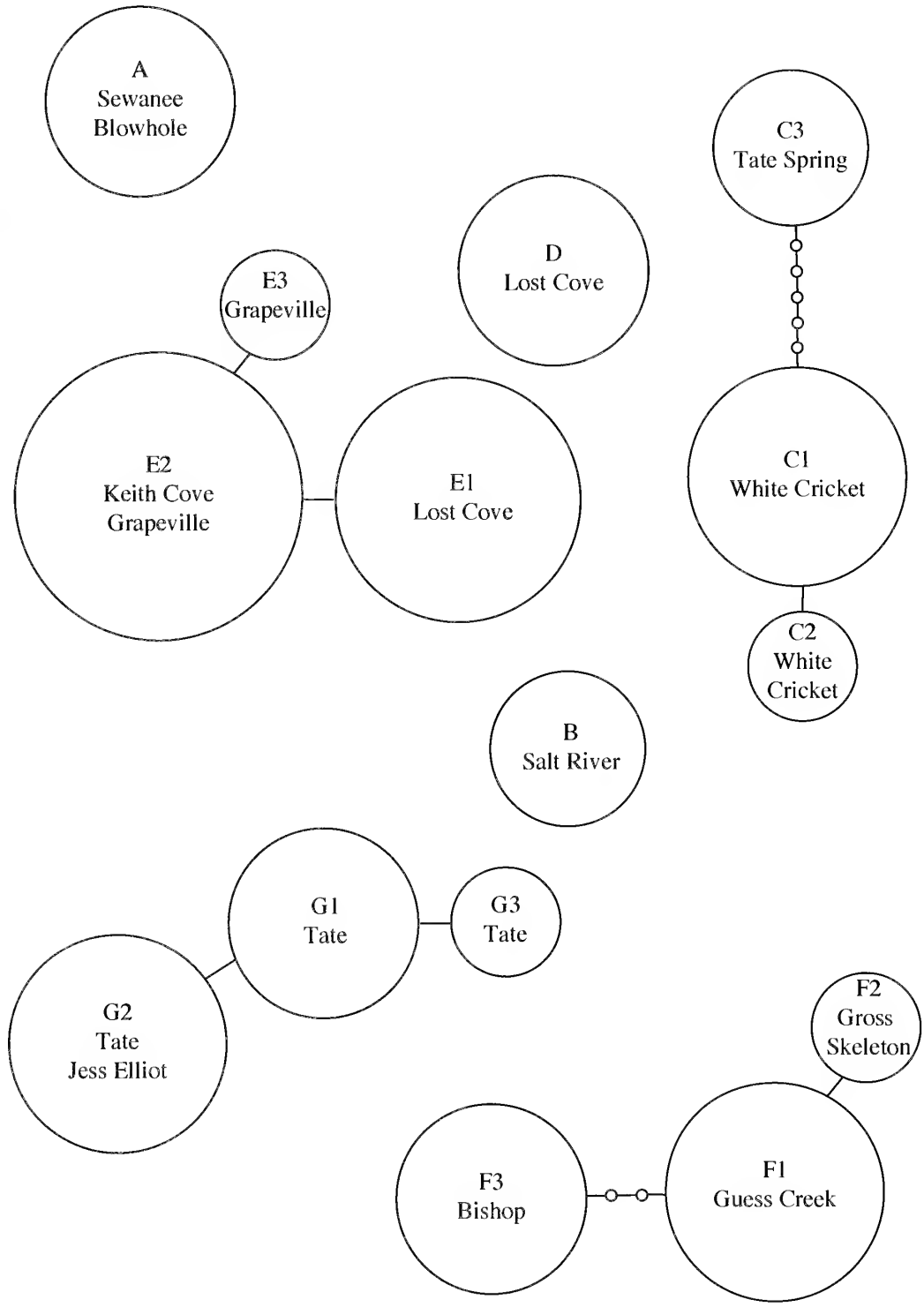

Figure 3.—Haplotype networks for *N. barri* COI, with locality information for each haplotype. The distribution of networks roughly corresponds to their geographic location (see Figure 4 for more details). The area of each circle is proportional to the number of sampled individuals with each haplotype. Unsampled and/or extinct haplotypes are indicated by small open circles. Unconnected networks differ by more than ten nucleotide substitutions. Lost Cove Cave is the only cave with individuals present in two different networks.

Genetic diversity and population structure in *N. barri*.—The significant genetic diversity found within *Nesticus barri* is partitioned into a number of geographically distinct subpopulations. We found no examples of shared haplotypes between caves that were more than 12 km apart and no examples of shared mitochondrial lineages at distances over 40 km (Figs. 4, 5). Given the general lack of shared haplotypes between caves it is not surprising that all F_{ST} comparisons showed significant population structure. We found complete congruence between the haplotype networks and primary Bayesian mitochondrial lineages (Figs. 3, 5). The mitochondrial lineages found in *N. barri* differ from one another by 2.1–4.5%,

Table 2.—Mean pairwise uncorrected 'p' distances between haplotypes within networks (on diagonal, in italics) and haplotypes from different networks (below diagonal).

	A	B	C	D	E	F	G
A	*0.000*						
B	0.027	*0.000*					
C	0.024	0.031	*0.007*				
D	0.021	0.025	0.023	*0.000*			
E	0.032	0.028	0.029	0.022	*0.002*		
F	0.042	0.032	0.041	0.034	0.033	*0.004*	
G	0.036	0.036	0.045	0.028	0.027	0.037	*0.002*

indicating isolation for significant periods of time (Table 2). Remarkably, caves less than 10 km apart may have spider populations with mitochondrial haplotypes that were placed into different haplotype networks. The observation that haplotypes were typically unique to a single cave (Table 1) indicates that there is currently little to no migration of spiders between caves. The observation that highly distinct mitochondrial lineages occupy geographically adjacent areas yet have not mixed suggests that migration has been limited for a very long time.

One notable result was the presence of spiders with haplotypes from two different networks (E and D; Fig. 3) in Lost Cove Cave. All of these spiders were collected on the same date, and within 100 m of one another. Spiders from network E (though with different haplotypes) were found in two other caves near Lost Cove Cave, whereas we did not collect spiders from network D from any other cave (Fig. 3). It is unclear whether the presence of spiders with significantly different haplotypes in this cave is a result of mixing due to migration, or to long-term coexistence and divergence.

Our results are consistent with the preliminary studies of *N. barri* by Hedin (1997b). Hedin (1997b) performed a TCS analysis on haplotypes from a mitochondrial region spanning partial 16S, complete tRNA-leucine, and partial NADH dehydrogenase subunit I genes from four *N. barri* individuals (one each from Salt River Cave, Lost Cove Cave, Bishop Cave, and Guess Creek Cave). Hedin (1997b) found that only the individuals from Bishop Cave and Guess Creek Cave were joined in a network. We found, similarly, that individuals from those two caves have CO1 haplotypes belonging to network F (Table 1), whereas spiders from the other two caves (Salt River and Lost Cove) belonged to distinct networks (B, and either D or E, respectively; Table 1).

Status of *N. valentinei*.—Gertsch (1984) described *Nesticus valentinei* as a new species from Heating Stove Cave in Marion County, Tennessee, on the northeastern edge of *N. barri*'s range. The entrance to Heating Stove Cave was evidently destroyed during the construction of an interstate highway (Hedin and Dellinger 2005). Hedin and Dellinger (2005) collected specimens from Tate Spring, which is presumably connected to Heating Stove Cave. These individuals were morphologically compared to other *N. barri* individuals and to the *N. valentinei* holotype; based on this comparison, these authors concluded that *N. valentinei* was not distinct from *N. barri*, and accordingly synonymized *N. valentinei* with *N. barri*.

We found that individuals from Tate Spring, though they exhibited a unique haplotype ('C3' in Table 1 and Fig. 5), are nested within *N. barri*, most closely related to specimens from nearby White Cricket Cave (Table 1, Figs. 3, 4). As such, our results provide independent corroboration of the synonymization of *N. valentinei* with *N. barri* as proposed by Hedin and Dellinger (2005).

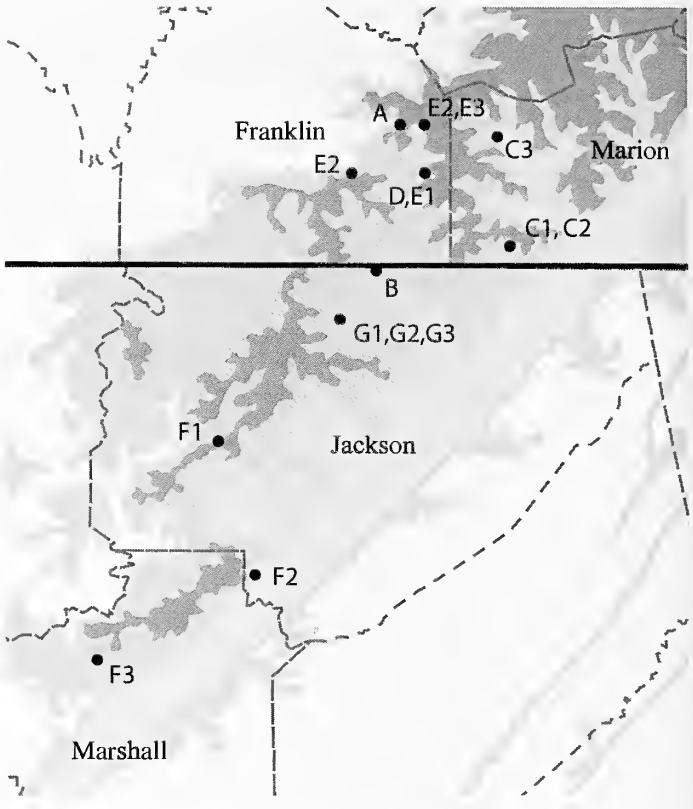

Figure 4.—Distribution of COI haplotypes and networks in *N. barri*. Haplotype labels correspond to those provided in Table 1. Approximate locations of sampled caves are indicated by black filled circles. Tate Cave and Jess Elliot Cave, whose entrances are close together, are indicated by a single filled circle (with haplotypes G1, G2, and G3).

Population structure of *Nesticus* from other areas and of stygobites of the Cumberland Plateau.—Because there have been no genetic studies on troglobites from the Cumberland Plateau, we are limited to comparing our results to studies on *Nesticus* species from other regions, and to stygobites from the Cumberland Plateau. Our findings in *N. barri* are consistent with those of Hedin (1997a) for the *Nesticus tennesseensis* complex. This complex contains seven species, some of which are surface species, some troglophilic, and some troglobitic (Hedin 1997a). Hedin (1997a) found that there was little to no mitochondrial gene flow between populations of these spiders, regardless of their habitat requirements. He also found significant genetic divergence on a small geographic scale. Cesaroni et al. (1981) examined three species of *Nesticus* in Italy using isozymes and found that genetic diversity was slightly less in the two cave dwelling species than in the surface dwelling species that they studied.

As *N. barri* is restricted to cave environments, these caves are effectively 'islands' of habitat. Though the southern Cumberland Plateau has one of the highest cave densities in the eastern United States (Christman and Culver 2001), significant genetic diversity is present across the range of this

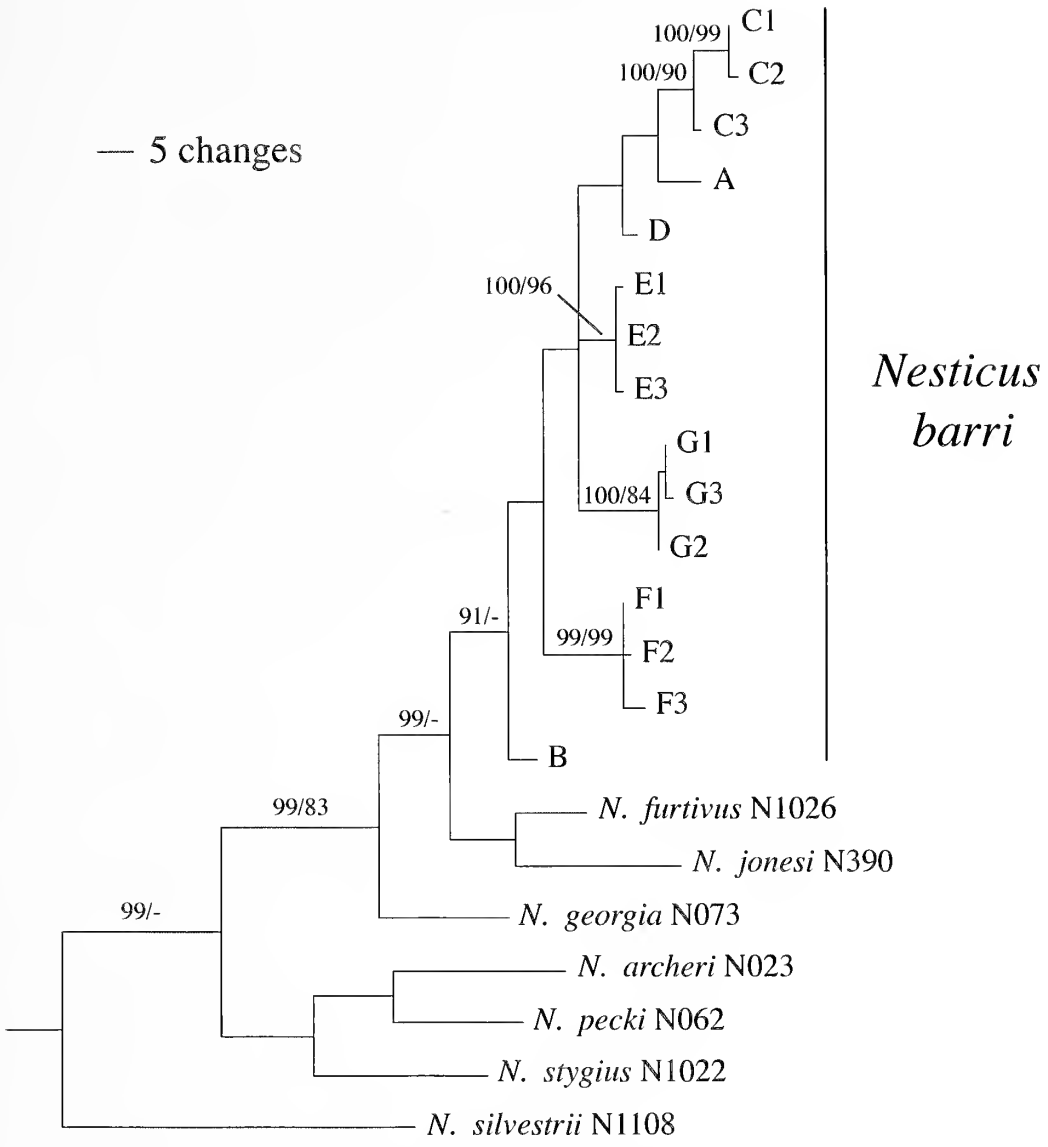

Figure 5.—Bayesian majority rule consensus tree based on cytochrome oxidase I sequences. *Nesticus barri* haplotypes correspond to those in Table 1. The tree is rooted with sequences from *Eidmanella* (not shown). Bayesian clade credibility values (from 4000 trees) greater than 90%, and neighbor-joining bootstrap values (from 1000 replicates) greater than 80%, are indicated above branches.

species, indicating that even with other 'islands' nearby, these spiders rarely migrate from one to another. Further studies are necessary to determine whether other troglobitic taxa (e.g. beetles, millipedes, flatworms) maintain population connectivity across the habitat 'islands' of the southern Cumberland Plateau.

The results in *N. barri* contrast with the two population genetie studies on cave crayfish whose ranges extend into the southern Cumberland Plateau. For both *Orconectes australis* and *Cambarus hamulatus* there was genetic evidence for large population sizes and extensive gene flow among caves (Buhay and Crandall 2005; Buhay et al. 2007). Greater population connectivity in both of these species was also evident in their haplotype networks, where a single network included all members of the species (Buhay and Crandall 2005; Buhay et al. 2007). Stygobites, such as these crayfish, may have a higher rate of gene flow between populations because they can migrate through underground aquifers that are inaccessible to troglobites (Porter 2007).

CONCLUSION

Nesticus barri shows significant genetic diversity on a small geographic scale. Female-based migration between caves appears to be extremely limited. The isolation and diversity of populations of *N. barri* has conservation implications because the loss of a single population or of several nearby populations could mean the loss of a distinct genetic lineage. This pattern contrasts with that found for stygobites from the same area, which show evidence for gene flow over significant distances. Further studies on other troglobitic taxa in this biodiverse region will clarify whether the pattern of population structure observed in *N. barri* is common, or whether it is unique to this cave spider.

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The genus *Taira*, with notes on tibial apophyses and descriptions of three new species (Araneae: Amaurobiidae)

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Abstract. Homologies of the tibial apophyses of *Taira* with those of other members of the subfamily Amaurobiinae are evaluated. The monophyly of the genus *Taira* and the phylogenetic relationships of its species are analyzed using parsimony. The genus *Taira* is supported by two putative synapomorphies: the presence of broad epigynal teeth and the distally originating tegular sclerite apophysis. A diagnosis and description of *Taira* and a key to its species are provided. Three new species are described from China: *Taira qiuae* new species (♂♀), *T. sichuanensis* new species (♂♀), and *T. zhui* new species (♂♀).

Keywords: Identification key, taxonomy, homology, phylogenetic relationships, China

Lehtinen (1967) created the new genus *Taira* for the species *Amaurobius flavidorsalis* Yaginuma 1964, described from Japan, compared it with other genera of Amaurobiidae, and placed *Taira* with the genus *Tamgrinia* Lehtinen 1967 in the tribe *Tairini* within the Holarctic subfamily Amaurobiinae Thorell 1870. Only four papers have been published on *Taira* since its establishment in 1967. Wang (2000) questioned the sister group relationship between *Taira* and *Tamgrinia* by comparing spinnerets, tracheae, and trichobothria of the family Amaurobiidae. Zhu et al. (2004) and Wang & Ran (2004) described two new species based on specimens from two closely situated localities in Libo County, Guizhou, China. A study by Zhang et al. (2008) concluded that *T. lunaris* Wang & Ran 2004 is a junior synonym of *T. liboensis* Zhu, Chen & Zhang 2004. In recent years, field work by Zhang et al. (2008) in southern China has yielded five more new species: *T. cangshan* Zhang, Zhu & Song 2008, *T. latilabiata* Zhang, Zhu & Song 2008, *T. obtusa* Zhang, Zhu & Song 2008, *T. concava* Zhang, Zhu & Song 2008, and *T. sulciformis* Zhang, Zhu & Song 2008, and a new combination, *T. decorata* (Yin & Bao 2001). As a result, a total of eleven species from Japan and southern China are included in *Taira*, including the three new species described here. In all previous studies, the diagnosis of the genus *Taira*, and therefore the distinction between *Taira* and other amaurobiids, was only vaguely defined. Zhang et al. (2008) provided a diagnosis for *Taira* that we argue was based on a misinterpretation of non-homologous features (i.e., tibial apophyses, see below). This study is focused on diagnosing and describing the genus *Taira*, discussing its monophyly, estimating the species relationships, and providing a key to the *Taira* species described so far. The three new species described here belong to a distinct group, which differs from other *Taira* by the widely separated, elongated spermathecae in females and the long RTA (except *T. sichuanensis*), presence of an intermediate apophysis between the RTA and dorsal tibial apophysis, and the uniquely modified conductor in males.

METHODS

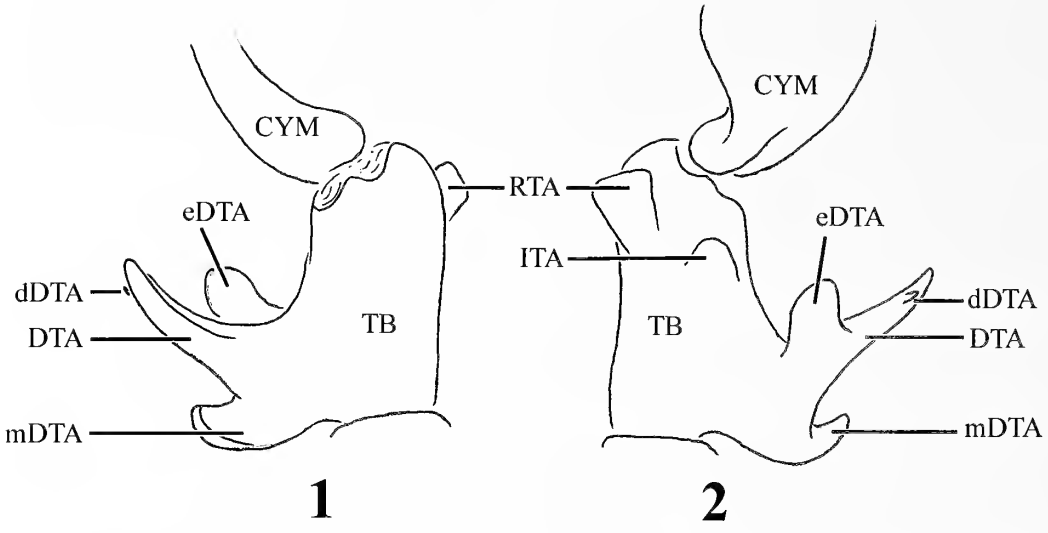
All measurements are in mm. Scale lines are 0.2 mm long except where indicated otherwise. Eye diameters are taken at the widest point. The length of body, prosoma, and opisthosoma do not include the length of the chelicerae or spinnerets. The distribution map was generated using GIS ArcView software, and the text files of the studied species are downloadable from Wang (2009). More type specimen photos of the species included in this paper can be viewed in Li & Wang (2009).

Anatomical abbreviations used in the text and figures: *Eyes*: AME–anterior median eyes; ALE–anterior lateral eyes; PLE–posterior lateral eyes; PME–posterior median eyes. *Female Genitalia*: CD–copulatory duct; EL–epigynal median lobe; ET–epigynal tooth; FD–fertilization duct; S–spermatheca. *Male palp*: C–conductor hyaline apophysis; C1–conductor apophysis I (from which hyaline apophysis arises); C2–conductor apophysis II, which is an extension of the conductor apophysis I; C3–conductor apophysis III; CYM–cymbium; dDTA–distal/subdistal tooth of DTA; DTA–dorsal tibial apophysis; E–embolus; eDTA–ectal branch of DTA; ITA–Intermediate tibial apophysis; MA–median apophysis; mDTA–mesal branch of DTA; RTA–retrolateral tibial apophysis; ST–subtegulum; T–tegulum; TB– tibia; TS–tegular sclerite; TSA–tegular sclerite apophysis.

Specimens studied in the current paper are deposited in the California Academy of Sciences, San Francisco (CAS); Hunan Normal University, Changsha, Hunan (HNU); Institute of Zoology, Chinese Academy of Sciences, Beijing (IZCAS); Museum of Hebei University, Baoding, Hebei (MHBU); Southwest University, Chongqing (SWUC); and Senckenberg Museum, Frankfurt (SMF).

AMAUROBIINAE TIBIAL APOPHYSES

Tibial apophyses of the subfamily Amaurobiinae are important characters in species identification, generic limitation, and, therefore, valuable tools for phylogenetic analyses. In previous publications, however, different arachnologists



Figures 1, 2.—Schematic drawings of *Taira* tibia (TB), showing the retrolateral tibial apophysis (RTA), dorsal tibial apophysis (DTA), intermediate tibial apophysis (ITA), external branch (eDTA) and mesal branch (mDTA) of DTA, and teeth (dDTA) on DTA. CYM = cymbium. 1. Prolateral view. 2. Retrolateral view.

have perceived and defined those apophyses in different ways and given them different names. In this study, we have conducted initial homology assessments of Amaurobiinae tibial apophyses based on similarities in their position and morphology. There are at least three apophyses on amaurobiine tibiae: a retrolateral tibial apophysis (RTA), a dorsal tibial apophysis (DTA) and an intermediate tibial apophysis (ITA), which originates between the RTA and DTA in most Amaurobiinae. The *Taira* DTA usually has branches on its external and mesal side, or teeth on its distal half; here we differentiate the external branch of the DTA (eDTA), mesal branch of the DTA (mDTA), and distal teeth of the DTA (dDTA) (Figs. 1, 2).

Retrolateral tibial apophysis (RTA)

The presence of the RTA, plus the presence of tarsal trichobothria and three or more metatarsal trichobothria, supports the monophyly of RTA-clade spiders (Coddington & Levi 1991; Griswold et al. 2005). The morphology of the RTA varies among amaurobiids. Rather than originating proximally or medially on tibia, extending along the tibial length, protruding distally as in coelotines (Wang 2002: figs. 44–46; Wang 2003; Wang & Jäger 2007; Xu & Li 2008), the RTA of most amaurobiines originates distally or medially on the tibia, and does not extend along the tibial length (Leech 1972; Yaginuma 1987; Thaler & Knoflach 1993). In *Taira*, the RTA varies from absent, as in *T. liboensis* (Zhang et al. 2008: figs. 31–33), long, sharply pointed, originating on the distal half of the tibia, as in *T. qiuae* new species (Figs. 19–21) and *T. zhui* new species (Figs. 33–35), to a small lobe, as in *T. sichuanensis* new species (Figs. 26–28) and five other remaining *Taira* species (i.e., *T. cangshan*, *T. concava*, *T. decorata*, *T. flavidorsalis*, and *T. sulciformis*) (Zhang et al. 2008: figs. 11, 16, 21, 26, 40). We follow Zhang et al. (2008) and treat this small apophysis as the RTA. Among those with a small RTA, *T. cangshan* has a RTA originating on the proximal half of the tibia; in the remaining species, it originates either medially or distally on the tibia. Except for *T. convava* and *T. sulciformis*, which have an RTA situated close to the DTA, other species have an RTA distinctly separated from the DTA. The RTA was labeled as the ectal process by Leech (1972: fig. 10) and Yaginuma (1987: figs. C, D, G, H).

Dorsal tibial apophysis (DTA)

The presence of a DTA, in combination with a hyaline conductor, is synapomorphic for the family Amaurobiidae (Griswold et al. 2005). The DTA is usually long with a slender distal end in *Amaurobius* and *Callobius* (Leech 1972: figs. 10, 11). In *Taira* the DTA is broad, modified with grooves, an external branch (eDTA) and mesal branch (mDTA), or with one or more distal teeth (dDTA) (Figs. 1, 2). eDTA is a branch protruding externally on the DTA toward the lateral side of the body, while mDTA is a branch protruding mesally on the DTA toward the middle of the body in natural condition (dorsal view of palp). The DTA was termed a "mesal process" by Leech (1972: fig. 10) and Yaginuma (1987. figs. G, H), an "innere apophyse" by Thaler (1990: figs. 7–10), a "prolateral-dorsal apophysis" by Thaler & Knoflach (1993: figs. 7–12), and a "prolateral apophysis" by Thaler & Knoflach (1991: figs. 3, 4). Zhang et al. (2008) treated the DTA as having two branches, iDTA (interior branch of DTA) and eDTA (exterior branch of DTA). The iDTA of Zhang et al. is in fact the DTA (rather than a branch on DTA), and the eDTA of Zhang et al. is the mDTA, which protrudes mesally toward the middle of the body (rather than on the external side of DTA). All *Taira* species have a long, deeply grooved DTA and a broad mDTA except *T. liboensis*, which has a less deeply grooved DTA and a broad eDTA, but no mDTA (Zhang et al. 2008: figs. 31–33). DTA of *T. cangshan* is less grooved and slightly larger than mDTA (Zhang et al. 2008: figs. 14–16).

Intermediate tibial apophysis (ITA)

The ITA is small, lobe-shaped, and present in most amaurobiines between the RTA and DTA. The ITA was referred to as a "dorsal process" by Leech (1972: fig. 10) and Yaginuma (1987: figs. G, H), as a "mittlere apophyse" by Thaler (1990: figs. 7–10), and as an "intermediate apophysis"

by Thaler & Knoflach (1993: figs. 7–12). The lateral tibial apophysis found in coelotines (Wang 2002: figs. 89, 107) also arises from the dorsal side of the RTA and its possible homology to the Amaurobiinae ITA needs further investigation. Most *Taira* species lack an ITA except for the three new species described in this study: *T. qiuae*, *T. sichuanensis*, and *T. zhui* (Figs. 20, 28, 35). We followed Zhang et al. (2008) and treated the small tibial apophysis of previous described *Taira* species as RTA, rather than ITA.

RELATIONSHIPS

Taxa

Representatives of other Amaurobiinae and Coelotinae are included as outgroup taxa to test the monophyly of *Taira* and to help root the tree: *Tamgrinia laticeps* (Schenkel 1936), *Coelotes atropos* (Walckenaer 1830), *Draconarius wudangensis* (Chen & Zhao 1997), *Callobius bennetti* (Blackwall 1846) and *Amaurobius fenestralis* (Ström 1768). We chose one *Tamgrinia* species to root the tree because it is cribellate and was treated as a member of the tribe *Tairini* together with *Taira* by Lehtinen (1967). *Tamgrinia* could be more closely related to Ageleninae and Coelotinae than to Amaurobiinae (Wang 2000; Wang & Zhu 2008; Wu et al. 2002). Two coelotine species were also chosen as outgroup taxa because they were previously placed in the same family with Amaurobiinae, they are well studied relative to other amaurobiids, and their relationship with Amaurobiinae has been explored in other studies (Spagna & Gillespie 2008; Wu et al. 2002). To reconstruct the relationships among the species, we compiled a data matrix that includes nine *Taira* species with both male and female described.

Characters

Twenty characters, mostly from genitalic structures, are used in the matrix. The female genitalia contributed nine characters, the male palp contributed nine, and the last two characters signify whether or not they are the cribellate spiders and whether the small hood of trichobothria is ridged:

Character 0: Epigynal teeth (0 = absent; 1 = present).

Character 1: Epigynal teeth, shape (0 = slender, longer than wide; 1 = broad, wider than long or with subequal length and width).

Character 2: Epigynal teeth, position (0 = situated anteriorly on anterior part of epigynum, with bases distinctly separated from epigastric furrow; 1 = situated posteriorly on posterior part of epigynum, with bases close to epigastric furrow). *Taira* species share the synapomorphy of having broad epigynal teeth. Similar to other amaurobiines, the epigynal teeth of *Taira* originate posteriorly near the epigastric furrow.

Character 3: Epigynal lobe (0 = absent; 1 = present).

Character 4: Epigynal lobe, shape (0 = epigynum with a small median lobe and two large lateral lobes; 1 = epigynum with a single, large, medially situated lobe).

Character 5: The single, large, medially situated epigynal lobe (0 = extending longitudinally, longer than wide; 1 = extending transversely, wider than long). An epigynal lobe is present in all *Taira* species, as well as in other Amaurobiinae. In *Callobius*, there are two broad lateral lobes and a small, anteriorly originated median lobe, but in *Amaurobius* and *Taira*, there is only one large lobe, which varies in size and shape. The size and shape of epigynal lobe could be species-specific and important in species diagnosis. The slightly elongated epigynal lobe is shared by *T. flavidorsalis* and *T. sulciformis*.

Character 6: Spermathecae, length (0 = long, with distinct heads arising distally; 1 = short, without heads). Another feature shared by *Taira* and other Amaurobiinae is the presence of short spermathecae, compared to the long ducts in most Coelotinae.

Character 7: Short spermathecae, separation (0 = separated by their width or less; 1 = separated by at least twice their width). Widely separated spermathecae apparently evolved in parallel in three species of the clade D and *T. cangshan* (Fig. 3).

Character 8: Short spermathecae, shape (0 = round; 1 = elongated, with the length twice the width). The short spermathecae in *Taira* are usually round or slightly elongated, but in three species of clade D (Fig. 3), (i.e., *T. qiuae*, *T. sichuanensis*, and *T. zhui*) they are elongated with the length at least twice the width.

Character 9: RTA (0 = extending along tibial length, protruding distally; 1 = not extending along tibial length). In Amaurobiinae, the RTA arises medially or distally on the tibia, rather than extending along the tibial length as in Coelotinae. The RTA is absent in *T. liboensis*, more or less long, with a sharply pointed distal end in *T. qiuae* and *T. zhui*, but small in other *Taira* species.

Character 10: DTA (0 = absent; 1 = present).

Character 11: ITA (0 = absent; 1 = present). The ITA arises between the RTA and DTA in Amaurobiinae, but only the three new species of the clade D (Fig. 3) have this apophysis in *Taira*.

Character 12: DTA, the broad, mesally protruding branch (mDTA) (0 = absent; 1 = present). Most *Taira* species have a broad, mesally protruding branch on the DTA. One species *T. liboensis* has a broad, externally protruding branch (eDTA) but lacks a mDTA. The three new species of clade D have small teeth on the DTA, but has neither an eDTA nor a mDTA.

	0 1 2 3 4 5 6 7 8 9	1 1 1 1 1 1 1 1 1 1 2 3 4 5 6 7 8 9
Tamgrinia laticeps	0 - - 0 - - 0 - - 0 1	? - - 1 0 - 0 1 0
Coelotes atropos	1 0 0 0 - - 0 - - 0 0	? - - 0 - - 0 0 0
Draconarius wudangensis	1 0 0 0 - - 0 - - 0 0	? - - 0 - - 0 0 0
Callobius bennetti	0 - - 1 0 - 1 0 0 1 1	1 0 0 1 0 - 0 1 1
Amaurobius fenestralis	1 0 1 1 1 1 1 0 0 1 1	1 0 0 1 0 - 0 1 1
Taira qiuae sp. nov.	1 1 1 1 1 1 1 1 1 1 1	1 1 0 1 1 1 1 1 1
T. flavidorsalis	1 1 1 1 1 0 1 0 0 1	1 0 1 0 1 1 0 0 1 1
T. cangshan	1 1 1 1 1 1 1 1 1 0	1 0 1 0 1 1 0 0 1 1
T. concava	1 1 1 1 1 1 1 1 0 0	1 0 1 0 1 1 0 0 1 1
T. decorata	1 1 1 1 1 1 1 1 0 0	- 1 0 1 0 1 1 0 0 1 1
T. liboensis	1 1 1 1 1 1 1 1 0 0	1 1 0 0 0 1 1 0 0 1 1
T. sulciformis	1 1 1 1 1 0 1 0 0 1	1 0 1 0 1 1 0 0 1 1
T. zhui sp. nov.	1 1 1 1 1 1 1 1 1 1 1	1 1 0 1 1 1 1 1 1
T. sichuanensis sp. nov.	1 1 1 1 1 1 1 1 1 2	1 1 0 1 1 1 1 1 1

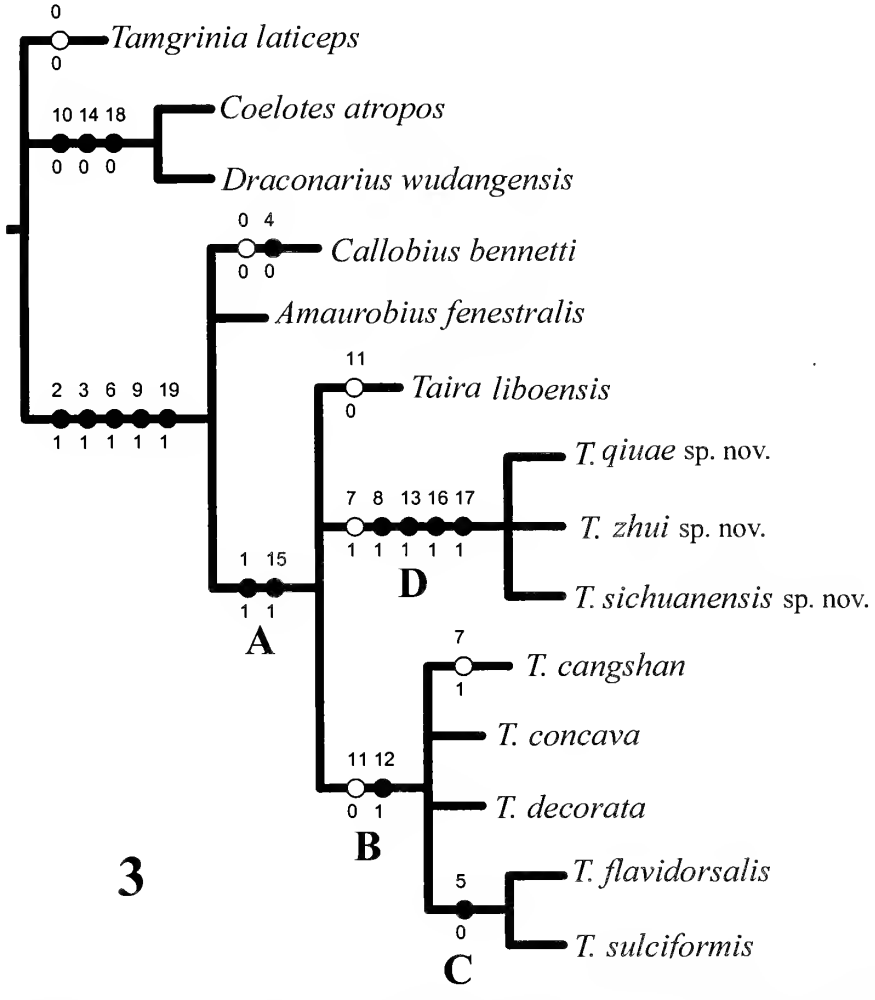


Figure 3.—The preferred cladogram of hypothesized *Taira* species relationships (L = 23, Ci = 90, Ri = 93); see text for discussion.

Character 13: DTA, distal teeth (0 = absent; 1 = present). DTA distal teeth are present in the three closely related new species of clade D.
Character 14: Tegular sclerite apophysis (0 = absent; 1 = present).
Character 15: Tegular sclerite apophysis, position (0 = proximal; 1 = distal).
Character 16: Distally originating tegular sclerite apophysis (0 = normal; 1 = strongly expanded anteriorly and covering most part of embolus). The presence of a tegular sclerite apophysis is another synapomorphy of Amaurobiinae, although in *Taira* it arises more distally and as a result is distinctly separated from the median apophysis by about the length of median apophysis. In three closely related D-clade *Taira* species, the tegular sclerite apophysis is strongly expanded anteriorly and leaves only the apex of the embolus visible in ventral view.
Character 17: Conductor modification (0 = absent; 1 = present). Unique to the three new *Taira* species at node D, the conductor is modified to have 2–3 strongly sclerotized apophyses (Figs 19–21: C1, C2, C3).
Character 18: Cribellum (0 = absent; 1 = present).
Character 19: Trichobothria, small hood (0 = smooth; 1 = ridged).

Results

We generated a data matrix and optimized characters using WinClada version 1.00.08 (Nixon, 1999) (Fig. 3). To perform the parsimony analyses, we used Hennig86 version 1.5 (Farris, 1988), with all characters treated as non-additive. The exact search algorithm (ie*;) was used, resulting in the eight most parsimonious trees. In the analysis, we arbitrarily used slow (Deltran) optimization, which favors parallelism over reversal. The preferred tree is shown in Fig. 3 (L = 23, Ci = 90, Ri = 93). Four of the eight resulting trees were excluded because they indicate the sister group relationship between *T. cangshan* and clade D, based on the widely separated spermathecae. The round-shaped spermathecae in *T. changshan*, which are similar to other *Taira*, differ from the elongated spermathecae of clade D taxa. We excluded two more trees because they show a sister group relationship between *T. liboensis* and clade B, supported by the absence of an ITA. Although having similar spermathecae and lacking an ITA, *T. liboensis* differs from others by the absence of an RTA, the absence of an mDTA, and the presence of an eDTA. Another tree shows the same *Taira* species relationship as the preferred tree but was excluded because it supports sister group relationship between *Amaurobius* and *Taira*. Our study focuses on *Taira* species relationships, so the characters indicating generic-level relationships are understudied.

The species of the genus *Taira* are united at node A by the presence of broad epigynal teeth (character 1, state 1) and the distally originating tegular sclerite apophysis (character 15, state 1). Three distinct species groups are found, and their relationships remain unresolved: *T. liboensis*, clade B species, and clade D species. Clade B includes five species: *T. cangshan*, *T. concava*, *T. decorata*, *T. flavidorsalis*, and *T. sulciformis*, based on the absence of an ITA (character 11, state 0, which is parallel in *T. liboensis*) and the presence of a broad, mesally protruding branch on the DTA (mDTA) (Character 12, state 1). The elongated epigynal median lobe (character 5, state 0) supports a sister group relationship between *T. flavidorsalis* and *T. sulciformis* at node C. Three new species (*T. qiuae*, *T. sichuanensis*, and *T. zhui*) are united at node D by the widely separated spermathecal bases (character 7, state 1), the long spermathecae, which can be twice as long as wide (character 8, state 1), the presence of teeth on distal and subdistal DTA (character 13, state 1), the anteriorly expanded tegular sclerite apophysis that hides most of the embolus (character 16, state 1), and the presence of 2–3 broad, strongly sclerotized conductor apophyses (character 17, state 1).

SYSTEMATICS

Family Amaurobiidae Thorell 1870
Subfamily Amaurobiinae Thorell 1870
Taira Lehtinen 1967

Taira Lehtinen 1967:266.
Type species: *Amaurobius flavidorsalis* Yaginuma 1964

Diagnosis.—The genus *Taira* can be distinguished from *Amaurobius* and related genera by two putative synapomorphies: the presence of broad epigynal teeth in females (Figs. 22, 29, 36) and the distally originating tegular sclerite apophysis in males (Figs. 19, 27, 34). An additional diagnostic character includes the branched or toothed dorsal tibial apophysis (Figs. 21, 26, 33).

Description.—Small to medium-sized cribellate spiders (Figs. 13, 14, 18, 24, 25, 31, 32, 38, 39), total length 4.18–6.43 (males) and 5.30–10.7 (females). Carapace elongate, dark brown, with distinct wide, light-colored, longitudinal median band and two wide, dark-colored, longitudinal lateral bands; cephalic area slightly narrowed, with cover of gray setae and sparsely distributed black setae; fovea longitudinal, deep. Anterior eye row straight or slightly recurved, posterior eye row strongly recurved, with anterior margin of PME distinctly posterior to posterior margin of PLE (Fig. 16); AME smallest, PME subequal to or slightly larger than AME, ALE largest, PLE subequal to or slightly smaller than ALE; AME separated from each other by less than their diameter, separated from ALE by 1–1.5 times AME diameter, PME separated from each other by 1.5–2 times PME diameter, widely separated from PLE by at least 2 times PME diameter; eyes with median ocular quadrangle wider in back than in front, longer than wide (Fig. 16). Clypeus high, approximately two times AME diameter, curved downward. Sternum longer than wide (width/length = 0.75–0.80), sparsely covered with black setae, anterior margin straight, lateral margins without extensions between coxae, posterior margin pointed, slightly separating coxae IV (Fig. 17). Chilum undivided, hairless. Chelicerae with 4 promarginal and 3 retromarginal teeth. Labium subequal or slightly longer than wide. Endites rectangular, anteriorly slightly pointed, laterally slightly depressed, with promarginal scopula, without serrula

(Fig. 15). Tibiae with two rows of trichobothria; metatarsi and tarsi with one row of trichobothria; trichobothria with large hood transversely striated, small hood longitudinally ridged (Fig. 5), tarsal organ with simple opening. Tarsi with three claws, paired superior claws with 8–10 teeth on each; scopulae absent; leg spination often varying among individuals, typical leg spination pattern (only surfaces bearing the spines listed, each leg segment was divided into four surfaces, dorsal, prolateral, ventral, retrolateral, then indicating the number of spines in the proximal, middle, and distal one-thirds of each segment): femur: I p0–0–2; II p0–0–2, r0–0–1; III p0–0–1, r0–0–1; IV p0–0–1; tibia: I p1–1–1, v2–2–2, r0-1-1; II p0–1–1, v0–2–2, r0-1-1; III p1–1–0, v1–1–2, r1-1-0; IV p0–0–1; v1–0–2; metatarsus: I p0-1-0, v2–2–2, r0-1-1; II p0-1-1, v2–2–2, r0-1-1; III p1–1–1, v2–2–2, r1–1–1; IV v0–0–2. Tracheal tubes simple, limited to opisthosoma, spiracle situated close to spinnerets and connected to atrium, from which two lateral and two median tubes arise (Fig. 4). Cribellum divided (Figs. 6, 7). ALS short, conical, two-segmented, apex of ALS with 2 major ampullate gland spigots and approximately 28–55 piriform gland spigots (Fig. 8); PMS short, one-segmented, with 1 minor ampullate gland spigot, 1 aciniform gland spigot, 1 cylindrical gland spigot, about 5–8 paracribellar spigots (Fig. 9); PLS with approximately 8–22 aciniform gland spigots, 2–3 cylindrical gland spigots, 1 "amaurobiid PLS spigot" on distal end, 2 paracribellar spigots beside "amaurobiid PLS spigot" (Fig. 10).

Female epigynum simple; atrium small or indistinct, with openings on anterior margin of epigynal median lobe; epigynal median lobe distinct; epigynal teeth short, broad, situated lateral of median lobe, close to but slightly separated from epigastric furrow; copulatory ducts small, originating medially, or indistinct in some species; spermathecae small, widely separated, round in most species (elongated only in *T. qiuae*, *T. sichuanensis* and *T. zhui*); fertilization ducts long, can be as long as spermathecae.

Male palp without patellar apophysis; retrolateral tibial apophysis (RTA) small, situated distally or medially, widely separated or close to dorsal tibial apophysis (DTA) (but long and bent dorsally in *T. qiuae* and *T. zhui*, and absent in *T. liboensis*); dorsal tibial apophysis large, with distinct groove, branched on its external and mesal surfaces, with mesal branch usually broad and large; intermediate tibial apophysis only observed in three clade D species; cymbium short, with distal end extending slightly beyond bulb, without distinct spines; proximal cymbium strongly constricted and concave to narrow base; conductor broad, arising from distal bulb, hyaline with sclerotized base (in *T. qiuae*, *T. sichuanensis*, and *T. zhui*, the conductor is modified to small, less sclerotized apophysis and broad, highly sclerotized, branched, beak-shaped apophyses); median apophysis long, with slender apex and broad base, arising from less sclerotized tegulum area; tegulum with distally originating tegular sclerite apophysis, which is widely separated from base of median apophysis; embolus broad, short, arising distally on prolateral tegulum.

Natural history.—Species of *Taira* build small cribellate webs (Figs. 40, 41), which are similar to *Amaurobius* webs. The spiders can be found on buildings, cliffs, trees, in caves, and on other substrates and favor shady, humid conditions. Individuals usually live together in high density, particularly in the late spring and early summer during which the adults are active. Although the adult female can also be found from July to August (personal observation), we collected specimens of *T. liboensis* from caves where adults are active in the summer (Wang & Ran 2004; Zhu et al. 2004).

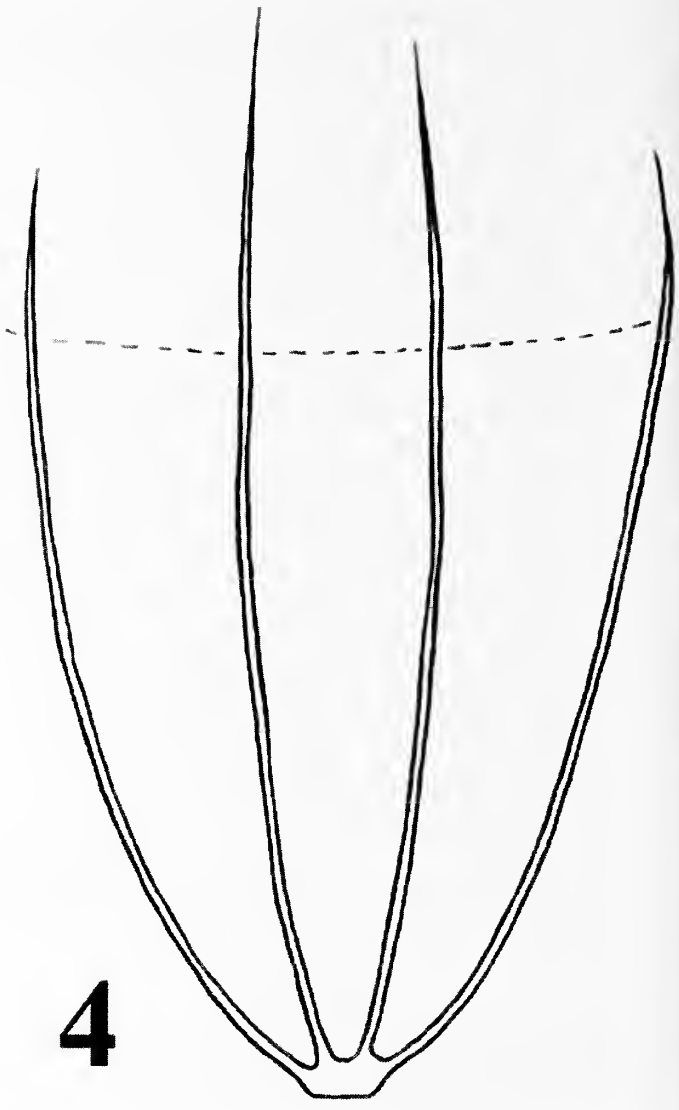
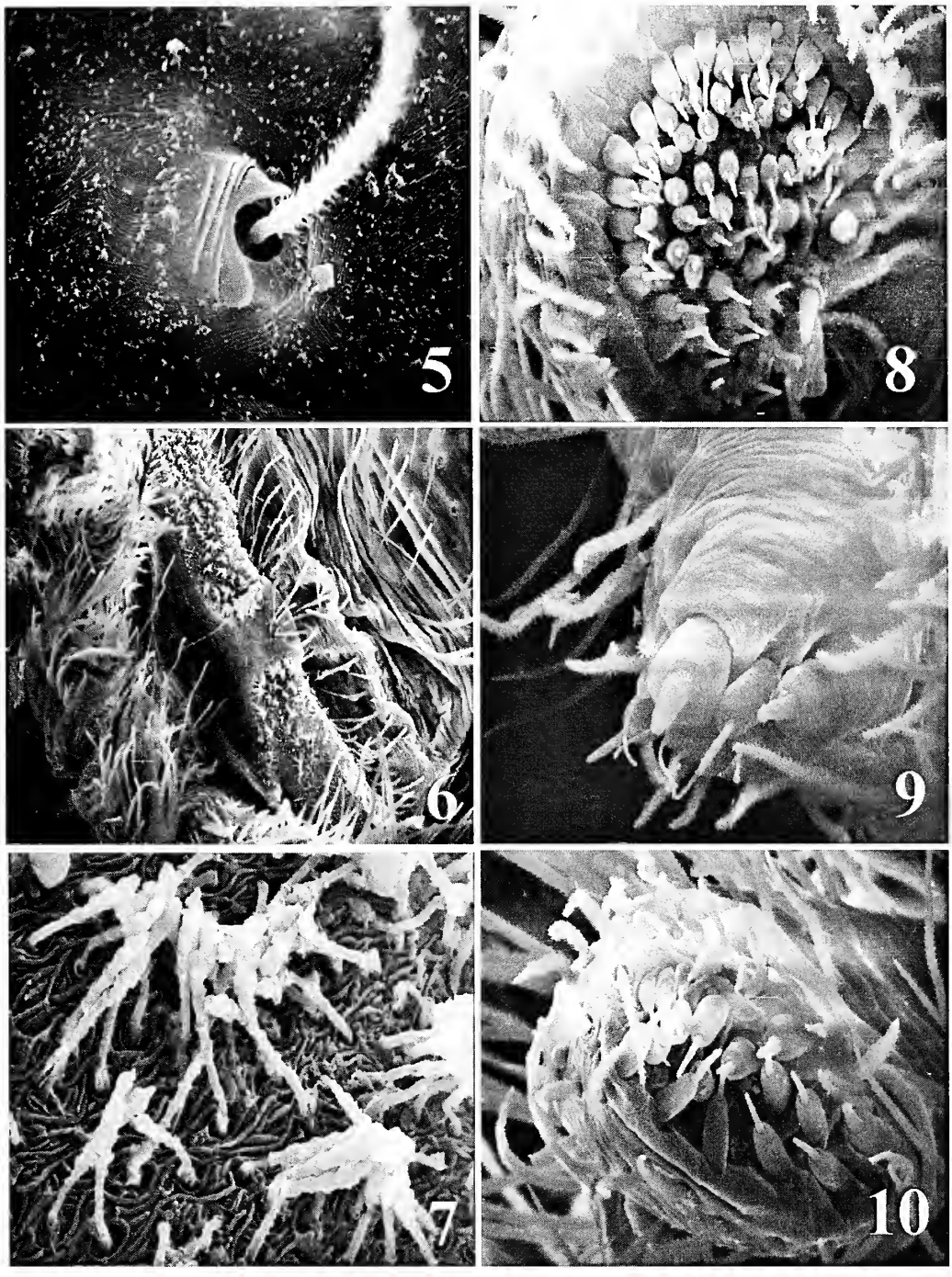


Figure 4.—*Taira decorata* (Yin & Bao 2001), female from Wuyi Mt., Fujian, China, trachea (dashed line refers to the position of epigastric furrow).

Composition.—Eleven species: *T. cangshan* Zhang, Zhu & Song 2008, *T. concava* Zhang, Zhu & Song 2008, *T. decorata* (Yin & Bao 2001), *T. flavidorsalis* (Yaginuma 1964), *T. latilabiata* Zhang, Zhu & Song 2008, *T. liboensis* Zhu, Chen & Zhang 2004, *T. obtusa* Zhang, Zhu & Song 2008, *T. qiuae* new species, *T. sichuanensis* new species, and *T. sulciformis* Zhang, Zhu & Song 2008, and *T. zhui* new species

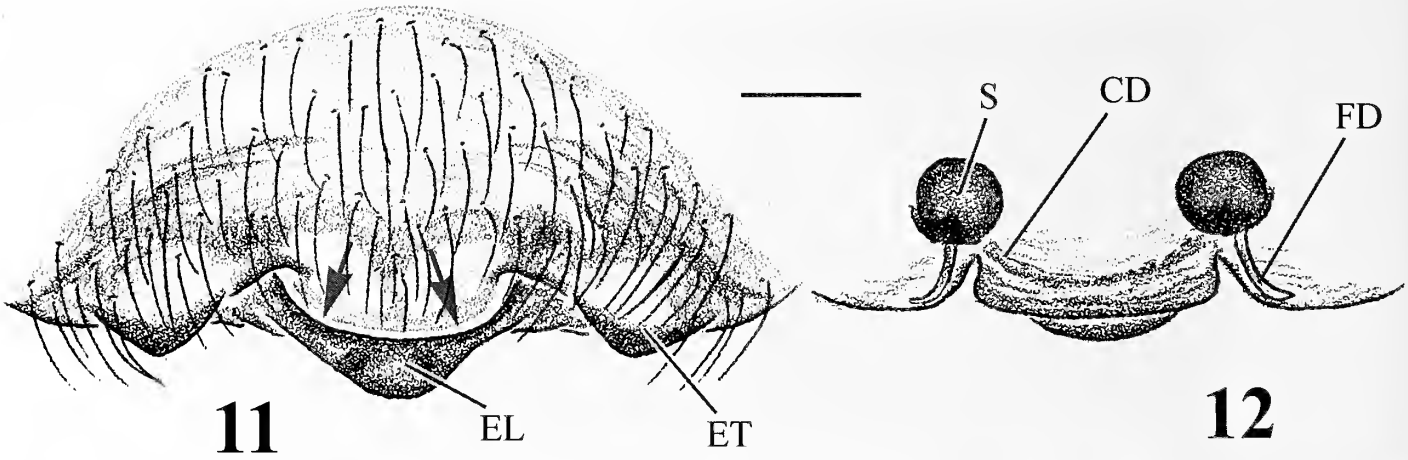
Distribution.—China, Japan (Fig. 42).



Figures 5–10.—*Taira decorata* (Yin & Bao 2001), female from Wuyi Mt., Fujian, China, SEM pictures. 5. Trichobothria. 6. Cribellum. 7. Cribellum, enlarged, showing the spigots. 8. ALS. 9. PMS. 10. PLS.

KEY TO SPECIES OF *TAIRA*

1. Male (those of *T. obtusa*, *T. latilabiata* unknown) 2
   Female 10
2. Conductor with additional, highly sclerotized apophyses (C1, C2 and C3 in Figs. 19–21); tegular sclerite apophysis (TSA) broad, anteriorly expanding, covering most of embolus from ventral view (Figs. 19–21, 26–28, 33–35) 3
   Conductor with single broad, hyaline apophysis; tegular sclerite apophysis small, embolus visible from ventral view (Zhang et al. 2008: figs. 9–11) 5
3. RTA long, bent distally, with a sharp distal end (Figs. 20, 35) 4
   RTA short, exhibiting only a small lobe (Figs. 27, 28) . . . . . *sichuanensis*
4. Median apophysis with sharp, long basal process (Fig. 34) . . . . . *zhui*
   Median apophysis with blunt, short basal process (Fig. 19) . . . . . *qiuae*



Figures 11, 12.—*Taira cangshan* Zhang, Zhu & Song 2008, female (CASENT9021382) from Lushui, Yunnan, China, epigynum (ventral and dorsal view).

5. RTA present; DTA with distinct branch on its mesal side; prolateral tegular lobe absent or indistinct (Zhang et al. 2008: fig. 10) . . . 6
RTA absent; DTA with distinct branch on its ectal side; tegulum with distinct prolateral lobe (Zhang et al. 2008: figs. 32, 33) . . . *liboensis*
6. DTA distinctly longer than its mesal branch; RTA arising from distal half of tibia (Zhang et al. 2008: figs. 9–11) . . . 7
DTA and its mesal branch about the same length; RTA arising from proximal half of tibia (Zhang et al. 2008: figs. 14–16) *cangshan*
7. RTA distinctly separated from DTA (Zhang et al. 2008: figs. 16, 26) . . . 8
RTA close to DTA (Zhang et al. 2008: figs. 21, 40) . . . 9
8. Distal embolus abruptly narrowed; tegular sclerite apophysis with slightly notched apex (Zhang et al. 2008: figs. 9, 10) *flavidorsalis*
Distal embolus as broad as its base; tegular sclerite apophysis with rounded apex (Zhang et al. 2008: fig. 25) . . . *decorata*
9. DTA slender; tegular sclerite apophysis round (Zhang et al. 2008: fig. 20) . . . *concava*
DTA broad; tegular sclerite apophysis blunt (Zhang et al. 2008: fig. 39) . . . *sulciformis*
10. Epigynal lobe with length and width subequal, or longer than wide (Zhang et al. 2008: figs. 7, 36) . . . 11
Epigynal lobe wider than long (Figs. 11, 22) . . . 12
11. Epigynal lobe widest anteriorly (Zhang et al. 2008: fig. 7) . . . *flavidorsalis*
Epigynal lobe widest medially (Zhang et al. 2008: fig. 36) . . . *sulciformis*
12. Spermathecal bases widely separated by at least two times their width (Figs. 12, 23, 37) . . . 13
Spermathecal bases slightly separated by less then their width (Zhang et al. 2008: figs. 18, 30) . . . 17
13. Spermathecae elongated, with length at least two times their width (Figs. 23, 37) . . . 14
Spermathecae round (Fig. 12) . . . 16
14. Spermathecae strongly converging anteriorly, with distal ends separated by only ¼ of proximal separation (Fig. 30) *sichuanensis*
Spermathecae slightly converging anteriorly, with distal ends separated by more than ½ of proximal separation (Figs. 23, 37) 15
15. Spermathecal bases distinctly folded (Fig. 37) . . . *zhui*
Spermathecal bases smooth, not folded (Fig. 23) . . . *qiuae*
16. Epigynal lobe distinctly curved; spermathecae separated by about 2 times their width (Fig. 12) . . . *cangshan*
Epigynal lobe not curved; spermathecae separated by about 3 times their width (Zhang et al. 2008: fig. 27) . . . *latilabiata*
17. Epigynal teeth and lateral margins of epigynal lobe widely separated by the width of epigynal teeth (Zhang et al. 2008: fig. 17) . . . *concava*
Epigynal teeth and lateral margins of epigynal lobe close together (Zhang et al. 2008: figs. 22, 29) . . . 18
18. Spermathecae separated by less than half of their width (Zhang et al. 2008: fig. 23) . . . *decorata*
Spermathecae separated by more than half of their width (Zhang et al. 2008: figs. 30, 35) . . . 19
19. Spermathecae with distinct, anterior extensions (Zhang et al. 2008: fig. 35) . . . *obtusa*
Spermathecae round, without anterior extensions (Zhang et al. 2008: fig. 30) . . . *liboensis*

Taira decorata (Yin & Bao 2001)
Figs. 4–10, 42

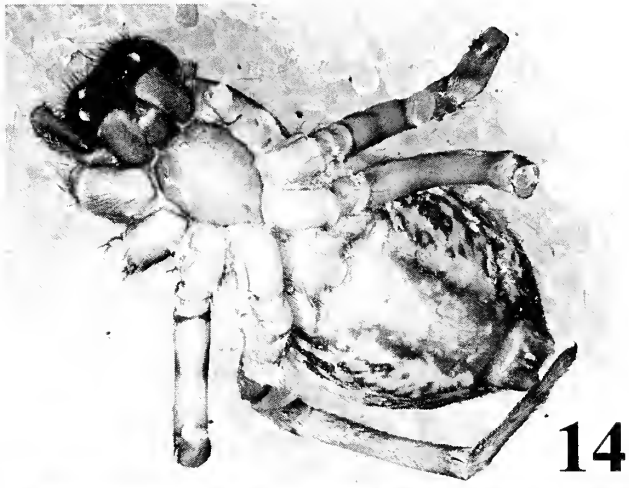
Titanoeca decorata Yin & Bao 2001:60, figs. 2a–e.
Taira decorata Zhang, Zhu & Song 2008:507, figs. 22–26.

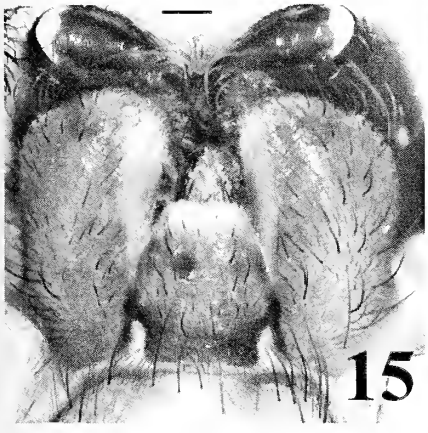
Remarks.—In addition to the genitalic illustrations by Zhang et al. (2008), in this study we also examined its tracheae (Fig. 4), trichobothria (Fig. 5), and spinnerets (Figs. 6–10), which are similar to *T. liboensis* of Wang (2000: figs. 16–18, 35).

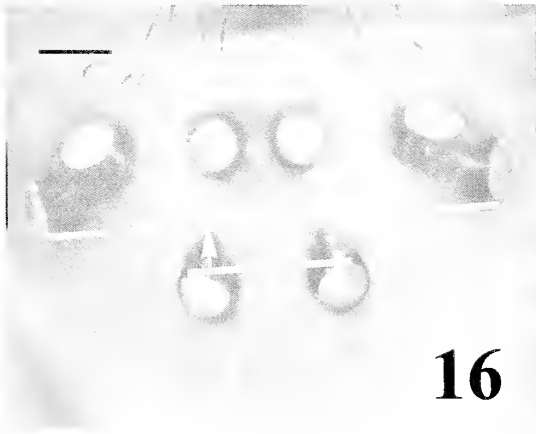
Taira cangshan Zhang, Zhu & Song 2008
Figs. 11–17, 42

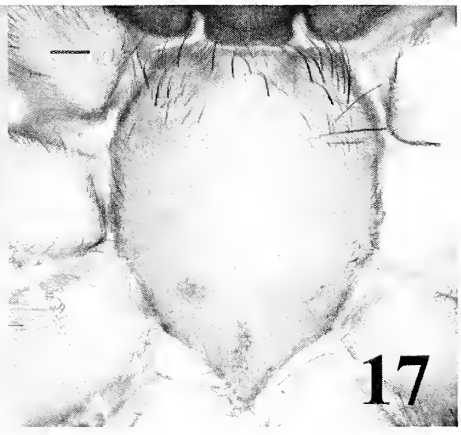
Taira cangshan Zhang et al. 2008:505, figs. 12–16.

Remarks.—In addition to the material examined by Zhang et al. (2008), more specimens were collected from other parts of Yunnan, China (1♀, Lushui County, Yaojiaping He at Pianma Road, 44.7 km, elev. 2516 m, 25.97479°N, 098.71027°E, disturbed forest, night collecting in forest and









Figures 13–17.—*Taira cangshan* Zhang, Zhu & Song 2008, female (CASENT9021382) from Lushui, Yunnan, China. 13. Habitus, dorsal view. 14. Habitus, ventral view. 15. Labium and endites. 16. Eyes, view between dorsal and front. 17. Sternum.

along roadcuts, 20 May 2005, C. Griswold & D. Kavanaugh, CAS, CASENT9021382; 1♀, same data, CAS, ENT9022279); 1♀, same data, HNU, ENT9022278; 1♀, same data, HNU, ENT9022357).

In this study, we have re-illustrated the epigynum and vulva. Our illustration of the epigynum shows a much narrower lobe than that of Zhang et al. (2008) because it is viewed from a slightly different angle (Figs. 11, 12). We also took photos of habitus, eyes, sternum, labium, and endites to display the general somatic structures of *Taira* (Figs. 13–17).

Taira liboensis Zhu, Chen & Zhang 2004
Figs. 18, 42

Taira liboensis Zhu et al. 2004:61, figs. 1A–F (female holotype and male paratype, in MHBU, examined). Zhang et al., 2008:509, figs. 29–33.

Taira lunaris Wang & Ran 2004:31, figs. 1–4 (female holotype and paratype, in IZCAS, examined). First synonymized by Zhang et al. 2008.

Remarks.—In addition to the material examined by Zhang et al. (2008) and Wang & Ran (2004), we collected more specimens from Guizhou, China (4♀, Guiyang City, Qianlin Park, 8 August 2007, Z.S. Zhang, SWUC; 1♀, Guiding County, Yanxia Town, Jingangdong Cave, 10 August 2007, Z.S. Zhang, SWUC; 1♂2♀, Guiding County, Yanxia Town, Dayandong Cave, 9 August 2007, Z.S. Zhang, SWUC).

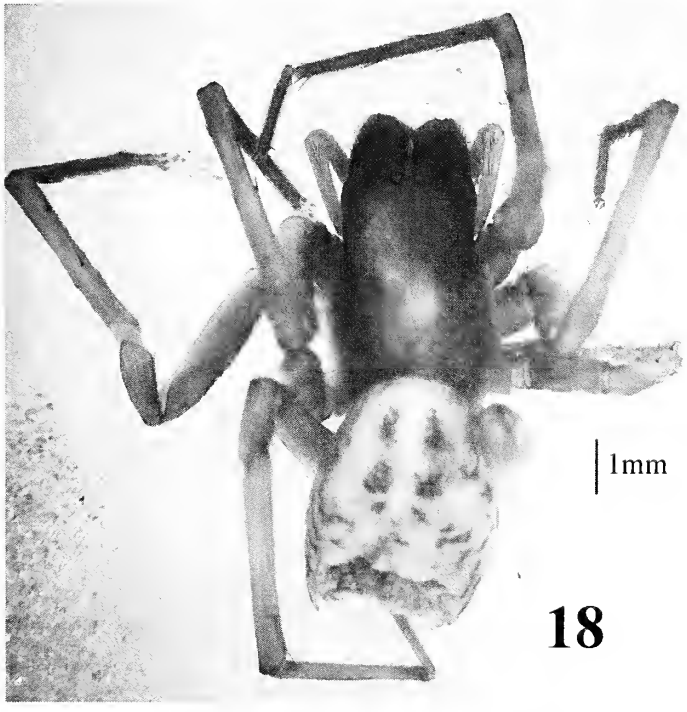
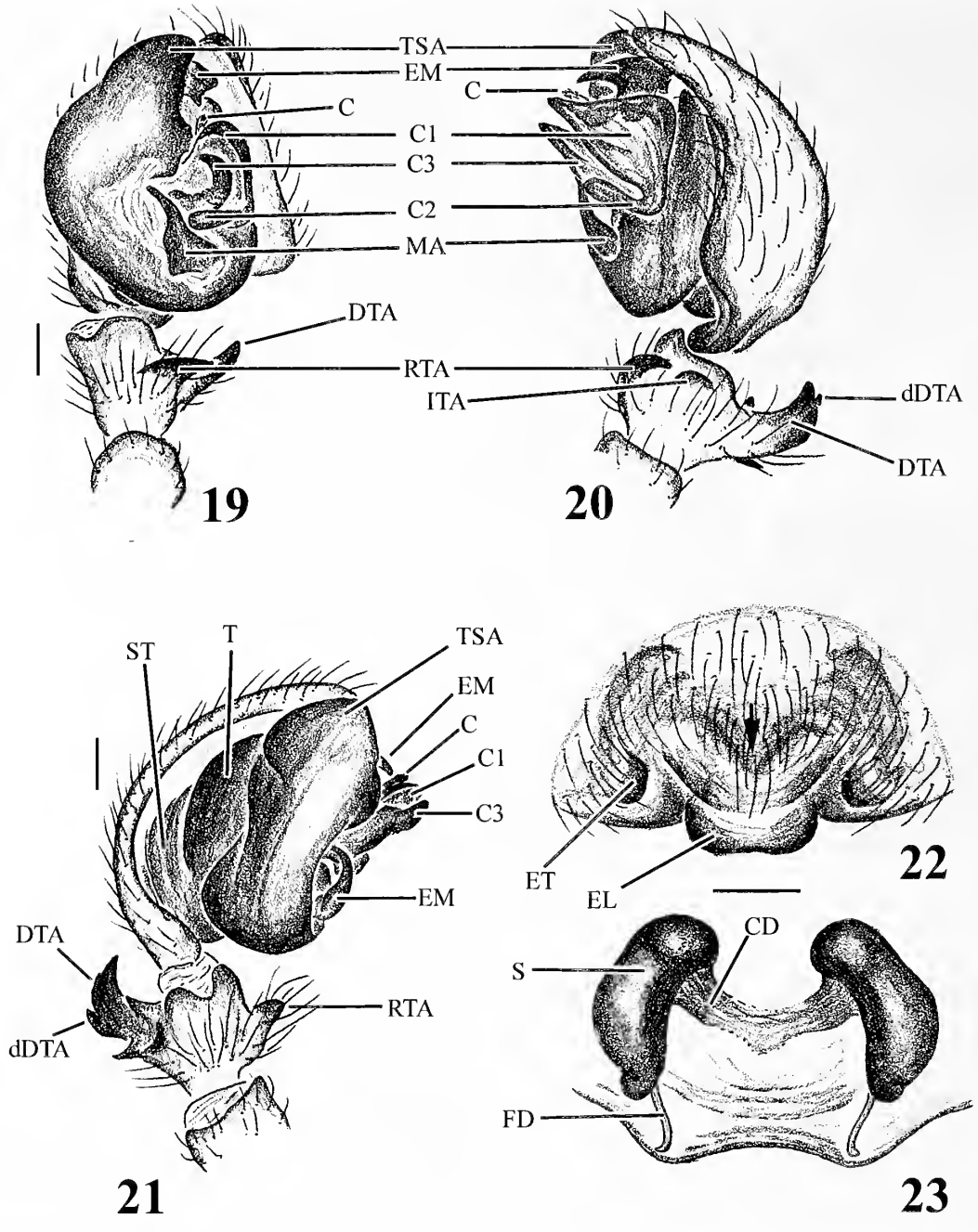


Figure 18.—*Taira liboensis* Zhu, Chen & Zhang 2004, female holotype of *T. lunaris* Wang & Ran, 2004 from Libo, Guizhou, China, habitus, dorsal view.



Figures 19–23.—*Taira qiuae* new species, male holotype and female paratype from Taibaishan, Shaanxi, China, drawings. 19. Palp, ventral view. 20. Palp, retrolateral view. 21. Palp, prolateral view. 22. Epigynum, ventral view (arrow points to copulatory opening). 23. Epigynum, dorsal view.

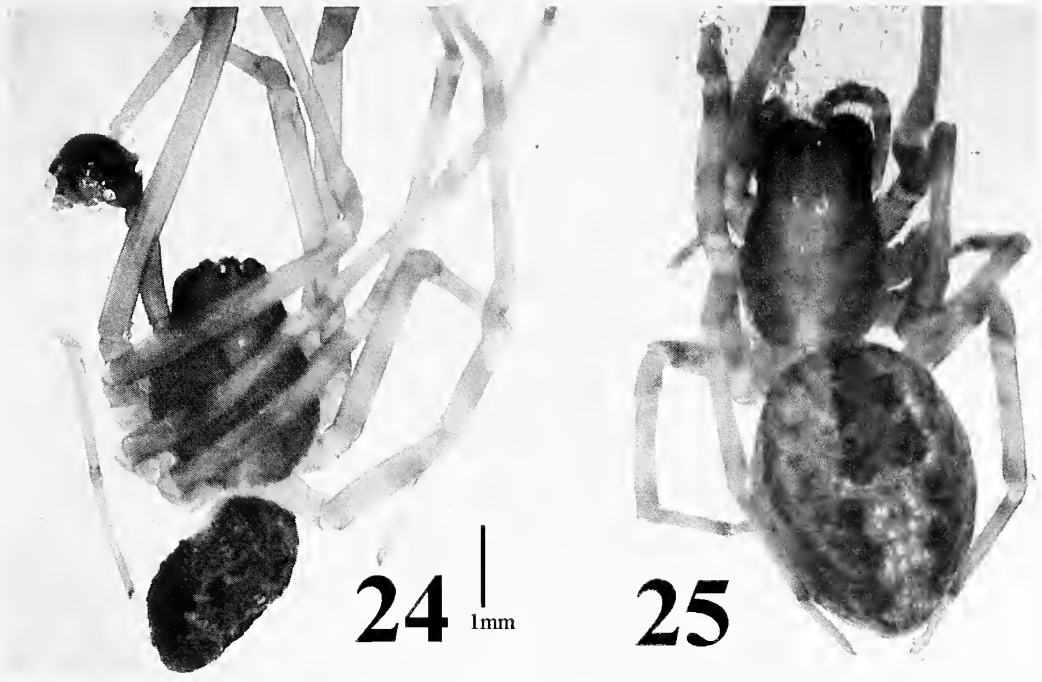
Habitus photos of the female holotype of *Taira lunaris* (=*T. liboensis*) were taken for the purpose of comparison with other *Taira* species (Fig. 18).

Taira qiuae new species
Figs. 19–25, 42

Types.—CHINA: *Shaanxi*, ♂ holotype, 4♀ paratypes from Taibai Shan, S. flanks, above Houzhenzi, secondary broad-leaf forest, 1300–1700 m, 8 June 1997, P. Jäger and B. Martens, deposited in SMF; 1♂ paratype from Taibai Shan, S. flanks, above Houzhenzi, secondary broad-leaf forest, 33°52′32.42″N, 107°48′34.73″E, 1700 m, sieving leaf litter, 7 June 1997, P. Jäger and B. Martens, deposited in SMF; 30♂32♀ paratypes from Taibai Shan, above Houzhenzi, 1250–1800 m, 23–25 May 2009, Z.S. Zhang, deposited in SWUC.

Etymology.—The specific name is in honor and memory of Professor Qiong-Hua Qiu (deceased), who advised and supported Xin-Ping Wang's work; noun (name) in genitive case.

Diagnosis.—This new species is similar to *T. zhui* new species in having a free standing, long RTA and the long, more or less anteriorly converging spermathecae but can be distinguished by the longer spermathecae, the smooth spermathecal bases (not folded) in females, the less extending



Figures 24, 25.—*Taira qiuae* new species, male holotype (24) and female paratype (25) from Taibaishan, Shaanxi, China, photos, habitus, dorsal view.

base of the median apophysis and the different shapes of the conductor apophyses (much larger C1 and strongly prolaterally curved C3) in male (Figs. 19–23).

Description.—*Male (holotype):* Medium-sized spider, total length 5.40 (Fig. 24). Carapace 3.00 long, 2.16 wide; opisthosoma 2.40 long, 1.65 wide. AME smallest, ALE largest, PME and PLE subequal in size (AME 0.07, ALE 0.14, PME 0.11, PLE 0.12); AME separated from each other by less than their diameter, widely separated from ALE by about 1.5 times AME diameter; PME separated from each other by slightly more than their diameter, from PLE by almost 2 times PME diameter; AME and PME widely separated by about 2 times AME diameter (AME–AME 0.05, AME–ALE 0.10, ALE–PLE 0.05, PME–PME 0.13, PME–PLE 0.18, AME–PME 0.15). Palpal RTA long, bent dorsally, with sharply pointed distal end (Fig. 20); dorsal tibial apophysis (DTA) large, toothed proximally and distally (Fig. 21); intermediate tibial apophysis (ITA) small, arising between the RTA and DTA (Fig. 20); conductor reduced to a slender, hyaline apophysis, which arises and hides behind the well-developed, strongly branched, beak-shaped conductor apophyses (C1, C2, C3 in Figs. 19–21); median apophysis long, with broad base and slender apex (Figs. 19, 20); tegular sclerite extending anteriorly and forming a broad apophysis on distal bulb, the latter covering most of embolus (Fig. 19); embolus broad, only the distal end visible from ventral view (Fig. 19).

Female (paratype): Medium-sized spider, total length 6.62 (Fig. 25). Carapace 2.77 long, 1.87 wide; opisthosoma 3.85 long, 2.82 wide. AME smallest, ALE largest, PME and PLE subequal (AME 0.07, ALE 0.13, PME 0.10, PLE 0.10); AME close together, separated from each other by less than their diameter, widely separated from ALE by about 1.5 times AME diameter; PME separated from each other by 1.5 times their diameter, from PLE by almost 2 times PME diameter; AME and PME widely separated by about 2 times AME diameter (AME–AME 0.05, AME–ALE 0.11, ALE–PLE 0.04, PME–PME 0.16, PME–PLE 0.18, AME–PME 0.15). Epigynum with a triangular plate anterior of median lobe; median lobe two times wider than long; epigynal teeth broad, separated from median lobe by approximately their width; copulatory ducts small but distinct, arising medially and extending laterally to spermathecae; spermathecae long, kidney-shaped, widely separated from each other anteriorly by at least their width and posteriorly by at least two times their width; fertilization ducts long, approximately half the length of spermathecae (Figs. 22, 23).

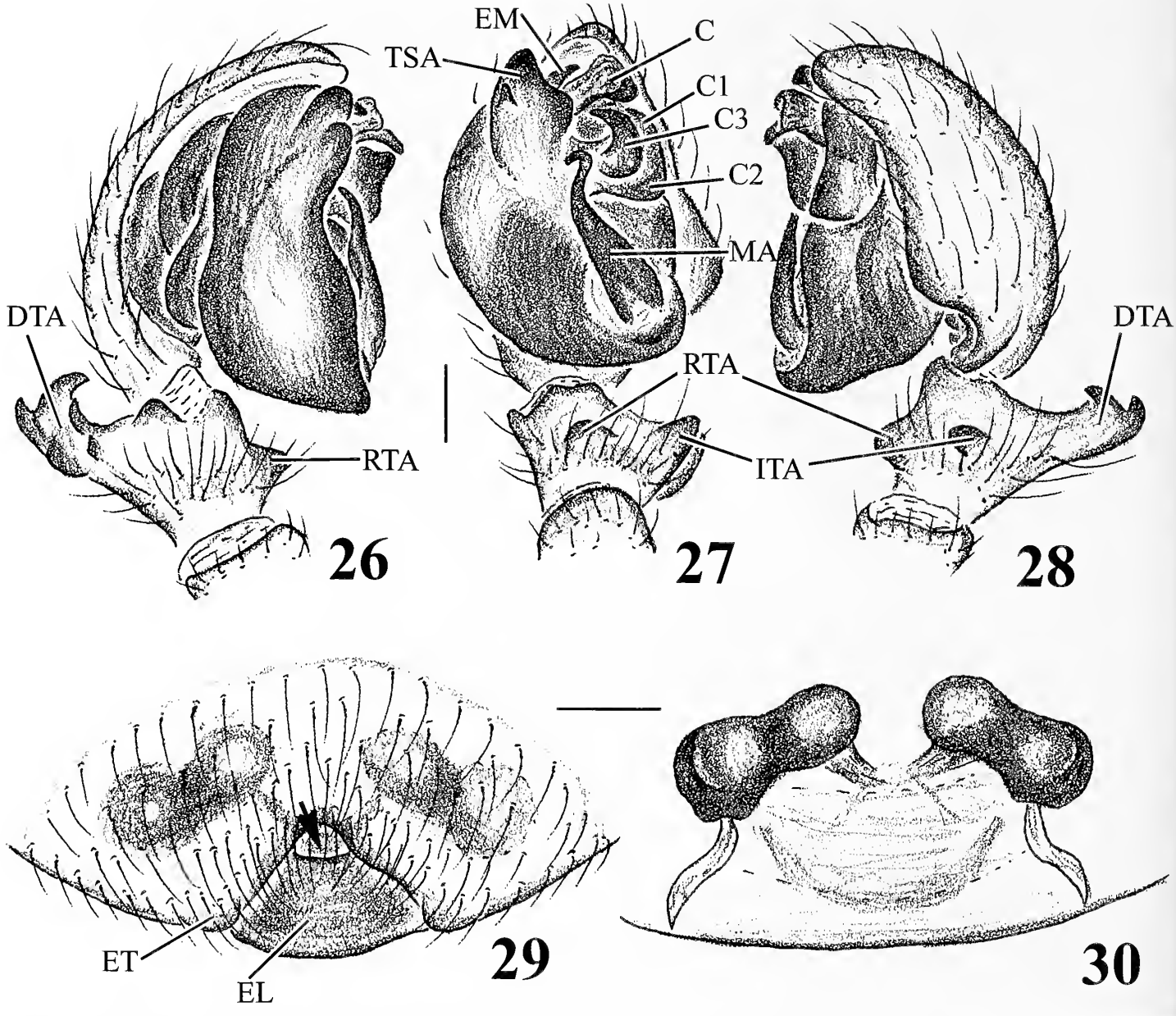
Distribution.—China (Shaanxi) (Fig. 42).

Taira sichuanensis new species
Figs. 26–32, 42

Types.—CHINA: *Sichuan*, ♂ holotype, 6♂12♀ paratypes from Changning County, Meidong Town, Gaojian Village, 28°18′5.0″N, 104°58′56.8″E, 700 m, 1 May, 2008, Z.S. Zhang & R.Y. Zuo, deposited in SWUC; 7♀ paratypes from the same locality, 5 June 2008, Z.S. Zhang, deposited in SWUC.

Etymology.—The specific name refers to the type locality, Sichuan Province, China; adjective.

Diagnosis.—This new species is similar to *T. qiuae* new species and *T. zhui* new species in having the distinct conductor apophyses, the anteriorly expanding tegular sclerite apophysis, and the elongated spermathecae but can be distinguished by the small RTA, the presence of a broad conductor, and the different shapes of conductor apophyses (much smaller C1 and C2) in males, and by the small epigynal teeth, the presence of a distinct atrial opening, and the relatively transversely extending spermathecae in females (Figs. 26–30).



Figures 26–30.—*Taira sichuanensis* new species, male holotype and female paratype from Changning, Sichuan, China. 26. Palp, prolateral view. 27. Palp, ventral view. 28. Palp, retrolateral view. 29. Epigynum, ventral view (arrow points to copulatory opening). 30. Epigynum, dorsal view.

Description.—*Male (holotype):* Medium-sized spider, total length 4.90 (Fig. 31). Carapace 2.10 long, 1.50 wide; opisthosoma 2.80 long, 1.60 wide. Median eyes subequal in size, ALE largest, PLE slightly smaller than ALE (AME 0.08, ALE 0.13, PME 0.08, PLE 0.10); AME separated from each other by less than their diameter, from ALE by about AME diameter; PME separated from each other by slightly more than 1.5 times their diameter, from PLE by almost 2 times PME diameter (AME–AME 0.05, AME–ALE 0.08, PME–PME 0.13, PME–PLE 0.15, ALE–PLE 0.05). Palpal RTA short, forming a small lobe; dorsal tibial apophysis (DTA) large, toothed distally; intermediate tibial apophysis (ITA) arising between the RTA and DTA; conductor broad, distally hyaline; conductor apophyses broad, strongly branched; median apophysis long, with broad base and slender apex; tegular sclerite extending anteriorly and forming a broad apophysis on distal bulb, the latter covering most of embolus; embolus broad, only the distal end visible from ventral view (Figs. 26–28).

Female (paratype): Medium-sized spider, total length 3.80 (Fig. 32). Carapace 1.50 long, 1.00 wide; opisthosoma 2.30 long, 1.50 wide. AME smallest, ALE largest, posterior eyes subequal in size (AME 0.05, ALE 0.10, PME 0.08, PLE 0.08); AME separated from each other by about their diameter, from ALE by about 1.5 times AME diameter; PME separated from each other by slightly more than their diameter, from PLE by almost 2 times PME diameter (AME–AME 0.05, AME–ALE 0.08, PME–PME 0.10, PME–PLE 0.15, ALE–PLE 0.05). Epigynum with a small median lobe; epigynal teeth small, originating on posterior margin of epigynum and close to median lobe; copulatory ducts small, arising medially between

Figures 31, 32.—*Taira sichuanensis* new species, male holotype (31) and female paratype (32) from Changning, Sichuan, China, photos, habitus, dorsal view.

spermathecae; spermathecae long, more or less extending transversely, separated from each other anteriorly by approximately their width and posteriorly by at least three times their width; fertilization ducts long, at least half the length of spermathecae (Figs. 29, 30).

Distribution.—China (Sichuan) (Fig. 42).

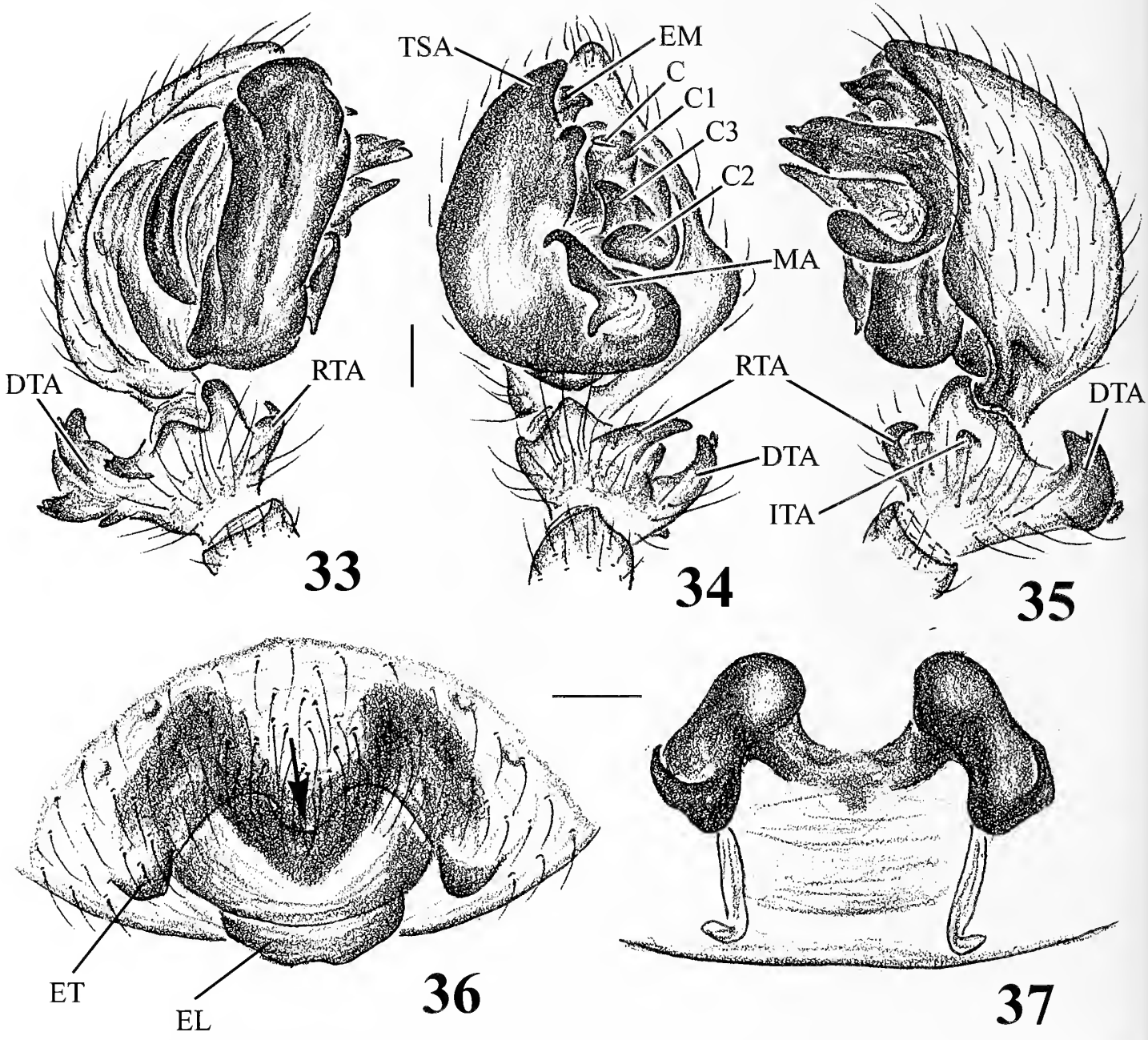
Taira zhui new species
Figs. 33–39, 42

Types.—CHINA: *Chongqing*, ♂ holotype, 2♂28♀ paratypes from Jinyunshan Natural Nature Reserve, 29°41′08″–29°52′03″N, 106°17′43″–106°24′50″E, 350–951 m, Beipei May 2008, R.Y. Zue & Z.H. Liu, deposited in SWUC; 1♂ paratype from same locality as above, 26 April 2008, Z. S. Zhang, deposited in SWUC. 6♂10♀ paratypes from same locality as above, 17 May 2009, Z.S. Zhang, deposited in SWUC.

Etymology.—The specific name is in honor of Professor Ming-Sheng Zhu, who advised and supported Zhi-Zheng Zhang's work; noun (name) in genitive case.

Diagnosis.—This new species is similar to *T. qiuae* new species in having a long RTA and long, anteriorly converging spermathecae but can be distinguished by the distinctly folded spermathecal bases in females, the distinctly extending base of the median apophysis and the different shapes of the conductor apophyses (much smaller C1 and slightly prolaterally curved C3) in males (Figs. 33–37).

Description.—*Male (holotype):* Medium-sized spiders, total length 4.60 (Fig. 38). Carapace 2.50 long, 1.70 wide; opisthosoma 2.30 long, 1.50 wide. AME smallest, 2/3 size of ALE, ALE largest; posterior eyes subequal in size, slightly smaller than ALE (AME 0.10, ALE 0.15, PME 0.13, PLE 0.13); AME close together, slightly separated from each other by 1/3 of their diameter, from ALE by slightly less than AME diameter; PME separated from each other by about their diameter, from PLE by almost 1.5 times PME diameter (AME–AME 0.03, AME–ALE 0.08, PME–PME 0.13, PME–PLE 0.18). Palpal RTA long, bent dorsally, with sharply pointed distal end; dorsal tibial apophysis (DTA) large, toothed proximally and distally; intermediate tibial apophysis (ITA) small, arising between the RTA and DTA; conductor reduced to a slender, hyaline apophysis, the latter arising from the distal end of the well-developed, strongly branched, beak-shaped conductor apophyses (C1, C2, C3); median apophysis long, with slender apex and broad base, the latter with a long, slender apophysis; tegular sclerite extending anteriorly and forming a broad apophysis on distal bulb, the latter covering most of embolus; embolus broad, only distal end visible from ventral view (Figs. 33–35).



Figures 33–37.—*Taira zhui* new species, male holotype and female paratype from Jinyunshan, Beipei, Chongqing, China, drawings. 33. Palp, prolateral view. 34. Palp, ventral view. 35. Palp, retrolateral view. 36. Epigynum, ventral view (arrow points to copulatory opening). 37. Epigynum, dorsal view.

Female (paratype): Medium-sized spider, total length 6.50 (Fig. 39). Carapace 2.60 long, 1.80 wide; opisthosoma 4.00 long, 3.00 wide. AME smallest, 2/3 size of ALE, ALE largest; posterior eyes subequal in size (AME 0.10, ALE 0.15, PME 0.13, PLE 0.13); AME close together, slightly separated from each other by 1/3 of their diameter, from ALE by slightly more than AME diameter; PME separated from each other by more than their diameter, from PLE by almost 2 times PME diameter (AME–AME 0.03, AME–ALE 0.13, PME–PME 0.18, PME–PLE 0.23, AME–PME 0.15. Epigynum with a triangular plate anterior of median lobe; median lobe two times wider than long; epigynal teeth broad, separated from median lobe by approximately their width; copulatory ducts small but distinct, arising medially and extending laterally to spermathecae; spermathecae long, kidney-shaped, widely separated from each other anteriorly by at least their width and posteriorly by at least two times their width; spermathecal bases with distinct folders looping around; fertilization ducts long, slightly longer than half the length of spermathecae (Figs. 36, 37).

Distribution.—China (Sichuan) (Fig. 42).

ACKNOWLEDGMENTS

The first author thanks Charles E. Griswold (CAS, San Francisco) and Norman I. Platnick (American Museum of Natural History, New York) for their advice and comments

Figures 38, 39.—*Taira zhui* new species, male holotype (38) and female paratype (39) from Jinyunshan, Beipei, Chongqing, China, photos, habitus, dorsal view.

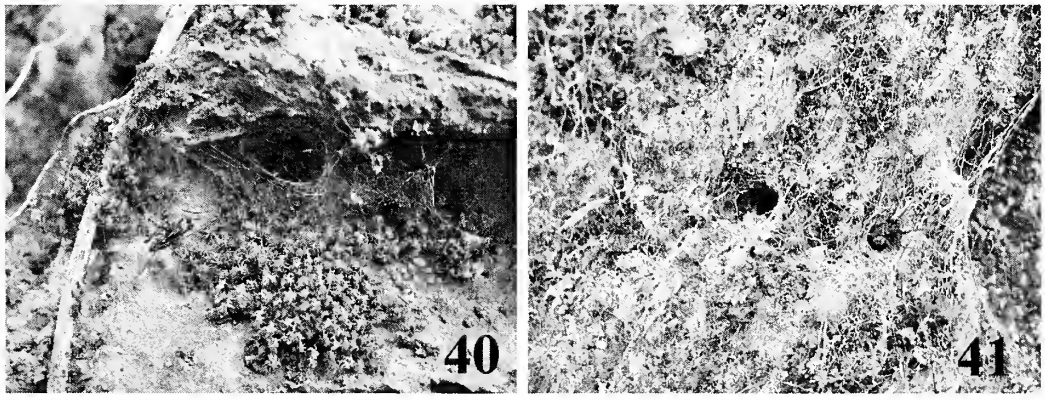


Figure 40, 41.—Habitat and web of *Taira*. 40. Male *T. sichuanensis* new species from Changning, Sichuan, China on the female web. 41. Female *T. obtusa* Zhang, Zhu & Song 2008 from Shennongjia, Hubei, China on the entrance of the web hole.

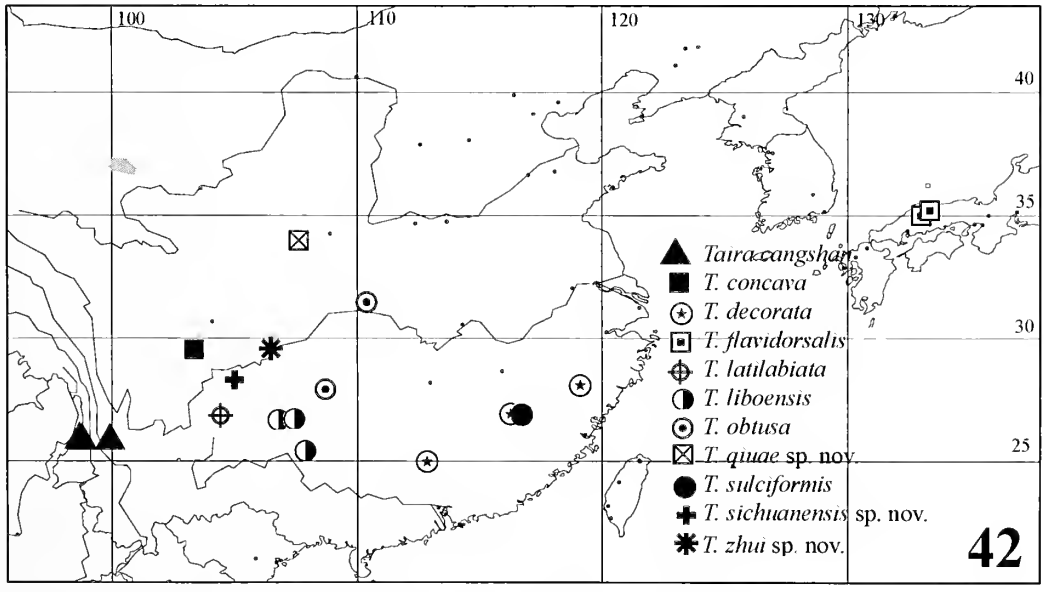


Figure 42.—Records of *Taira* speeies in China and Japan.

on the paper. Charles E. Griswold loaned the Yunnan Gaoligongshan material studied, which are based upon work supported by the China Natural History Project of the California Academy of Sciences and the US National Science Foundation grant DEB 0103795 (Peter Fritsch, Principal Investigator; David Kavanaugh, Nina Jablonski, and the late Joe Slowinski, co-Principal Investigators). Jeremy Miller (CAS) provided *T. cangshan* habitus photos and Ming-Sheng Zhu (MHBU) loaned the type specimens and gave permission to use the SEM photos taken in his laboratory. The expedition of P. Jäger to Shaanxi, China in 1997 was supported financially by the Stifterverband für die Deutsche Wissenschaft, Essen, Germany. Zhi-Sheng Zhang's work was supported by the Ministry of Science and Technology of the People's Republic of China (MOST grant nos. 2006FY120100 and 2005DKA21404), the Chinese post-doctoral Science Fund (20080431265), the Natural Science Foundation Project of Chongqing (CSTC 2008BB7088) and the post-doctoral program of Southwest University of China. We particularly thank the following individuals for reading and providing critical comments to the manuscript: Shu-Qiang Li (IZCAS, Beijing), Xiang Xu (HNU, Changsha), and Ming-Sheng Zhu (Hebei University, Baoding).

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A new species and new records of *Hentzia* (Araneae: Salticidae: Dendryphantinae) from the United States

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Abstract. *Hentzia alamosa* (Salticidae) is described from Big Bend Ranch State Park in Presidio County, Texas, and a closely related species, *Hentzia fimbriata* (F.O. Pickard-Cambridge 1901) is recorded for the first time from the USA. Both species are also closely related to *Hentzia palmarum* (Hentz 1832), the type species for the genus. New locality records provided by the Texas A & M University Insect Collection (TAMUIC) for *H. palmarum* are also included.

Keywords: Jumping spiders, Texas, Arizona

The genus *Hentzia* is primarily circum-Caribbean-Gulf of Mexico in nature, eentered in Cuba, where seven of the 20 known species have been collected (Richman 1989). However, the genus has several outlier species in western North Ameriea. One species, *Hentzia pima* Richman 1989, was described from the Baboquivari Mountains of Arizona, *Hentzia fimbriata* (F.O. Piekard-Cambridge 1901) reaehes the west coast of Mexico in Nayarit, and *H. poenitens* (Chamberlin 1924) is known only from the Gulf Coast of Sonora (Richman 1989). *Hentzia palmarum* (Hentz 1832) and *H. mitrata* (Hentz 1846) are both known from eastern Texas, with *H. palmarum* reaching western Texas in the panhandle (Richman 1989). Since the revision of the genus (Riehman 1989), specimens have been collected in southern Arizona and southwestern Texas that have expanded our understanding of the distribution of the genus. In this paper I am bringing the genus up to date by describing a new species from the region of Big Bend in Presidio County, Texas, adding several records for the Mexican species *H. fimbriata* from Arizona, and also adding new records of *H. palmarum* from south-central Texas. All of these species are in the *palmarum* species group, with the males of the new species and *H. fimbriata* resembling this widespread eastern species.

METHODS

Methods used for this description were described by Richman (1989). All specimens examined for the description of the new speeies were measured using an Olympus® binocular dissecting microscope with a measuring reticle in a 20× eyepiece calibrated with a stage micrometer of 1 mm divided into hundredths. All measurements are in mm. Specimens of the new species are deposited in the colleetions of Texas A & M University, College Station, Texas; the Florida State Collection of Arthropods, Gainesville, Florida; and the Arthropod Museum at New Mexico State University, Las Cruees, New Mexico. Anatomical photographs were made with an Optronics Magnifer-SP® electronic camera attached to a Leica MZ 16® binocular microscope and Dell® laptop computer at the Biological Control Insectary, Department of Entomology, Plant Pathology and Weed Science, New Mexieo State University.

TAXONOMY

Family Salticidae Blackwell 1841

Genus *Hentzia* Marx 1883

Attus Walckenaer 1805 (applied to nearly all salticids – junior synonym of *Salticus* Latreille 1804)

Epiblemum Hentz 1832 (applied to *Salticus* as well as *Hentzia*)

Hentzia Marx 1883, type species *palmarum* (Hentz) 1832.

Wala Keyserling 1885, type species *palmarum* (Hentz) 1832. Synonomy: Bryant 1940.

Anoka Peckham and Perckham 1893, type species *vernalis* Peckham and Peckham 1893. Synonomy: Bryant 1940.

Parahentzia Bryant 1943, type species *mandibularis* Bryant 1943. Synonomy: Richman 1989.

Maeviobeata Caporiacco 1847, type species *charitonovi* Caporiacco 1947 (= *Anoka parallela* Peckham and Peckham 1894.) Synonomy: Richman 1989.

Type species.—*Epiblemum palmarum* Hentz 1832, original designation

Hentzia alamosa new species

Figs. 1–10, 17

Type material.—Female holotype, USA: *Texas*: Cuevas Amarillas, Big Bend Ranch State Park, Presidio County, Texas (29°29′41.5″N 104° 06′ 00″W, 1094.5 m), 28 March 2004, D.B. Richman, beating cottonwood along wash east of caves, deposited in the collection of Texas A & M Insect Collection (TAMUIC), College Station, Texas. Male and female paratypes: same data as holotype. Male paratype deposited in TAMUIC; female paratype deposited in the Florida State Collection of Arthropods, Gainesville, Florida.

Other material.—USA: *Texas*: 2 females, Ojito Adentro, Big Bend Ranch State Park, Presidio County (29°29′28.8″N, 104°03′42″W, 1162 m), 14 October 2000 (TAMUIC) and 27 March 2004 (Arthropod Museum, New Mexico State University - NMSU), D.B. Richman. Beating cottonwoods.

Distribution.—Known only from Big Bend area.

Etymology.—The name is taken from the Spanish alamo for cottonwood, the trees on which this species has so far been collected.

Diagnosis.—Males of this species would key out to *Hentzia palmarum* in Richman (1989), but the females have a very distinctive flattened to normal U-shaped atrium above the bell-like central structure (Figs. 4, 5, 9, 10). *Hentzia palmarum* has either two separate openings or these are connected as an upside-down, U-shaped depression (Figs. 11–15 and Richman (1989, figs. 24, 26).

Females so far collected, with the exception of one from Ojito Adentro (TAMUIC), which had three sets of distinct paired brown spots on the dorsum, lack a pattern on their abdomen except for a few tiny spots and occasionally vague

Figure 1.—Habitat of *Hentzia alamosa* new species near Cuevas Amarillas, Big Bend Ranch State Park, Presidio County, Texas. Adults were collected in the cottonwood trees in the background.

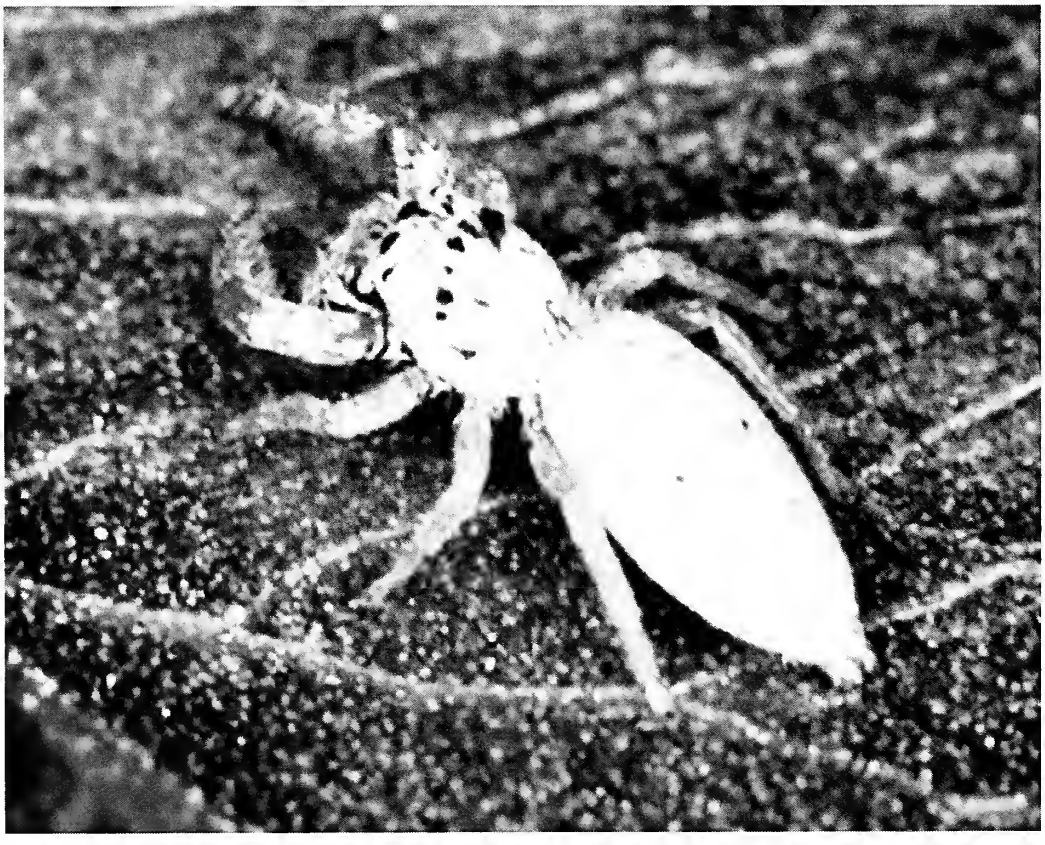


Figure 2.—Female holotype of *Hentzia alamosa* new species from Big Bend Ranch State Park, Presidio County, Texas. Note light legs and general lack of distinct markings.

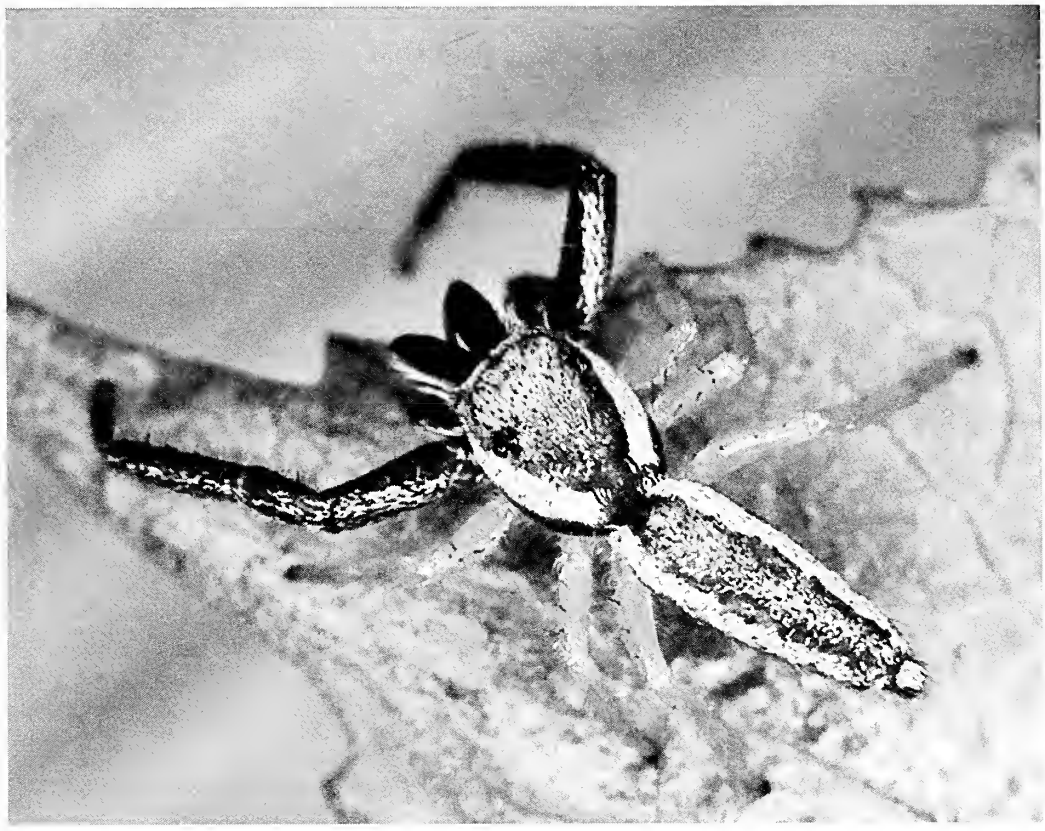


Figure 3.—Male allotype of *Hentzia alamosa* new species from Big Bend Ranch State Park, Presidio County, Texas. Note white band on dorsal abdomen anterior to spinnerets.

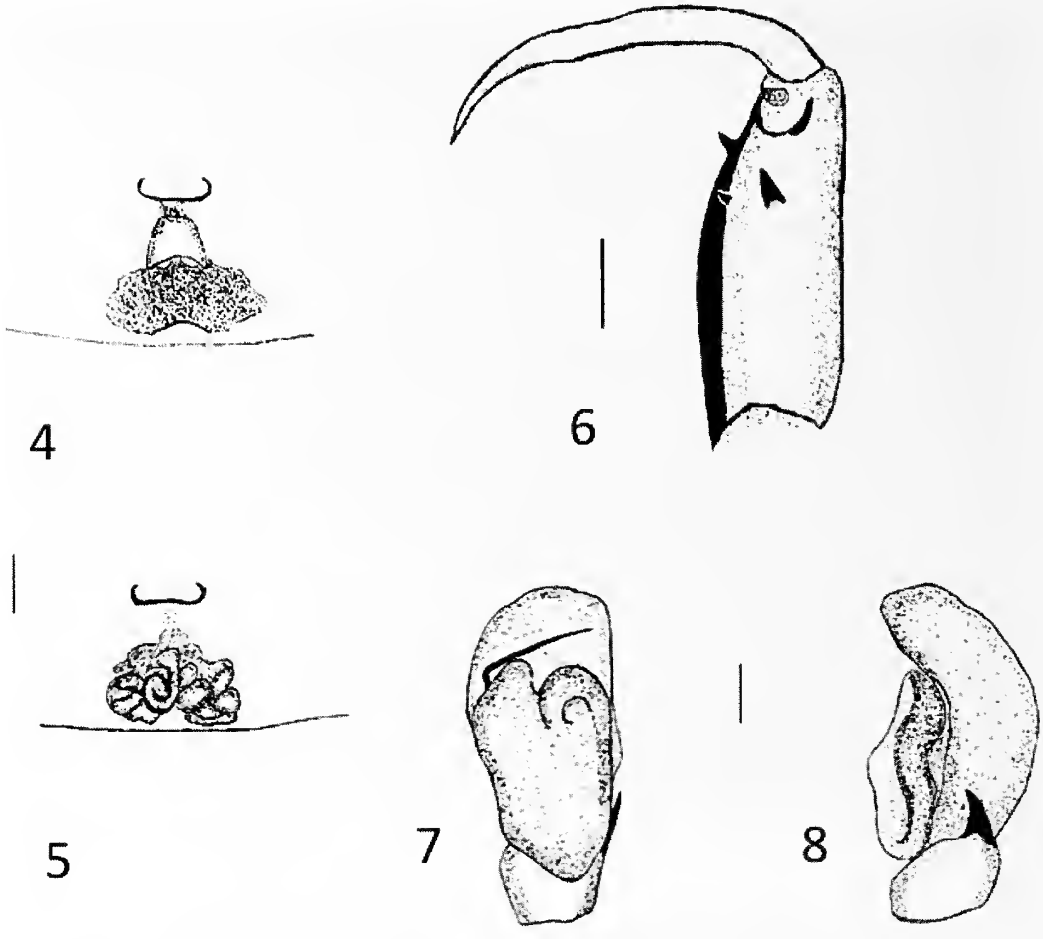
streaks, whereas most *H. palmarum* females have at least a faint, but distinct, set of blotches and chevrons (see Kaston 1978; Richman 1989). Females of *H. alamosa* also have all pale legs, whereas in *H. palmarum* females the front pair is darker than the rest. The male chelicerae (Figs. 6, 17) differ from those of *H. fimbriata*, in which the teeth are evenly spaced (Richman 1989, fig. 37), and more closely resembled those of *H. palmarum*. However, in *H. palmarum* the retromarginal tooth is usually slightly more proximal than the proximal promarginal tooth (Richman 1989, figs. 18, 19), while in *H. alamosa* the proximal promarginal and retromarginal teeth are almost exactly in line when viewed ventrally (Fig. 17). The one male collected also had a very light band on the tip of its abdomen, which has not been seen in *H. palmarum*. This is the first *Hentzia* reported from the Chihuhuan Desert, and the type locality is approximately 385 km southwest of the nearest known records for *Hentzia palmarum* in Edwards County, Texas.

Female.—Female holotype from Presidio County, Texas: Total length 4.2, carapace length 1.9, carapace width 1.6. Ventral spines on first tibiae 2-2-2. Leg formula 1423. Chelicerae with 2 promarginal teeth and one larger retromarginal tooth. Body almost unicolored yellowish, with two dark speckles (4–6 on paratype females) on the dorsum of the abdomen [very faint slanted bands laterally in paratype female from Cuavas Amarillas, and one female from Ojito Adentro had dark brown markings similar to those found on females of *Hentzia mitrata* (Hentz) (see Richman 1989, fig. 30)]. Chelicerae red-brown, endites lighter red-brown with pale distal portion. Sternum brown anteriorly, fading to yellow toward the posterior. Legs and palpi pale yellow.

Male.—Male allotype (paratype) from Presidio County, Texas. Total length 4.5, carapace length 2.0, carapace width 1.7. Leg formula 1423. General description close to *H. palmarum*, with 2 promarginal teeth and one larger retromarginal tooth, all acute and the latter almost exactly in line with the proximal promarginal tooth (Figs. 6, 17). Abdominal pattern distinctive, with light band (appearing as spot) at tip of abdomen. However, as only one male is known this may not be a diagnostic character.

Natural History.—This species seems to be closely associated with tall trees, especially, if not exclusively, cottonwoods (Fig. 1). Attempts to collect it on associated trees and shrubs along the wash at Cuevas Amarillas on the same date as the types failed, despite numerous attempts. Males are only known from March and females from March and October. Adults may be found (like *H. palmarum*) throughout the year.

Remarks.—An illustration by Kaston (1948, fig. 1814) bares some slight resemblance to the epigynum of this species, but resembles the epigynum of *H. fimbriata* even more closely. On the other hand illustrations of the epigynum of *H. palmarum* in Peckham & Peckham (1909, plate 42, fig. 1b) and in Chickering (1944, fig. 42), as well as unpublished drawings by Wayne Maddison (see Proszynski 2007), all agree with the illustrations of Richman (1989). It is not certain exactly what species Kaston was actually illustrating, since none of the specimens examined for the revision of the genus (Richman



Figures 4–8.—*Hentzia alamosa* new species. 4, 5. Female holotype epigynum; 4. Ventral view; 5. Dorsal view. 6–8 Male allotype; 6. Ventral chelicera; 7, 8. Left palp; 7. Ventral view; 8. Retrolateral view. Scales = 0.1 mm for female epigynum and male palp; 0.2 mm for male chelicera.

1989) appeared to match this drawing, which was presumably of a female from Connecticut.

Hentzia fimbriata (F.O. Pickard-Cambridge 1901)
Fig. 16

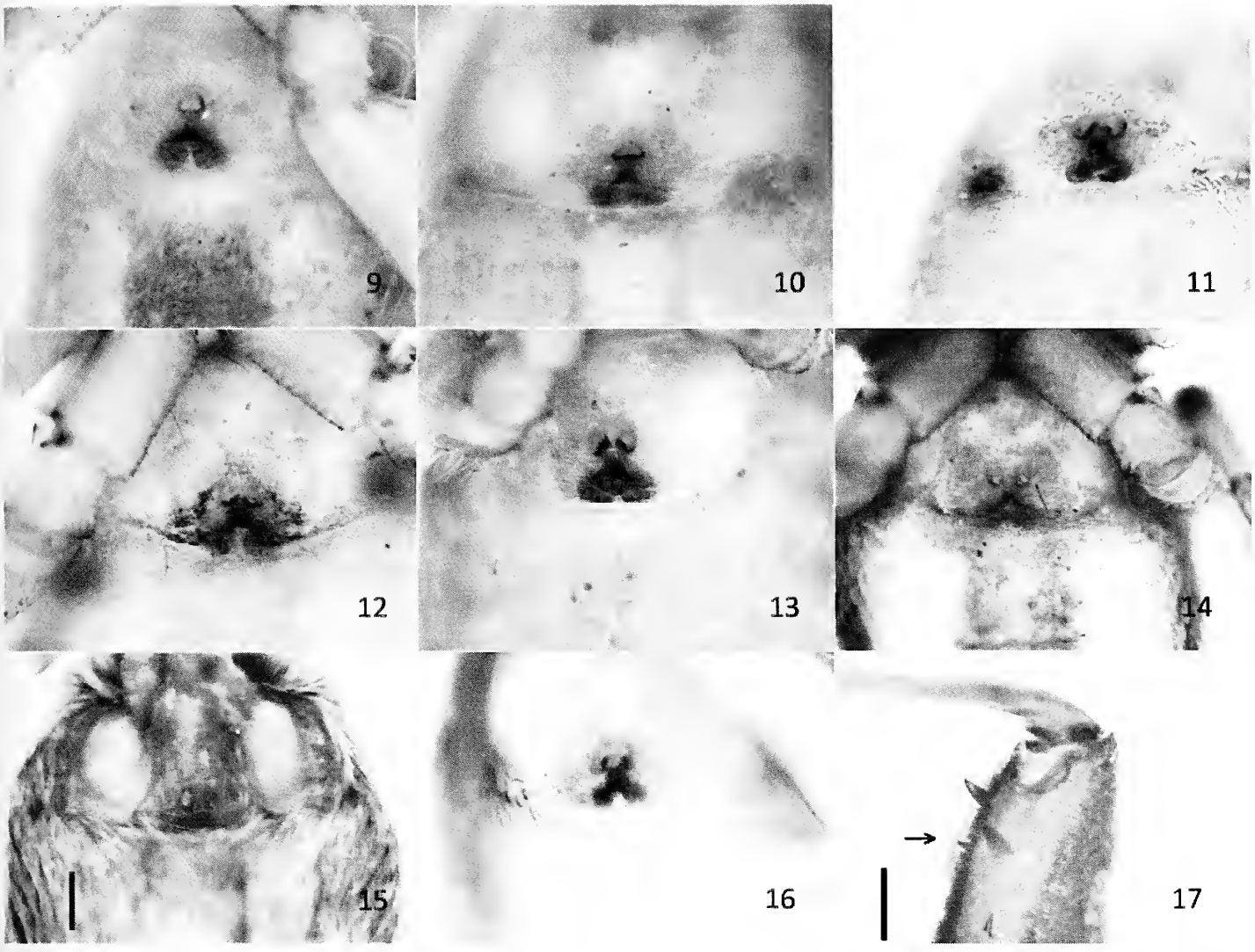
This species was adequately described by Richman (1989, pp. 306–307, figs. 37–43), but a few specimens have been collected in the United States, far north of the previously northernmost known record in Nayarit, Mexico. Based on the specimens examined it is possible that *H. pima* Richman 1989 is a junior synonym of *H. fimbriata*, as the photograph of the epigynum (Fig. 17) of a female collected from east of Sycamore Canyon resembles fig. 65 in Richman (1989), except for the openings, which in the Sycamore Canyon female are typical for *H. fimbriata*. The dorsal pattern of the abdomen of *H. pima* (Richman 1989, fig. 64) is very similar to that of the female from near Sycamore Canyon. The males collected in Sycamore Canyon and Florida Canyon closely match those of *H. fimbriata*. I suspect that *H. pima* is based on an slightly aberrant female, especially because of the relatively close geographical proximity of the type specimen from the Baboquivari Mountains. However, more material is needed either to verify *H. pima* as a separate species or to synonymize it with *H. fimbriata*.

New Records.—USA: *Arizona*: 1 female, Santa Cruz County, Coronado National Forest, 3–6 km east of Sycamore Canyon in Pajarito Mountains (ca 31°20′08″N, 111°08′46″W, ca 1372 m), 29 July 1999, D.B. Richman (beating oak) (NMSU AM 832); 1 male, Sycamore Canyon, Hank and Yank Springs (ca 31°25′39″N, 111°11′33″W), 18 August 1992, W. Maddison, G.B.Edwards & M. McMahon (92-045); 1 male, Pima Co.: Santa Rita Mountains, Florida Canyon, Florida Station (ca 31° 46′28″ N, 110 °52′04″W), 13 April 1991, W. Maddison (91-014); 1 female, Pima Co., Santa Rita Mountains, Florida Canyon, Florida Station (ca 31° 46′28″N, 110 °52′04″W), 3 March 1994, female (presumably *H. fimbriata*, not identified) collected by W. Maddison (94-008) (last three records all in W. Maddison collection, University of British Columbia, Vancouver, British Columbia, Canada).

Hentzia palmarum (Hentz 1831)
Figs. 11–15

In the process of comparing this species to specimens of *Hentzia alamosa* new species from Big Bend Ranch State Park, Presidio County, Texas, several new records were discovered. For a description of this species see Richman (1989, pp. 296–302, figs. 16–27).

New Records.—USA: *Texas*: 1 male, 4 females, Edwards County, near Rock Springs (30°01′29″N, 100°12′21″W), January 1994; 3 females, Mason County, near Mason (ca 30°44′56″N, 99°13′50″W), January 1994; 1 male, 5 females, Zavala County, near Nueces (ca 28°47′18″N, 99°49′09″W), January 1994. All



Figures 9–17.—*Hentzia* species. 9, 10. *Hentzia alamosa* new species, female paratype epigyna, ventral views; 9. From Cuevas Amarillas, Big Bend State Park, Presidio County, Texas; 10. From Ojito Adentro, Big Bend State Park, Presidio County, Texas. 11–15. *Hentzia palmarum* (Hentz) female epigyna, ventral views; 11. From Archbold Biological Station, Highland County, Florida; 12. From Madina County, Texas; 13. From Travis County, Texas; 14. From Zavala County, Texas; 15. From Edwards County, Texas. 16. *Hentzia fimbriata* female epigynum, ventral view, near Sycamore Canyon, Santa Cruz County, Arizona. 17. *Hentzia alamosa* new species male chelicerae, male allotype (paratype), from Cuevas Amarillas, Big Bend State Park, Presidio County, Texas. Note two acute promarginal teeth and one acute retromarginal tooth, similar to that of male *Hentzia palmarum*, but with the proximal teeth nearly in line. All epigynal photos to same scale; both bars = 0.2 mm.

specimens were taken from irrigation tubes in pecan orchards by J. W. Stewart. All specimens deposited in TAMUIC.

Discussion.—The scattered distribution of most *Hentzia* species in the western United States and Mexico, usually in riparian areas, suggests speciation events by the founder effect, with a few individuals being accidentally introduced (perhaps by storms) to isolated favorable habitats. The other possibility may be isolation of populations of more widely spread species (such as *H. palmarum* or *H. fimbriata*) in refugia because of desert expansion, followed by subsequent speciation events. The presence of *Hentzia fimbriata* in Sycamore Canyon is an exception to the isolated populations in other parts of the southwesten USA and northwestern Mexico, as this species is widespread in Mexico. It is likely that its distribution follows the Sierra Madre and the watersheds of Mexican rivers draining into the Gulf of California. Sycamore Canyon, which drains into the Rio Altar in the Rio de la Concepcion drainage in Sonora, and where the U.S. specimens of *H. fimbriata* have been collected, is unusual in the variety of jumping spiders found there that are primarily associated with other geographical ranges. These include *Zygoballus rufipes* Peckham & Peckham 1885 (Mexico and eastern United States), *Phidippus tux* Pinter 1970 (central Mexico), and *Sarinda hentzi* (Peckham & Peckham 1892) (eastern United States) (all collected by the author). Because of the similar morphological features, future research on the genus *Hentzia* should include mitochondrial DNA analysis of as many species as possible in order to clear up the actual phylogenetic source and relationships of these species, especially in the *palmarum* species group. This is unfortunately beyond the scope of this paper and would require collection of fresh material from Cuba and Mexico, as well as the USA, even if limited to the *palmarum* group.

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A review of the coelotine genus *Eurocoelotes* (Araneae: Amaurobiidae)

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Abstract. The genus *Eurocoelotes*, established in 2002 with fourteen species from Europe, including two new species, *E. halanensis* sp. nov. (♀ only) from Mali Halan, Croatia and *E. paramicrolepidus* sp. nov. (♂ only) from Peloponnisos, Greece, is reviewed. Each species is described with a focus on the male palp and the female epigynum. A key to species is provided. Except for *E. deltshevi* (Dinitrov 1996) and *E. drenskii* (Deltshev 1990), specimens of which were not available, we have provided illustrations for the male palp and the female epigynum of all species. In general, the male *Eurocoelotes* has a short cymbial furrow, a broad conductor dorsal apophysis, a spoon-shaped median apophysis, and a prolaterally originating embolus, but lacks a patellar apophysis. The female *Eurocoelotes* usually has laterally arising epigynal teeth, a large, anteriorly situated atrium, large copulatory ducts, and short, slightly longitudinally extending spermathecae. Exceptions include: *E. falciger* (Kulczyński 1897), which has a long cymbial furrow and a proximally originating embolus, *E. anoplus* (Kulczyński 1897) and *E. gasperinii* (Simon 1891), which have proximally originating emboli, *E. brevispinus* (Deltshev & Dimitrov 1996), which has a distinct patellar apophysis, *E. microlepidus* (de Blauwe 1973) and *E. paramicrolepidus*, which have a tiny patellar apophysis, and *E. xinpingwangi* Deltshev 2009, which has no epigynal teeth.

Keywords: Spider, Europe, new species

Europe is home to at least four genera of the spider subfamily Coelotinae F.O. Pickard-Cambridge 1898: *Coelotes* Blackwall 1841, *Eurocoelotes* Wang 2002, *Pireneitega* Kishida 1955 and *Urocoras* Ovtchinnikov 1999. Twelve species are currently included in *Eurocoelotes* (Platnick 2009; Wang 2009). The type species *Eurocoelotes inermis* (L. Koch 1855) was first described as a member of *Amaurobius*. Later, L. Koch (1868) transferred it to *Coelotes*, where it remained until transferred, although it was also occasionally referred to as *Amaurobius* (Miller 1971). *E. inermis* is the most widely distributed *Eurocoelotes*. Researchers have examined specimens from France, Switzerland, Poland, Germany, Austria, former Yugoslavia, and Bulgaria, while other species have been restricted to limited areas of southeastern Europe from Italy, former Yugoslavia to Bulgaria. Major studies of *Eurocoelotes* have been carried out by Simon (1891, in Gasperini 1891), Kulczyński (1897, in Chyzer & Kulczyński 1897 1906), Drensky (1915, 1942), de Blauwe (1973), Deltshev (1990, 2009), Dimitrov & Deltshev (1996), Dimitrov (1996) and Wang (2002). Simon (1891, in Gasperini 1891) described a unique speeies from Croatia, *E. gasperinii* (Simon, 1891), that has distinct, short macrosetae distally on the femur (Fig. 22). In addition to a redescription of *E. inermis*, Kulczyński (1897, in Chyzer & Kulczyński 1897, 1906) described three more species, *E. anoplus* (Kulczyński 1897), *E. falciger* (Kulczyński 1897), and *E. karlinskii* (Kulczyński 1906). de Blauwe (1973) treated six *Eurocoelotes* species, including a new species, from the Mediterranean region, [i.e., *E. anoplus*, *E. gasperinii*, *E. inermis*, *E. karlinskii*, and *E. microlepidus* (de Blauwe 1973)]. Another species, *Coelotes longimanus* de Blauwe 1973, was shown to be a junior synonym of *E. anoplus* by Brignoli (1977b). Drensky (1915, 1942) and Deltshev (1990) worked on seven species from Bulgaria, [i.e., *E. drenskii* Deltshev 1990, *E. falciger*, *E. inermis*, *E. jurinitschi* (Drensky 1915), *E. karlinskii*, *E. kulczynskii* (Drensky 1915), and *E. microlepidus*.] Detailed vulva structures were not described until Deltshev (1990) illustrated the vulva of some species. Deltshev (1990) also collected and described both sexes of four species from Bulgaria. More work was done in recent years with the description of three new species: *E. brevispinus* (Deltshev & Dimitrov 1996), *E. deltshevi* (Dinitrov 1996), and *E. xinpingwangi* Deltshev 2009.

In this study, all of the species are revised, with a particular focus on the description of their genitalic structures. Two new species, *E. halanensis* sp. nov. from Croatia and *E. paramicrolepidus* sp. nov. from Greece, are described. We have provided illustrations of the male palp and female epigynum for all the described species, except *E. deltshevi* and *E. drenskii*, because specimens are not available. Descriptions focus on genitalia. The phylogenetic relationships of *Eurocoelotes* species were not analyzed, but will be done in the near future in an analysis that will include all coelotine speeies.

METHODS

Measurements are in mm. Scale lines are 0.2 mm long. Eye diameters are taken at the widest point. The total body length does not include the length of the chelicerae or spinnerets. The species descriptions focus only on the male palp and female epigynum. Elevations are in m above msl. Due to the limitation of available specimens from this region, this study is based mainly on the examination of type specimens, which were loaned from the following museums: AMNH–American Museum of Natural History, New York, USA (N.I. Platnick); AMNH-CU–Cornell University Collection loaned to the AMNH (N.I. Platnick); CAS–California Academy of Sciences, San Francisco, USA (C.E. Griswold); COLL. DELTSHEV–Collection of C.D. Deltshev (C.D. Deltshev); COLL. UBICK–Collection of D. Ubick (D. Ubick); HEC–Hope Entomological Collections, Oxford, UK (M. Akinson); IZS–Institute of Zoology, Sofia, Bulgaria (C.D. Deltshev); MCB–Museo de Bergamo, Bergamo, Italy (P. Pantini); MCV–Musée Civique d'Histoire Naturelle de Verone, Verona, Italy (R.

Salmaso); MCZ–Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA (L. Leibensperger); MNHN–Musée National d'Histoire Naturelle, Paris, France (C. Rollard); NHMB–Naturhistorisches Museum Basel, Basel, Switzerland (A. Hänggi); SMF–Senckenberg Museum, Frankfurt, Germany (M. Grasshoff, J. Martens, P. Jäger); SMNH–Swedish Museum of Natural History, Stockholm, Sweden (T. Kronestedt); USNM–National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (J. Coddington); ZMB–Museum fur Naturkunde, Zentralinstitut der Humboldt-Universitut zu Berlin, Berlin, Germany (J. Dunlop and Sh. Nawai).

Abbreviations used in the text are: *Eyes:* AME–anterior median eyes; ALE–anterior lateral eyes; PLE–posterior lateral eyes; PME–posterior median eyes. *Epigynum:* A–atrium; CD–copulatory duct; EH–epigynal hood; ET–epigynal tooth; FD–fertilization duct; S–spermathecae; SB–spermathecal base; SS–spermathecal stalk; SH–spermathecal head. *Palp:* C–conductor; CDA–conductor dorsal apophysis; CL–conductor basal lamella; CF–cymbial furrow; E–embolus; EB–embolic base; LTA–Lateral tibial apophysis; MA–median apophysis; PA–patellar apophysis; RTA–retrolateral tibial apophysis; ST–subtegulum; T–tegulum; TS–tegular sclerite. Elevations are in m above msl.

SYSTEMATICS

Family Amaurobiidae Thorell 1870
Subfamily Coelotinae F.O. Pickard-Cambridge 1898
Genus *Eurocoelotes* Wang

Eurocoelotes Wang 2002:73.

Type species.—Type species *Amaurobius inermis* L. Koch 1855.

Diagnosis.—The genus *Eurocoelotes* resembles *Coelotes* in having a conductor dorsal apophysis, round spoon-shaped median apophysis, laterally arising epigynal teeth, and slightly longitudinally elongated spermathecae. Both genera have three promarginal and three retromarginal cheliceral teeth. But they differ as follows: 1) *Coelotes* has a large, broad patellar apophysis, which is as long as, or at least half of the patellar length. The patellar apophysis of *Eurocoelotes* is usually absent, but may be small, much less than half of the patellar length (e.g., *E. brevispinus* and *E. drenskii*) (Fig. 10) or tiny (*E. microlepidus* and *E. paramicrolepidus* sp. nov.) (Figs. 52, 55); 2) *Eurocoelotes* has a large, anteriorly situated atrium and large copulatory ducts (Figs. 25, 26), while *Coelotes* has a reduced, slit-shaped atrium and small copulatory ducts. *Eurocoelotes* also resembles *Coelotes* in having similar eyes and RTA. ALE largest, PME and PLE subequal in size, slightly smaller than ALE, AME slightly smaller than posterior eyes, but *E. inermis*, *E. jurinitschii*, *E. karlinskii* and *E. kulczynskii* with much smaller AME (Fig. 58). Similar to *Coelotes*, RTA in *Eurocoelotes* extends more than half of the tibial length (Figs. 5, 28).

Description.—See descriptions of type species by L. Koch (1855), Kulczyński (1906), Drensky (1942), de Blauwe (1973), Deltshev (1990), and Wang (2002).

Relationships.—Remain unresolved with *Coelotes* and two other lineages (Wang 2002).

Distribution.—Europe (Fig. 59).

Composition.—Fourteen species, including two new species described in this study.

KEY TO SPECIES OF THE GENUS *EUROCOELOTES*

1. Male 2
   Female 14
2. Patellar apophysis present (Figs. 10, 52, 55) 3
   Patellar apophysis absent (Fig. 5) 6
3. Patellar apophysis relatively large, distinctly extending beyond distal patella (Figs. 9, 10) ..... *brevispinus*
   Patellar apophysis relatively small, not extending beyond distal patella (Figs. 52, 55) 4
4. Conductor broad distally, abruptly curved ..... *drenskii*
   Conductor slender distally, smoothly curved (Figs. 50–55) 5
5. RTA strongly extending distally; conductor slightly coiled distally; median apophysis broad, with retrolateral margin longer than prolateral margin; embolic base smooth, not notched (Figs. 50–52) ..... *microlepidus*
   RTA slightly extending distally; conductor distinctly coiled distally, shaped like a semi-circle; median apophysis small, with subequal retrolateral and prolateral margins; embolic base with slightly notched retrolateral margin (Figs. 53–55) ..... *paramicrolepidus*
6. Embolus prolateral in origin (Figs. 27, 36, 40, 46) 7
   Embolus proximal or retrolateral in origin (Figs. 4, 14, 18) 12
7. Median apophysis large, with length of retrolateral margin more than twice the prolateral margin (Figs. 27, 36, 40) 8
   Median apophysis small, with length of retrolateral margin less than twice the prolateral margin (Fig. 46) 10
8. Conductor with subdistal, prolaterally directed tooth (Figs. 27, 29) ..... *inermis*
   Conductor without subdistal tooth (Figs. 36, 41) 9
9. Lateral tibial apophysis broad, wider than long; conductor slightly notched distally (Figs. 35–37) ..... *jurinitschi*
   Lateral tibial apophysis small, subequal in width and length; conductor not notched distally (Figs. 40–42) ..... *karlinskii*
10. Conductor slender ..... *xinpingwangi*
    Conductor broad (Fig. 46) 11
11. Lateral tibial apophysis small, not bifurcate (Fig. 47) ..... *kulczynskii*
    Lateral tibial apophysis broad, slightly bifurcate ..... *deltshevi*
12. Palpal femur with several short, distal macrosetae and one long, strong median spine on dorsal side (Fig. 22) ..... *gasperinii*
    Palpal femur without macrosetae 13

13. Embolus retrolateral in origin, extremely long, extending posteriorly to proximal tibia, anteriorly coiling beyond distal bulb; conductor long (Figs. 13–15) ..... *falciger*
    Embolus proximal in origin, moderately long, extending posteriorly to distal tibia, anteriorly not coiling beyond distal bulb; conductor short (Figs. 3–5) ..... *anoplus*
14. Epigynal teeth absent (Fig. 56) ..... *xinpingwangi*
    Epigynal teeth present (Figs. 1, 6, 11) 15
15. Epigynal teeth arising between the atrium and epigastric furrow (Figs. 1, 11, 23, 25) 16
    Epigynal teeth arising from lateral atrium, or slightly posterior atrium (Figs. 6, 16, 33, 38, 43, 48) 19
16. Atria distinctly separated; copulatory ducts distinctly separated (Figs. 23, 24) ..... *halanensis*
    Atrium with single opening; copulatory ducts connected with each other (Figs. 1, 11, 25) 17
17. Copulatory ducts extending laterally, then converging medially (Fig. 12) ..... *falciger*
    Copulatory ducts extending medially between spermathecae (Figs. 2, 26) 18
18. Spermathecae anteriorly converging, close together (Fig. 2) ..... *anoplus*
    Spermathecae anteriorly diverging, widely separated (Figs. 26, 32) ..... *inermis*
19. Copulatory ducts originating anteriorly, extending laterally, converging medially, connecting to spermathecae laterally (Fig. 44) ..... *kulczynskii*
    Copulatory ducts originating anteriorly or medially, extending and connecting to spermathecae anteriorly or medially (Figs. 7, 17, 34, 39, 49) 20
20. Copulatory ducts originating anteriorly, extending posteriorly, connecting to the spermathecae anteriorly (Figs. 7, 34) 21
    Copulatory ducts originating medially, extending and connecting to the spermathecae medially (Figs. 17, 39, 49) 23
21. Epigynal teeth shorter than the atrial length (Fig. 6) ..... *brevispinus*
    Epigynal teeth subequal or longer than the atrial length (Fig. 33) 22
22. Epigynal teeth longer than the atrial length, extending posteriorly close to the epigastric furrow (Fig. 33) ..... *jurinitschi*
    Epigynal teeth subequal to the atrial length, extending posteriorly and separated from the epigastric furrow by about their length ..... *delshevi*
23. Spermathecae separated by at least three times their width (Fig. 49) ..... *microlepidus*
    Spermathecae separated by about their width (Figs. 17, 39) 24
24. Atrium subequal in length and width; epigynal teeth shorter than the atrial length; spermathecae anteriorly diverging (Figs. 16, 17) ..... *gasperinii*
    Atrium wider than long; epigynal teeth about the atrial width; spermathecae slightly anteriorly converging (Figs. 38, 39) *karlinskii*

EUROCOELOTES SPECIES DESCRIPTIONS

Eurocoelotes anoplus (Kulczyński 1897)
(Figs. 1–5, 58)

Coelotes anoplus Kulczyński 1897 (in Chyzer & Kulczyński 1897):162, fig. 17 (female lectotype from Croatia, in MNHN, examined).

Amaurobius anoplus: Kulczyński 1906:468, figs. 5, 42.

Coelotes anoplus: Kolosvary 1938:63; Wiehle 1964:650, figs. 32a, 34–37 only.

Coelotes longimanus de Blauwe 1973:54, figs. 46–48 (male holotype from Castelnuovo, Istrie, Croatia, in MNHN, examined). (First synonymized by Brignoli 1977b:26).

Coelotes anoplus: Blauwe 1973:25, fig. 23; Brignoli 1977b:26; Polenec 1985:102, fig. 3.

Eurocoelotes anoplus: Wang 2002:75.

Material examined.—*Lectotype:* CROATIA: 1♀ (MNHN, 15271).

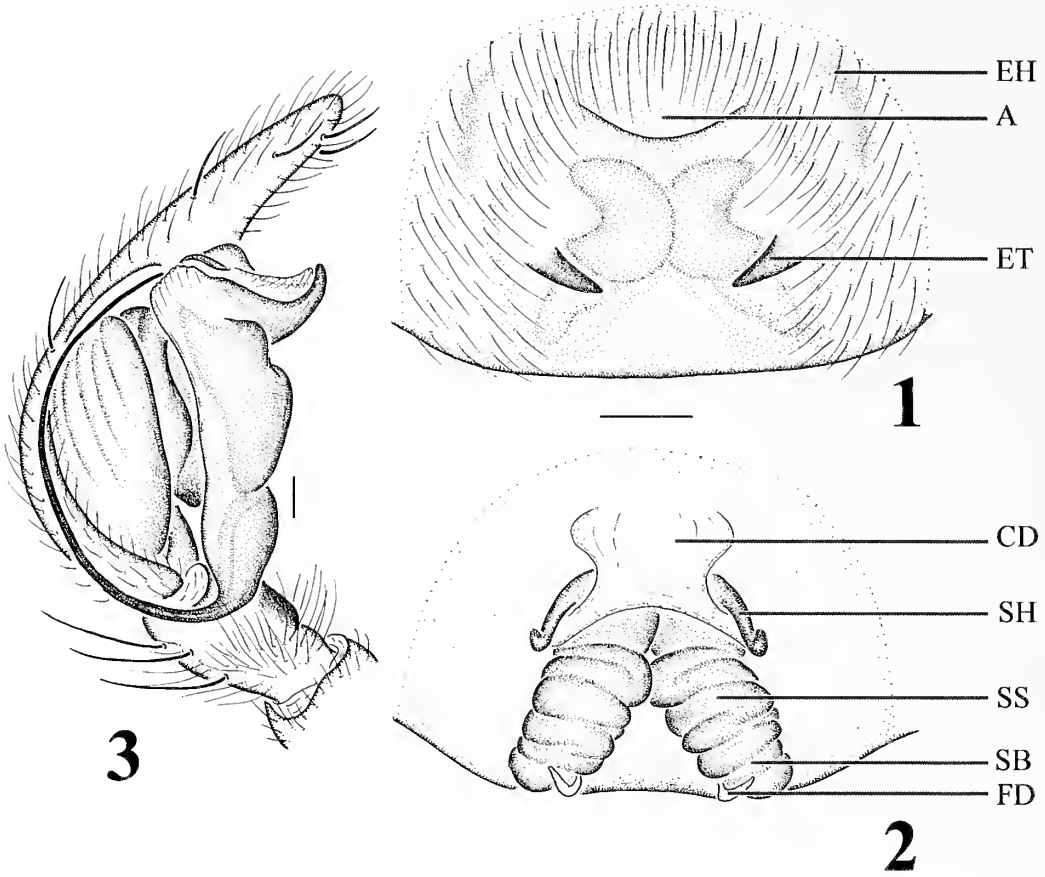
Other material examined: SLOVENIA: 1♀2♂ (H. Wiehle, SMF, 20632*l*1; 20633*l*2). CROATIA: Karlobag, P. so fra Karlobag e Gospić, 900 m, August 10, 1970, 3 females (B. Valle, MCB); Istria, N. slope Mt. Učka, 1100 m, June 23, 1962, 5♀ (H. L. Levi, MCZ); Istrie, Castelnuovo, ♂ holotype of *Coelotes longimanus* (E. Simon, MNHN, B 2011, 4.641). ITALY: Friuli-Venezia Giulia, Duino Aurisina, Collina di S. Pelagio, 140 m, September 14, 1963, 1♂ (Bianchi Valle, MCB); Friuli-Venezia Giulia, Prepotto, Santuario di Castelmonte, September 13, 1963, 1♀ (B. Valle, MCB); Veneto, Virrorio Veneto, Sella di Fadalto, September 4, 1964, 1♂ (B. Valle, MCB); Polcenigo, Pordenone, December 29, 1969, 1♀ (Zanetti, MCV).

Diagnosis.—The male of this species resembles *E. gasperinii* in having a similar conductor, proximally originating embolus, and broad median apophysis, but can be distinguished by the absence of numerous short macrosetae dorsodistally on the palpal femur. The female can be easily recognized by the epigynal teeth that arise between the atrium and epigastric furrow, the short, anteriorly originating copulatory ducts, and the spermathecae that are posteriorly widely separated, anteriorly converging and contiguous (Figs. 1–5).

Description.—See Kulczyński (1897 in Chyzer & Kulczyński), Kulczyński (1906), de Blauwe (1973).

Female: Epigynal teeth short, arising posteriorly between atrium and epigastric furrow, separated by approximately atrial width; atrium anteriorly originated, anterior and lateral margins indistinct, separated from epigastric furrow by 2–3 times its length; copulatory ducts small, anteriorly originating, slightly extending posteriorly; spermathecae broad, posteriorly widely separated by about their width, anteriorly extending and converging, contiguous; spermathecal heads long, slightly extending posteriorly and laterally (Figs. 1, 2).

Male: Patellar apophysis absent; RTA distinctly extended distally; lateral tibial apophysis broad; cymbial furrow slightly less than half of cymbial length; conductor short, with slightly curved apex, with broad dorsal apophysis, small basal lamella; median apophysis broad, spoon-shaped, retrolateral margin extending more than twice the length of prolateral margin; embolus long, filiform, proximal in origin, extending posteri-



Figures 1–3.—*Eurocoelotes anoplus* (Kulczyński). 1, 2. Female epigynum, ventral and dorsal view. 3. Male palp, prolateral view.

orly beyond tarsus/tibia junction to distal part of tibia, anteriorly not coiled beyond distal part of bulb (Figs. 3–5).

Distribution.—Former Yugoslavia, Italy, Austria.

Eurocoelotes brevispinus (Deltshev & Dimitrov 1996) (Figs. 6–10, 58)

Coelotes brevispinus Deltshev & Dimitrov 1996:77, figs. 1–4 (1 male and 1 female paratypes from Hambar dere, Slavyanka, Bulgaria, in IZS, examined).

Eurocoelotes brevispinus: Wang 2002:76.

Material examined.—*Paratypes:* BULGARIA: Slavyanka, Hambar dere, 1200 m, May 15, 1993, 1♂1♀ (Coll. Deltshev).

Diagnosis.—This species resembles *E. deltshevi* in having large, anteriorly situated copulatory ducts, broad lateral tibial apophysis, a similar conductor, and small median apophysis, but can be distinguished by the small atrium (length and width subequal), short epigynal teeth (shorter than atrial length) in female and the presence of a patellar apophysis in male (Figs. 6–10).

Description.—See Deltshev & Dimitrov (1996).

Female: Epigynal teeth short, arising from lateral atrium, widely separated by more than atrial width; atrium anteriorly situated, small, length and width subequal, separated from epigastric furrow by 1.5 to 2 times its length, with distinct lateral margins but indistinct anterior margin; copulatory ducts originating anteriorly, extending and diverging posteriorly into two distinct tubes; spermathecae broad, round, distinctly separated by about half of their width; spermathecal heads long, slender, medially originating, extending laterally (Figs. 6, 7).

Male: Patellar apophysis present, sharply pointed distally; RTA distinctly extended distally; lateral tibial apophysis broad; cymbial furrow about 1/3 of cymbial length; conductor short, broad, apex slightly curved, with a broad dorsal apophysis, a small basal lamella; median apophysis small, round, spoon-shaped, retrolateral margin approximately the same size as the prolateral margin; embolus short, filiform, prolateral in origin (Figs. 8–10).

Distribution.—Bulgaria.

Eurocoelotes deltshevi (Dimitrov 1996)

Coelotes sp.: Dimitrov 1993:74, fig. 1.

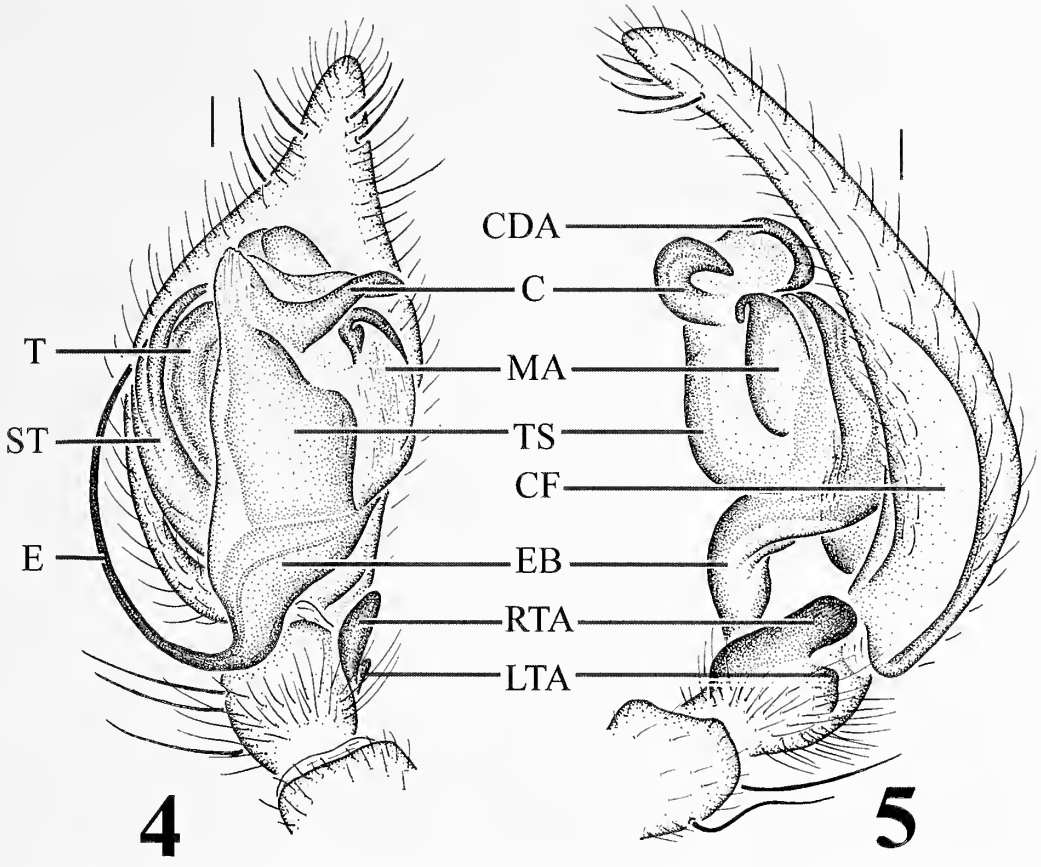
Coelotes deltshevi Dimitrov 1996:159, figs. 1–6 (male and female types from Bulgaria, deposited in IZS and Naturhistorisches Museum, Wien, not examined).

Eurocoelotes deltshevi Wang 2002:76.

Diagnosis.—This species resembles *E. brevispinus* in having large, anteriorly situated copulatory ducts, broad lateral tibial apophysis, a similar conductor, and small median apophysis, but can be distinguished by the broad atrium (wider than long), long epigynal teeth (at least as long as atrial length) in female and the absence of a patellar apophysis in male.

Description.—See Dimitrov (1996).

Female: Epigynal teeth as long as or longer than atrium length, arising from posterolateral atrium, separated by about atrial width; atrium large, anteriorly situated, wider than long, separated from epigastric furrow by at least its length;



Figures 4, 5.—*Eurocoelotes anoplus* (Kulczyński), male palp, ventral and retrolateral view.

copulatory ducts originating anteriorly, extending posteriorly, distinctly separated; spermathecae round, slightly extending anteriorly, distinctly separated; spermathecal heads medially originated.

Male: Patellar apophysis absent; RTA more than half of tibial length, slightly extending distally; lateral tibial apophysis broad, bifurcate; cymbial furrow slightly less than half of cymbial length; conductor short, broad, slightly extending anteriorly, with broad dorsal apophysis, small basal lamella; median apophysis small, round, spoon-shaped, retrolateral margin approximately the same size as prolateral margin; embolus short, filiform, prolateral in origin.

Distribution.—Bulgaria.

Eurocoelotes drenskii (Deltshev 1990)

Coelotes drenskii Deltshev, 1990:30, fig. 1 (male type from Bulgaria, in IZS, not examined).

Eurocoelotes drenskii: Wang 2002:76.

Diagnosis.—The male resembles *E. brevispinus* by having a patellar apophysis, a broad lateral tibial apophysis, and a small median apophysis, but can be distinguished by the blunt patellar apophysis and the abruptly curved conductor.

Description.—See Deltshev (1990).

Male: Patellar apophysis short, with blunt distal end; RTA slightly extending distally; lateral tibial apophysis broad; cymbial furrow about 1/3 of cymbial length; conductor broad, slightly extending anteriorly, abruptly curved distally, with broad dorsal apophysis, small basal lamella; median apophysis small, round, spoon-shaped, retrolateral margin approximately same size as prolateral margin; embolus short, filiform, prolateral in origin.

Female: Unknown.

Distribution.—Bulgaria.

Eurocoelotes falciger (Kulczyński 1897)
(Figs. 11–15, 58)

Coelotes falciger Kulczyński 1897 (in Chyzer & Kulczyński 1897):161, fig. 12 (types not examined).

Amaurobius falciger: Kulczyński 1906:467, figs. 8, 41.

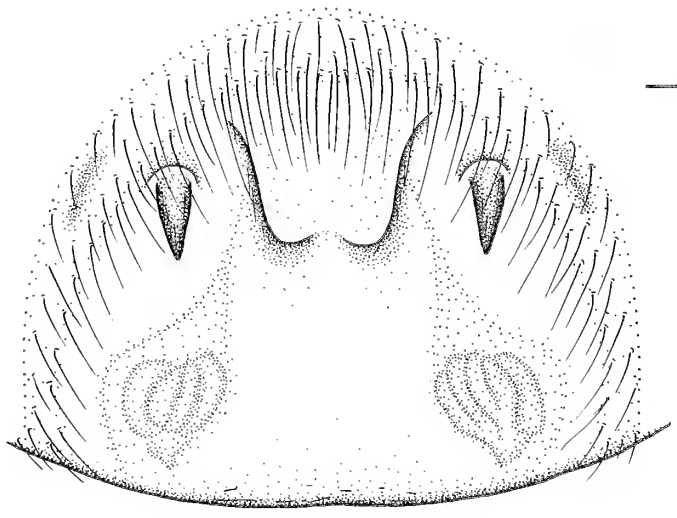
Coelotes intermedius Rosca 1935:250, figs. 9, 10 (first synonymized by Weiss and Andrei, 1989). Rosca 1937:205, fig. 11.

Coelotes falciger: Drensky 1942:43, figs. 5i, 6b; Brignoli 1977a:948, figs. 9–12; Weiss & Andrei 1989:338; Deltshev 1990:31, figs. 2.1–2.3.

Eurocoelotes falciger: Wang 2002:76.

Material examined.—BULGARIA: Varna, November 4, 1971, 1♂1♀ (Valle & Moretti, MCB); Black Sea, Albena, October 30, 1994, 2♂1♀ (V. Popov, Coll. Deltchev). GREECE: Cyrecie, Loannine, 1200–1500 m, passo ketere vers E., October 19, 1974, 2♀ (Vigue, MCV); Epiro, Katara (Loannina), 1600 m , September 30, 1966, 1♂ (P. Brignoli, MCV); Epiro, Malakasi (Trikkala), 1200 m, September 28, 1966, 1♀ (P. Brignoli, MCV). EUROPE: Label not readable, 4♀ (ZMB).

Diagnosis.—The male can be easily recognized from other *Eurocoelotes* by the cymbial furrow that extends more than 2/3 of cymbial length, the slender, long conductor, and the embolus that originates retrolaterally, extending posteriorly



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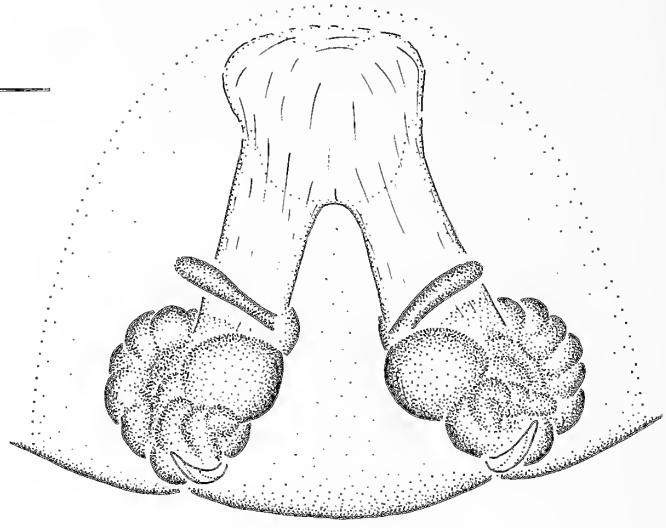

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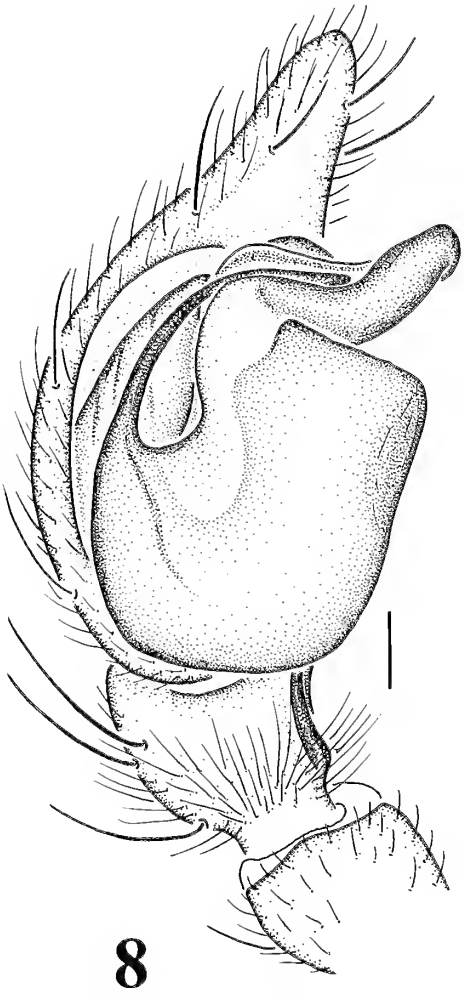

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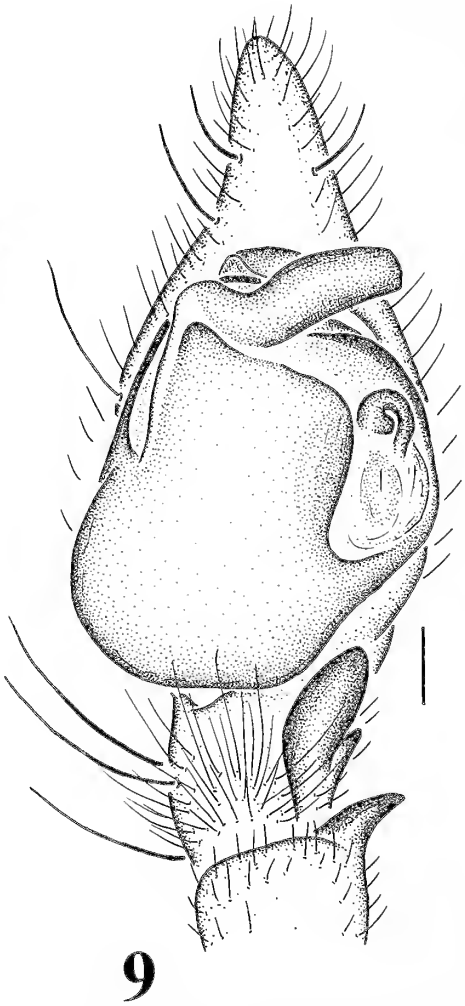

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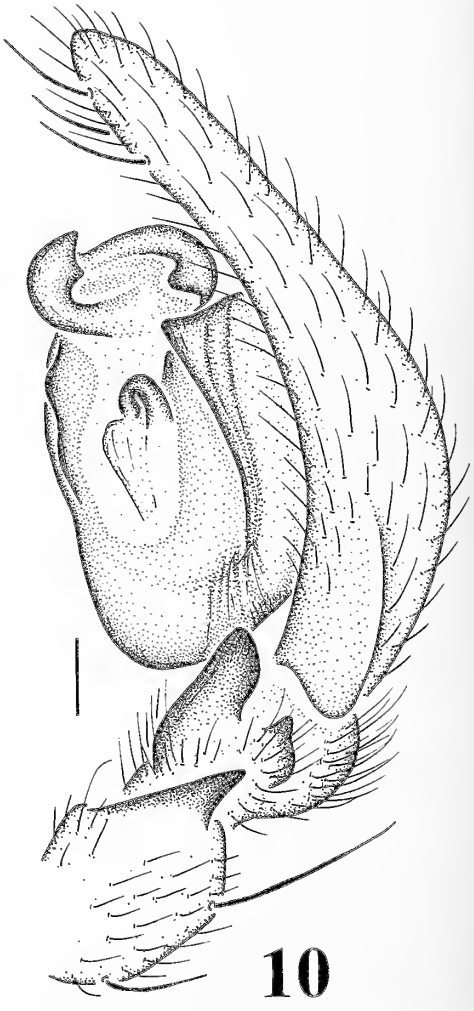

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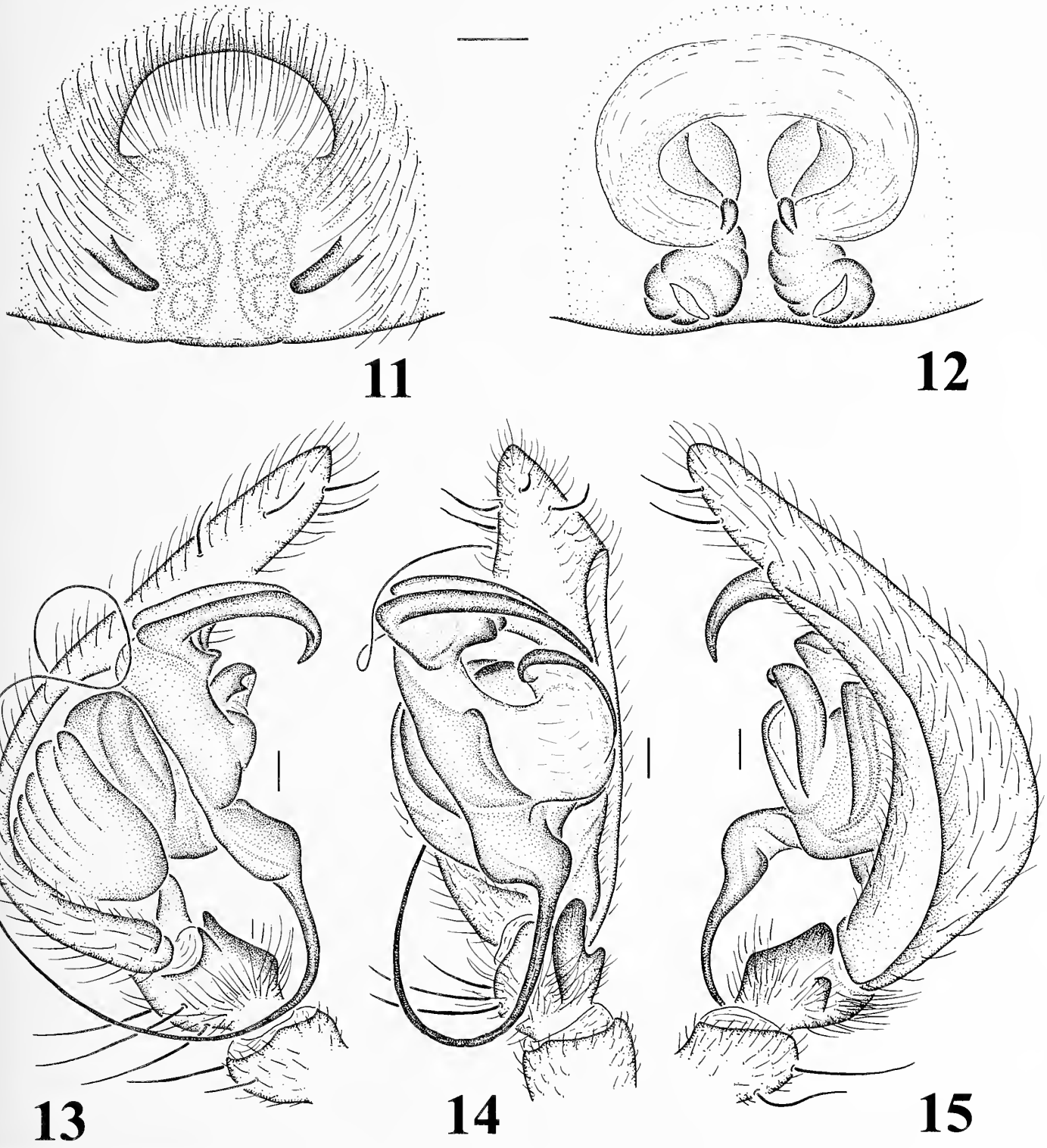
Figures 6–10.—*Eurocoelotes brevispinus* (Deltshev & Dimitrov). 6, 7. Female epigynum, ventral and dorsal view. 8–10. Male palp, prolateral, ventral and retrolateral view.

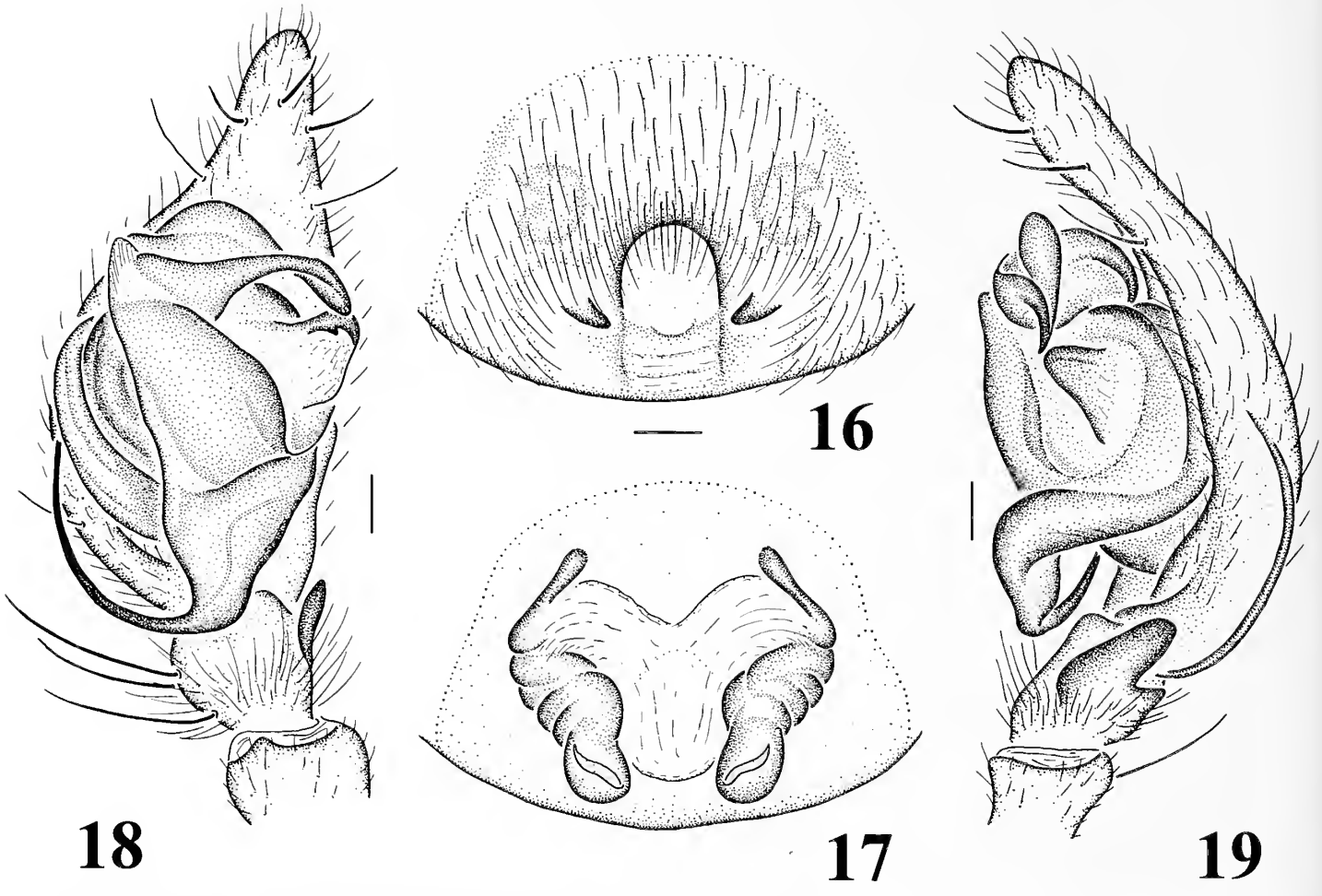


Figures 11–15.—*Eurocoelotes falciger* (Kulczyński). 11, 12. Female epigynum, ventral and dorsal view. 13–15. Male palp, prolateral, ventral and retrolateral view.

to proximal part of tibia and anteriorly coiled beyond distal part of bulb. The female can be easily recognized by the broad atrium (wider than long), the posteriorly arising epigynal teeth (between atrium and epigastric furrow), and the copulatory ducts that anteriorly originate, extending and connecting to spermathecae laterally (Figs. 11–15).

Description.—See Chyzer & Kulczyński (1897, 1906) and Deltshev (1990).

Female: Epigynal teeth short, arising between atrium and epigastric furrow, separated by approximately atrial width; atrium anteriorly originated, large, wider than long, separated from epigastric furrow by approximately 1.5 times its length;



Figures 16–19.—*Eurocoelotes gasperinii* (Simon). 16, 17. Female epigynum, ventral and dorsal view. 18, 19. Male palp, ventral and retrolateral view.

copulatory ducts large, originating anteriorly, extending laterally, converging and connecting to spermathecae laterally; spermathecae broad, round, slightly extending and converging anteriorly; seprmathecal heads small, arising distally (Figs. 11–12).

Male: Patellar apophysis absent; RTA slightly extending distally; lateral tibial apophysis small; cymbial furrow more than 2/3 of cymbial length; conductor long, slender, with a short dorsal apophysis, a small basal lamella; median apophysis broad, spoon-shaped, retrolateral margin at least twice the length of prolateral margin; embolus long, filiform, retrolateral in origin, extending posteriorly beyond tarsus/tibia junction to proximal part of tibia, anteriorly coiled beyond distal part of bulb (Figs. 13–15).

Distribution.—Bulgaria, Greece, Hungary, Romania, former Yugoslavia.

Eurocoelotes gasperinii (Simon 1891)
(Figs. 16–22, 58)

Coelotes gasperinii Simon 1891 (in Gasperini 1891):41 (male lectotype and female paralectotype from Dalmatia, Croatia, in MNHN, examined). Simon 1893:254, fig. 255.
Amaurobius gasperinii: Kulczyński 1906:462, figs. 7, 43, 62.
Coelotes gasperinii: de Blauwe 1973:35, figs. 30–33.
Eurocoelotes gasperinii: Wang 2002:76.

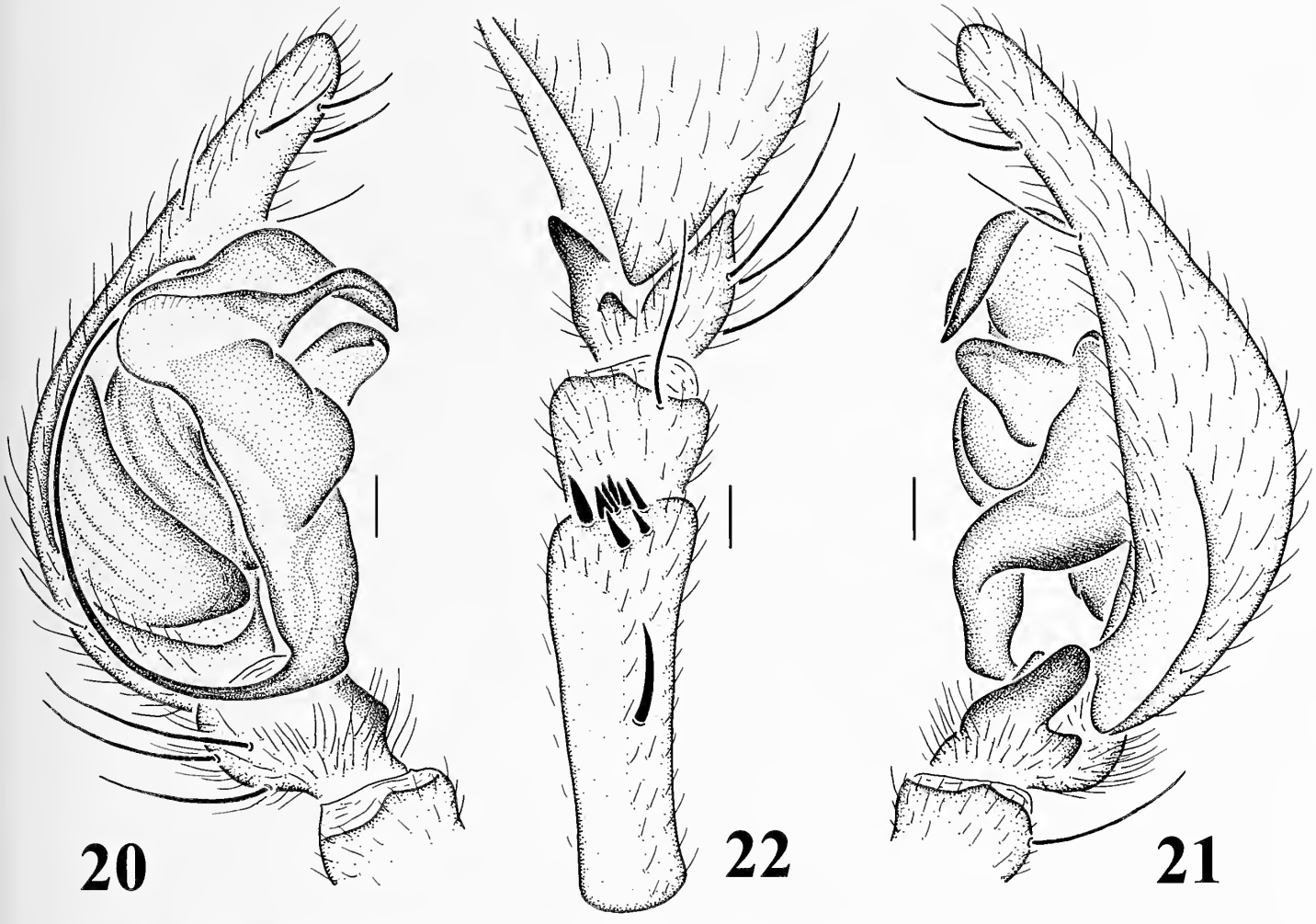
Material examined.—*Lectotype:* CROATIA: Dalmatia, ♂ lectotype and ♀ paralectotype (E. Simon, MNHN, Bocal 2.011, tube n 6341).

Other material examined: CROATIA: P. so Vagani, August 13, 1970, 1♂ (MCB); Dalmatia, Otok Šipan, Dubrava, June 22, 1974, 1♀ (D. Ljubić, COLL. UBICK).

Diagnosis.—The female resembles *E. inermis* by having medially extending copulatory ducts and the anteriorly diverging spermathecae, but can be distinguished by atrium situated at level posterior to epigynal hoods and separated from epigastric furrow by its length (in *E. inermis*, atrium situated at level of epigastric hoods and separated from epigastric furrow by at least 1.5 times its length) and copulatory ducts that extend between spermathecae (in *E. inermis* the copulatory ducts extend slightly anterior to spermathecae). Male resembles *E. anoplus* but can be distinguished by presence of approximately eight short macrosetae distally on palpal femur (Figs. 16–22).

Description.—See Simon (1891 in Gasperini 1891), Kulczyński (1906), de Blauwe (1973).

Female: Epigynal teeth short, situated slightly posteriorly of atrium, separated by slightly more than atrial width; atrium medially situated at level posterior to epigynal hoods, with subequal length and width, separated from epigastric furrow by approximately its length; copulatory ducts large, medially



Figures 20–22.—*Eurocoelotes gasperinii* (Simon). 20, 21. Male palp, prolateral and retrolateral view. 22. Male palp, dorsal view.

originating, extending medially between spermathecae; spermathecae with bases separated by their width, stalks extending anteriorly and diverging; spermathecal heads arising distally, extending slightly and converging anteriorly (Figs. 16, 17).

Male: Femur with approximately eight short macrosetae on dorsal side of distal femur and another long seta on dorsal side of middle femur; patellar apophysis absent; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow slightly less than half of cymbial length; conductor short, apex slightly curved, with broad dorsal apophysis, small basal lamella; median apophysis broad, spoon-shaped, retrolateral margin at least twice the length of prolateral margin; embolus long, filiform, proximal in origin, extending posteriorly beyond tarsus/tibia junction to distal part of tibia, anteriorly not coiled beyond distal part of bulb (Figs. 18–22).

Distribution.—Croatia.

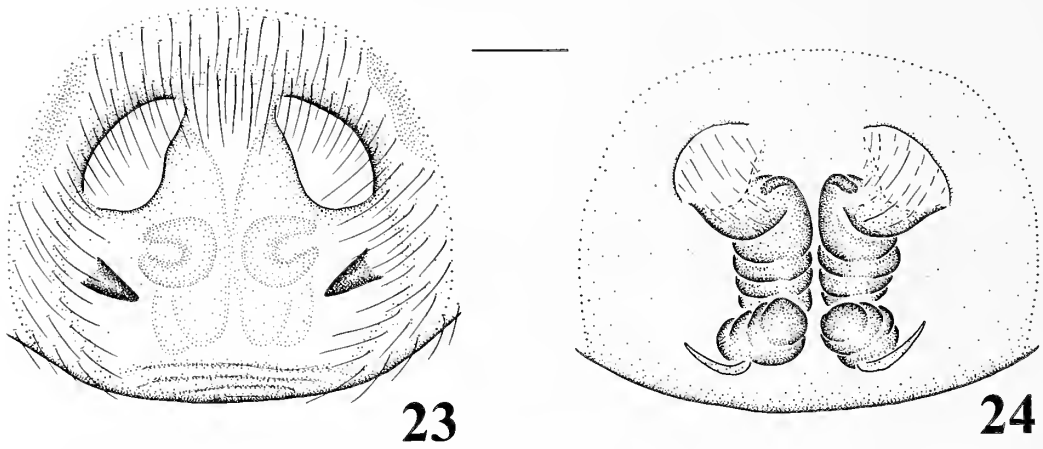
Eurocoelotes halanensis new species
(Figs. 23, 24, 58)

Material examined.—*Holotype:* CROATIA: ♀, Halan, Mali, July 2, 1970 (Valle, MCB).

Etymology.—The specific name refers to its type locality.

Diagnosis.—The female of this species can be easily recognized by the distinctly separated atrial openings and copulatory ducts, the posteriorly arising epigynal teeth, and the contiguous spermathecae (Figs. 23, 24).

Description.—*Female* (holotype): Large-sized coelotine. Total length 10.9. Carapace 5.50 long, 4.20 wide. Abdomen 5.38 long, 3.40 wide. AME and PME subequal in size, ALE largest, PLE slightly smaller than ALE (AME 0.18, ALE 0.25, PME 0.17, PLE 0.20) (Fig. 58); anterior eyes equally separated by approximately 2/3 of AME diameter, PME separated from each other by slightly more than their diameter, widely separated from PLE by twice PME diameter (AME–AME 0.12, AME–ALE 0.12, PME–PME 0.25, PME–PLE 0.34, AME–PME 0.20). Promargin of chelicera with three teeth, retromargin three. Epigynal teeth short, arising posteriorly between atrium and epigastric furrow, separated by about atrial width; atria anteriorly situated, with two distinct copulatory openings, separated from epigastric furrow by about 1.5 times its length; copulatory ducts relatively small, anteriorly originating, slightly extending posteriorly, distinctly separated; spermathecae closely set, with bases small, round, stalks extending anteriorly; spermathecal heads small, arising distally (Figs. 23, 24).



Figures 23, 24.—*Eurocoelotes halanensis* new species, female epigynum, ventral and dorsal view.

Male: Unknown.

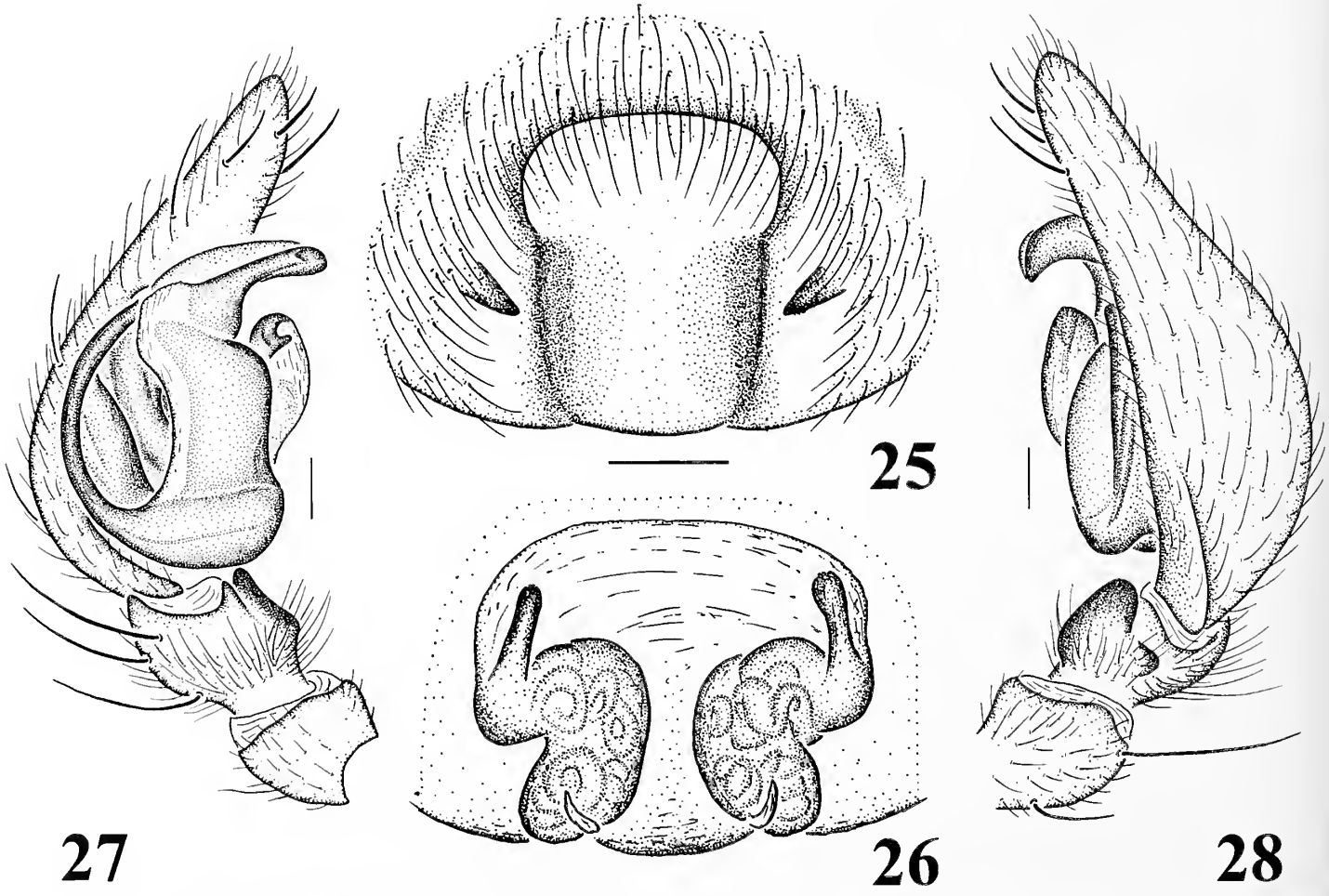
Distribution.—Croatia.

Eurocoelotes inermis (L. Koch 1855)
(Figs. 25–32, 58)

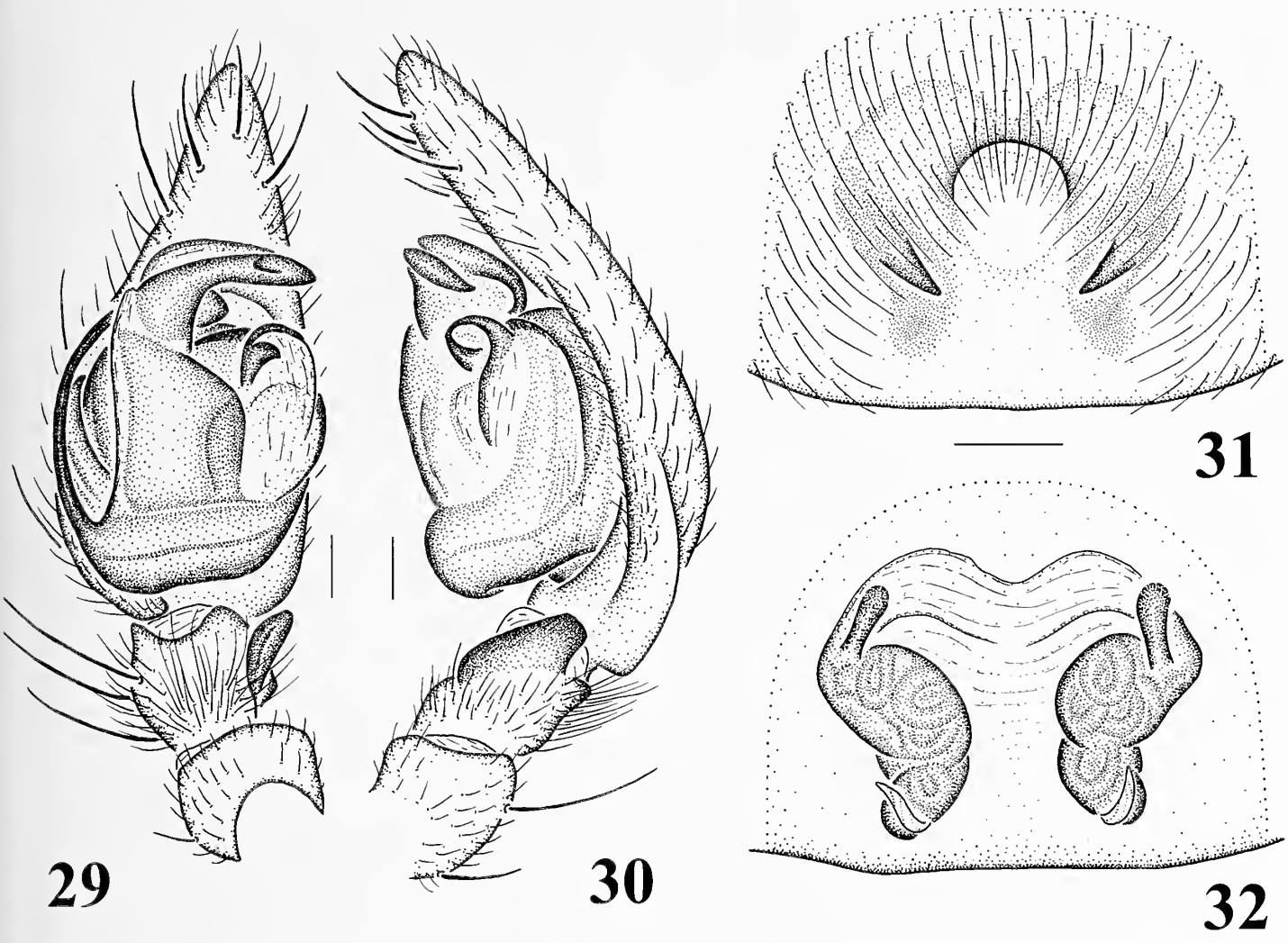
Amaurobius inermis L. Koch 1855:161, fig. 1 (female neotype from Krakow, Poland, in SMNH, examined).

Coelotes inermis: L. Koch 1868:33, figs. 15, 16; Kulczyński 1887:341, fig. 57; Becker 1896:189, fig. 1; Chyzer and Kulczyński 1897:157, fig. 16; Bösenberg 1902:222, fig. 315; Kulczyński 1906:464, figs. 2, 59; Dahl 1931:26, figs. 42, 43; Simon 1937:983, 987, 1037, figs. 1508, 1516; Drensky 1942:42, figs. 5k, 6a; Loksa 1969:106, figs. 73D, 75B.

Amaurobius inermis: Miller 1971:175, figs. 13–15.



Figures 25–28.—*Eurocoelotes inermis* (Koch) from Fra Gospić e Karlobag, Croatia. 25, 26. Female epigynum, ventral and dorsal view. 27, 28. Male palp, ventral and retrolateral view.



Figures 29–32.—*Eurocoelotes inermis* (Koch). 29, 30 (from Fra Gospić e Karlobag, Croatia). Male palp, prolateral and retrolateral view. 31, 32 (from Europe, no detailed location). Female epigynum, showing variation, ventral and dorsal view.

Coelotes inermis: de Blauwe 1973:39, figs. 34–36; Deltshev 1990:33, fig. 3; Heimer and Nentwig 1991:356, fig. 925; Roberts 1995:250; Buchar et al. 1995:120, fig. 35; Bellmann, 1997:136; Roberts 1998:267; Ovtchinnikov 1999:74, figs. 32, 33.

Eurocoelotes inermis: Wang 2002:76, figs. 211–226; Trotta 2005:161, fig. 202.

Material examined.—*Neotype:* POLAND: Krakow, 1♀ (SMNH, Coll.Thorell, 227/1383a).

Other material examined: POLAND: Roztocze Nat. Pk., Bukowa Gora, June 20, 1987, 3♀ (B. & H. Malkin, AMNH); Pachow, Pow. Wadowice. Woj. Krakowskie, September 8, 1974, 1♀ (B. Malkin & M. Mlynarski, AMNH). ITALY: Friuli-Venezia Giulia, Arta Terme, 440 m, September 7, 1963, 1♀ (Bianchi Valle, MCB); Friuli-Venezia Giulia, Paluzza, 600 m, September 9, 1963 (Bianchi Valle, MCB); Bolzano, Planca di Sotto, August 25, 1972, 1♀ (Rallo, MCV); Bressanone, Tonte Plose, 1200 m, June 18, 1972, 1♀ (Oppi, MCV); Basel, 1♂1♀ no detailed label (AMNH). FRANCE: no detailed label, 2♀ (MCZ). SWITZERLAND: Umgebung Basel, Franmatt VII, 6♂17♀ (Keine Angaben, NHMB); Alpes, 1♂1♀ (SMNH, Coll. Thorell, 227/1383b); Basel, 1♂1♀ (Schenkel, AMNH); Predigerholz, SW of Neumunchenstein, 340 m, September 2, 1973, under bark of oak log, 1♀ (B. Malkin & H. & I. Hurlimann, AMNH); Solothurn, Oensingen-Schloss, June 13, 1980, 1♀ (B. & H. Malkin, AMNH); Ruttenen, May 1–2, 1980, 2♂ (B. Malkin, AMNH); Ruttenen, May 1976, 1♀ (B. Malkin, AMNH); Oberdorf, August 1973, 1♀ (B. Malkin, AMNH). BULGARIA: C. Balkan, C. Tuzha, 1500 m, August 10, 1996, 2♂ (C. Deltshev, Coll. Deltshev). BOSNIA: Passo di Kupras, August 11, 1970, 1♂ (MCB); August 11, 1970, 1♂ (Mc Brigamo, MCV). SLOVENIA: Kranj forest, 1960, 3♂3♀ (A. Polenec, MCZ). CROATIA: Fra Gospić e Karlobag, August 17, 1969, 1♂3♀ (Bianchi Valle, MCB); Zagreb, Medvednica, Horvatovih 500 Stuba, 650 m beech litter, September 2–10, 1994, 1♂1♀ (D. Ljubić, COLL. UBICK). GERMANY: Nurnberg, 1♂1♀ (type specimens were collected from this locality) (SMNH, Coll. Thorell, 227/1383c); Between Deutz and Siegen, August 9, 1964, 1♀ (USNM); 1♂ (HEC, B.438, t.100); Neiderwall a Rhein, 1♀ (CAS); Hessen, June 28–29, 1958, 2♀ (H. & L. Levi, MCZ); Saxony, Tharandt, ca 13 air km SW of Dresden, Fichtenwald (=spruce forest), 3♂1♀ (ZMB); Saxony, Osterzgebirge (=East Erz Mountains), Seyde, ca 32 air km SSW of Dresden, Vienweide (=pasture), November 21, 1967, 1♀ female (ZMB, Kat. -Nr. 28823). AUSTRIA: Graz,

September, 1875, 1♂ (H. Emerton, MCZ). EUROPE (no detailed label): 2♂ (AMNH-CU, Lot.581, Sub.499). 1♂2♀ (Marx Collection, USNM, No. No. 242); 1♂, 2♀, 1♀, 1♂, 3♀, 2♀, 1♂1♀, 1♀ (ZMB); 2♀ (ZMB, Dahl 1162); 3 (ZMB, Kat. -Nr. 14016); 1♂1♀ (ZMB, 5169); 8♂ (ZMB, Kat. -Nr. 14094); 1♂1♀ (ZMB, 4698); 2♂4♀ (ZMB, 14473); 4♂ (ZMB, Kat. -Nr. 14013); 1♂1♀ (ZMB, Dahl 2073); 1♂2♀ (ZMB, 4696); 1♂ (ZMB, Dahl 877); 1♂ (ZMB, 14478); 1♀ (ZMB, Dahl 928); 3♂ (ZMB, 14471); 2♂ (ZMB, 14475); 1♀ (ZMB, Dahl, 926); 1♀ (ZMB, 1218); 1♀ (ZMB, Dahl 1636); 1♂ (ZMB, Dahl 2120); 1♀ (ZMB, Dahl 2358); 1♀ (ZMB, Dahl 1343); 1♀ (ZMB, 14480); 6 (ZMB, 14018); 1♂ (ZMB, 14480); 6♂ (ZMB, 14018); 1♂ (ZMB, 14470); 1♀ (ZMB, Dahl 2074); 1♀ (ZMB, Dahl 2119); 2♂ (ZMB, 14476); 2♀ (ZMB, Dahl, 2135); 1♂ (ZMB, 14477); 1♂ (ZMB, 14474); 4♂ (ZMB, 14472).

Diagnosis.—The female resembles *E. gasperinii* by having medially extending copulatory ducts and anteriorly diverging spermathecae but can be distinguished by the atrium, which is separated from epigastric furrow by at least two times its length (in *E. gasperinii*, the atrium is separated from epigastric furrow by its length) and the copulatory ducts that extend slightly anterior to spermathecae (in *E. gasperinii*, the copulatory ducts are limited between spermathecae). The male resembles *E. karlinskii* by having a similar conductor and a prolaterally originating embolus, but can be distinguished by the presence of a prolaterally directed tooth on subdistal conductor and a slightly notched embolic base (Figs. 25–30).

Description.—See L. Koch (1855), Kulczyński (1897 in Chyzer & Kulczyński 1897) and de Blauwe (1973), Deltshev (1990).

Female: Epigynal teeth short, arising between atrium and epigastric furrow, closer to atrium than to epigastric furrow, separated by slightly more than atrial width; atrium small (length and width subequal) or large (wider than long), anteriorly originated, separated from epigastric furrow by approximately 1.5 times its length (specimens with large atrium) or 2–3 times (specimens with small atrium); copulatory ducts large, anteriorly originating, extending medially between spermathecae, extending slightly to anterior spermathecae; spermathecae with bases separated by their width, stalks broad, anteriorly extending and diverging; spermathecal heads long, originating distally (Figs. 25, 26, 31, 32).

Male: Patellar apophysis absent; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow approximately 1/3 of cymbial length; conductor short, with subdistal, prolaterally directed tooth, broad dorsal apophysis, and small basal lamella; median apophysis broad, spoon-shaped, retrolateral margin twice the length of prolateral margin; embolus short, filiform, prolateral in origin (Figs. 27–30).

Notes.—Female epigynal atrium may vary in size, although vulva and male palp show consistent structures. Specimens examined from Thorell's collection at SMNH (female neotype from Krakow, Poland) have relatively large atria.

Distribution.—France, Poland, Germany, Switzerland, Italy, Austria, Bosnia, Slavenia, Croatia, Bulgaria.

Eurocoelotes jurinitschi (Drensky 1915)
(Figs. 33–37, 58)

Amaurobius jurinitschi Drensky 1915:155, 175, fig. 1 (types not examined).
Amaurobius j. flavus Drensky 1915:156.
Amaurobius j. niger Drensky 1915:156.
Coelotes jurinitschi Drensky 1942:42, fig. 5f; Deltshev 1990:33, figs. 4.1–4.4.
Eurocoelotes jurinitschi Wang 2002:76.

Material examined.—BULGARIA: Vitosha Mountain, Bistritsa, 1200 m, August 10, 1985, 3♂2♀ (L. Penev, Coll. Deltshev); Pirin mnt, Aramijska polyana, 1400 m, July 14, 1984, 1♀ (Coll. Deltshev).

Diagnosis.—The female resembles *E. deltshevi* in having long epigynal teeth, anteriorly extending copulatory ducts, broad lateral tibial apophysis, and a similar conductor but can be distinguished by the small atrium (length and width subequal), the longer epigynal teeth (almost reaching epigastric furrow), the contiguous copulatory ducts and spermathecae in female, and the broad median apophysis in male (Figs. 33–37).

Description.—See Drensky (1915) and Deltshev (1990).

Female: Epigynal teeth long, arising laterally of atrium, separated by more than atrial width, extending posteriorly and almost reaching epigastric furrow; atrium small, anteriorly situated, length and width subequal, with distinct septum, separated from epigastric furrow by about 1.5 times its length; copulatory ducts originating anteriorly, extending posteriorly; spermathecal bases small, round, separated by about their width, stalks broad, slightly extending and converging anteriorly, contiguous; spermathecal heads slender, long, medially originating, extending laterally (Figs. 33, 34).

Male: Patellar apophysis absent; RTA distinctly extending distally; lateral tibial apophysis broad; cymbial furrow about 1/3 of cymbial length; conductor short, slightly notched distally, with broad dorsal apophysis, small basal lamella; median apophysis spoon-shaped, retrolateral margin at least two times longer than prolateral margin; embolus short, filiform, prolateral in origin (Figs. 35–37).

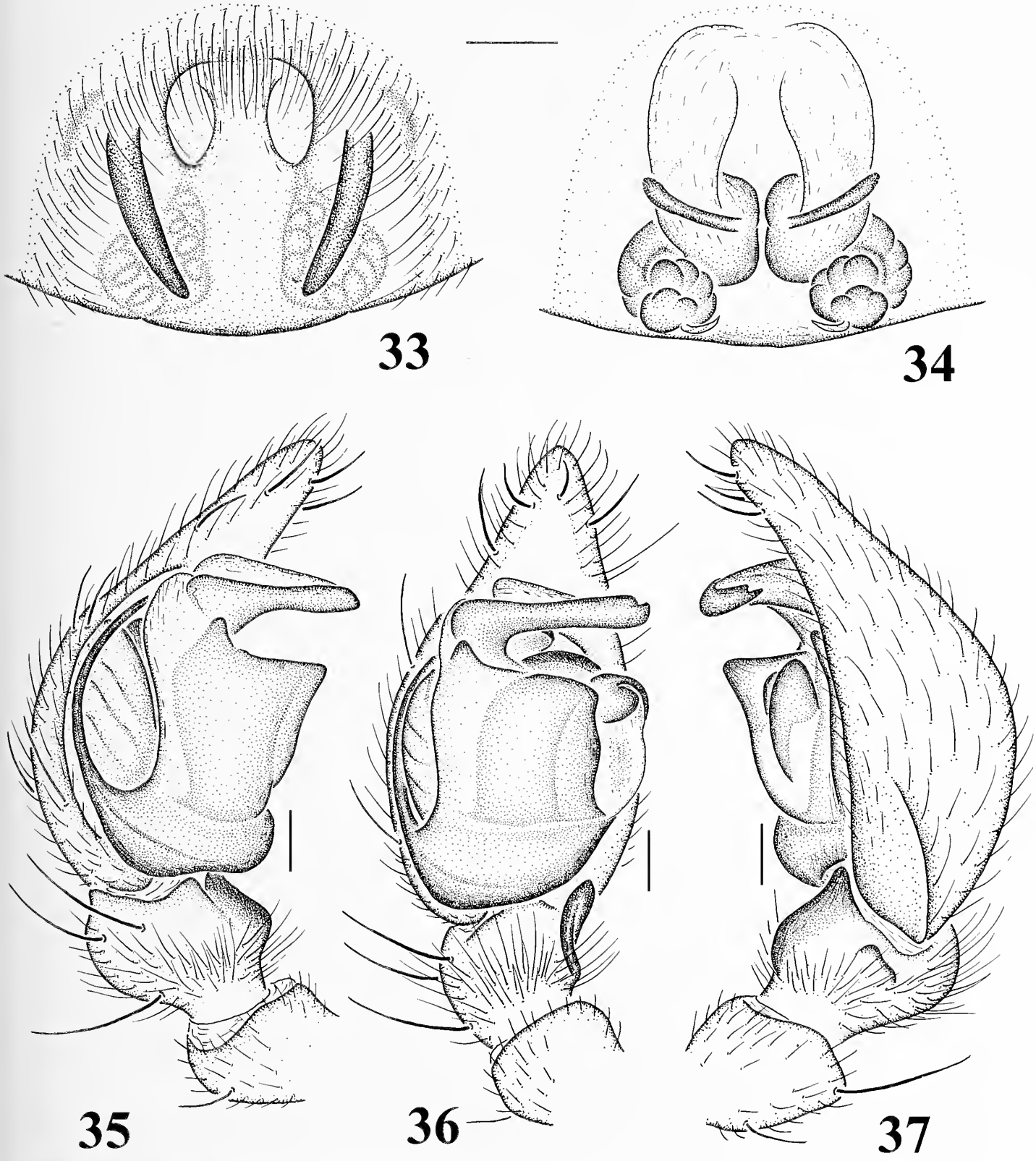
Distribution.—Bulgaria.

Eurocoelotes karlinskii (Kulczyński 1906)
(Figs. 38–42, 58)

Amaurobius karlinskii Kulczyński 1906:469, fig. 3 (types not examined).
Coelotes karlinskii: Kolosvary 1938:18, figs. g, h; Drensky 1942:42, figs. 5g, 7c, 8; Vasiliu, 1971:101, fig. 1; de Blauwe 1973:47, fig. 43; Deltshev 1990:36, fig. 5.1–5.4
Eurocoelotes karlinskii: Wang 2002:76.

Material examined.—BULGARIA: Vitosha Mountain, Bosnek, 1400 m, September 20, 1984, 3♂1♀ (L. Penev, Coll. Deltshev); detailed location not readable, April 1916, 1♂2♀ (ZMB). MONTENEGRO: Crno Jezero, 1400–1500 m, August 8–10, 1967, 1♂5♀ (B. Malkin, AMNH); 1400–1500 m, August 8–10, 1967, 2♂3♀ (B. Malkin, AMNH); 1400–1500 m, August 8–10, 1967, 5♂5♀ (B. Malkin, AMNH). EUROPE: detailed location not readable, June, 1909, 1♂ (ZMB).

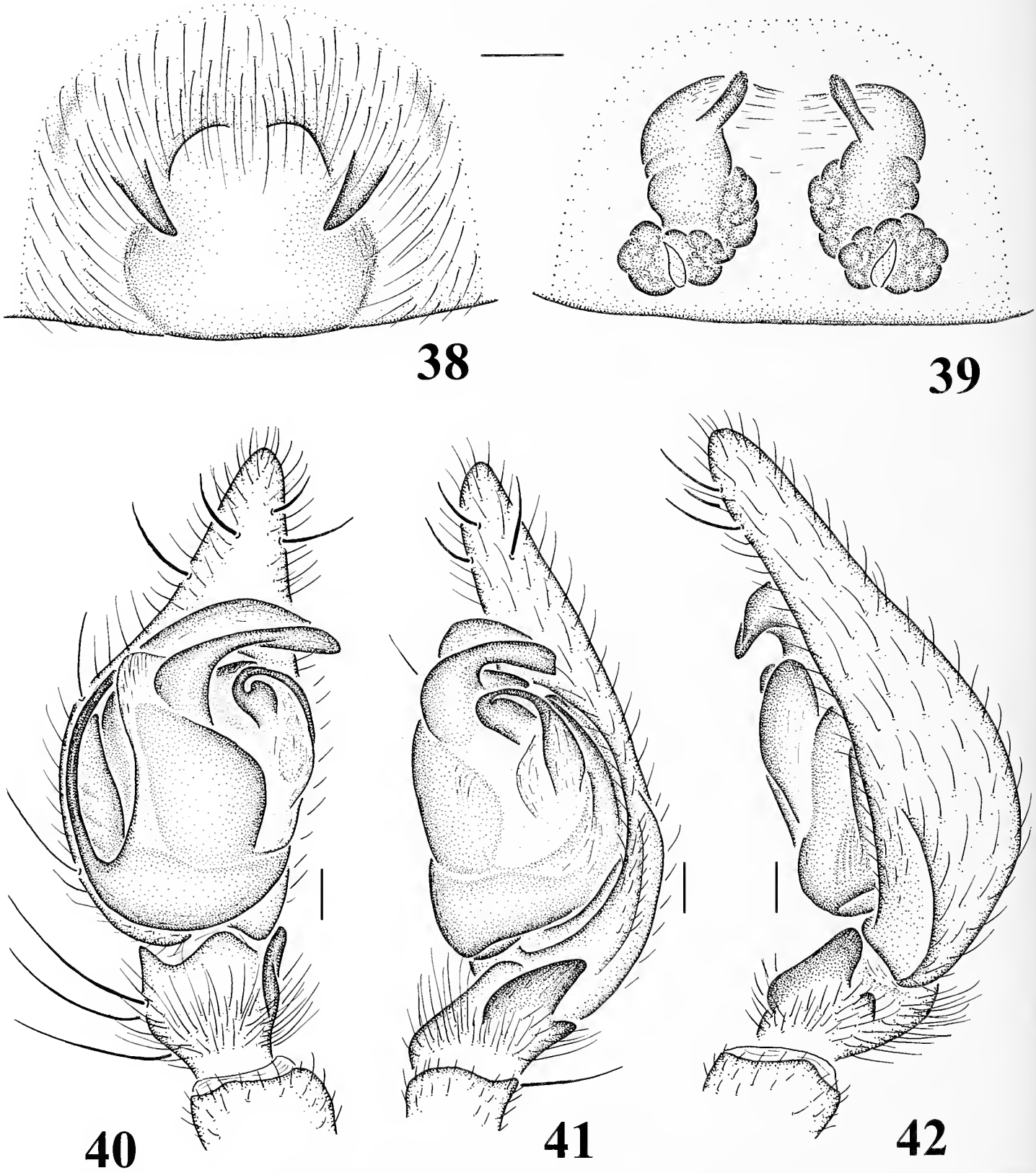
Diagnosis.—This species resembles *E. inermis* by having a similar conductor, prolaterally originating embolus, and medially extending copulatory ducts, but can be distinguished by the absence of a subdistal tooth on conductor, the smooth embolic base in male, and the anteriorly arising epigynal teeth and the anteriorly converging spermathecae in female (Figs. 38–42).



Figures 33–37.—*Eurocoelotes jurinitschi* (Drensky). 33, 34. Female epigynum, ventral and dorsal view. 35–37. Male palp, prolateral, ventral and retrolateral view.

Description.—See Kulczyński (1906), de Blauwe (1973), Deltshev (1990).

Female: Epigynal teeth short, arising laterally of atrium, separated by slightly more than atrial width; atrium small, originating anteriorly, separated from epigastric furrow by approximately 1.5–2 times its length; copulatory ducts small, medially originating, extending medially between spermathecae; spermathecae with bases small, round, separated by their



Figures 38–42.—*Eurocoelotes karlinskii* (Kulczyński). 38, 39. Female epigynum, ventral and dorsal view. 40–42. Male palp, prolateral, ventral and retrolateral view.

width, stalks broad, anteriorly extending, slightly converging and then parallel to each other; spermathecal heads originating distally, extending and slightly converging anteriorly (Figs. 38, 39).

Male: Patellar apophysis absent; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow about 1/3 of cymbial length; conductor short, with broad dorsal apophysis, small basal lamella; median apophysis broad,

spoon-shaped, retrolateral margin at least two times longer than prolateral margin; embolus short, filiform, prolateral in origin (Figs. 40–42).

Distribution.—Montenegro, Bulgaria.

Eurocoelotes kulczynski (Drensky 1915)
(Figs. 43–47, 58)

Amaurobius kulczynsky Drensky 1915:154, 175, fig. 2.2 (types not examined).

Amaurobius kulczynskii: Drensky 1939:86.

Coelotes kulczynskii: Drensky 1942:41, fig. 5e. Deltshev 1990:36, figs. 6.1–6.6 (types examined, male described for the first time).

Eurocoelotes kulczynski: Wang 2002:76.

Material examined.—BULGARIA: Pirin Mountain, Prevala, 2400 m, July 21, 1981, 1♂1♀ (C. Deltshev, Coll. Deltshev); Vitosha Mountain, ca Aleko, 1800 m, September 7, 1985, 3♂2♀ (L. Penev, Coll. Deltshev); Rilskii Monastir, Rila Mts. 1100–1300 m, July 17–21, 1972, 2♀ (B. Malkin, AMNH).

Diagnosis.—The female can be easily recognized by the tiny epigynal teeth, the large atrium separated from epigastric furrow by about its length, and the laterally extending copulatory ducts (Figs. 43, 44). The male resembles *E. deltshevi* and *E. brevispinus* in having a broad lateral tibial apophysis and a small median apophysis, but can be distinguished from *E. brevispinus* by the absence of a patellar apophysis, and from *E. deltshevi* by the non-bifurcate lateral tibial apophysis and the presence of a slightly sclerotized ridge on distal patella (Figs. 45–47).

Description.—See Drensky (1915) and Deltshev (1990).

Female: Epigynal teeth tiny, arising slightly posteriorly of atrium, separated by slightly more than atrial width; atrium large, originating anteriorly, length and width subequal, separated from epigastric furrow by approximately its length; copulatory ducts large, originating anteriorly, extending laterally and posteriorly, connecting to spermathecae laterally; spermathecal bases broad, round, slightly separated, stalks broad, contiguous; spermathecal heads arising anteriorly, extending laterally (Figs. 43, 44).

Male: Patellar apophysis absent, with slightly sclerotized ridge; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow less than 1/3 of cymbial length; conductor short, broad, slightly curved distally, with broad dorsal apophysis, small basal lamella; median apophysis small, spoon-shaped, retrolateral margin and prolateral margin subequal in length; embolus short, filiform, prolateral in origin (Figs. 45–47).

Distribution.—Bulgaria.

Eurocoelotes microlepidus (de Blauwe 1973)
(Figs. 48–52, 58)

Coelotes microlepidus de Blauwe 1973:67, fig. 57 (female holotype from Montecchio, Italy, in MCV, examined). Deltshev 1990:38, figs. 7.1–7.4, 8.1–8.2.

Eurocoelotes microlepidus: Wang 2002:76.

Material examined.—ITALY: Montecchio, May 2, 1968, ♀ holotype (G. Osella, MCV); Trento, Vallata di Ledro, May 15, 1971, 1♀ (G. Osella, MCV). BULGARIA: Zemen Gorge, 500 m, October 29, 1976, 2♂2♀ (G. Blagoev, Coll. Deltshev). EUROPE: label not readable, June, 1908, 1♀ (ZMB).

Diagnosis.—The female resembles *E. gasperinii* by having small epigynal teeth arising slightly posteriorly to atrium, medially originating and extending copulatory ducts, and widely separated spermathecae, but can be distinguished by the slightly wider than long atrium and the parallel extending spermathecae (Figs. 48, 49). The male resembles *E. paramicrolepidus* in having a small patellar apophysis and coiling conductor, but can be distinguished by the relatively broad median apophysis (retrolateral margin longer than prolateral margin) and the shorter conductor (slightly coiled distally, not shaped like a semicircle) (Figs. 50–52).

Description.—See de Blauwe (1973) and Deltshev (1990).

Female: Epigynal teeth short, arising posterolaterally of atrium, separated by atrial width; atrium small, slightly wider than long; copulatory ducts large, anteriorly originating, extending posteriorly between spermathecae; spermathecae slender, long, widely separated by at least three times their width; spermathecal heads small, originating distally (Figs. 48, 49).

Male: Patellar apophysis small; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow about 1/3 of cymbial length; conductor broad, slightly coiled distally, with broad dorsal apophysis, small basal lamella; median apophysis broad, spoon-shaped, retrolateral margin extending more than twice the prolateral margin; embolus short, filiform, prolateral in origin (Figs. 50–52).

Distribution.—Italy, Bulgaria, Macedonia.

Eurocoelotes paramicrolepidus new species
(Figs. 53–55, 58)

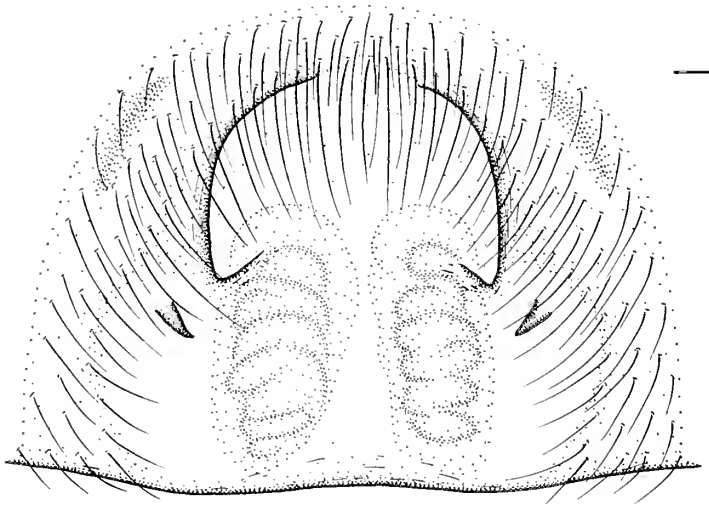
Material examined.—*Holotype:* GREECE: ♂, Peloponnisos, Camp Dimitri Mitropulos, about 10 km W of Vitina, Tripolis-Olimpia, 1000–1100 m, June 15–18, 1981, B. & H. Malkin (AMNH).

Etymology.—The specific name refers to its similarity to *E. microlepidus*.

Diagnosis.—The male resembles *E. microlepidus* in having a small patellar apophysis and distally coiled conductor, but can be distinguished by the small median apophysis and a distal conductor that is semicircular in shape (Figs. 53–55).

Description.—*Male (holotype):* Medium sized coelotine. Total length about 8.50. Carapace 4.40 long, 2.80 wide. Abdomen damaged. AME and PME subequal, ALE largest, PLE slightly smaller than ALE (AME 0.15, ALE 0.20, PME 0.16, PLE 0.18); AME separated from each other by approximately 2/3 of AME diameter, from ALE by approximately 1/3 of AME diameter; PME separated from each other by their diameter; from PLE by slightly less than 1.5 times PME diameter (AME–AME 0.09, AME–ALE 0.05, PME–PME 0.16, PME–PLE 0.22, AME–PME 0.14). Promargin of chelicera with three teeth, retromargin three. Patellar apophysis tiny; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow slightly less than half of cymbial length; conductor broad, long, coiled distally to a semicircle shape, with broad dorsal apophysis, small basal lamella; median apophysis small, spoon-shaped, retrolateral margin slightly longer than prolateral margin; embolus short, filiform, originating between prolateral and proximal (Figs. 53–55).

Female: Unknown.



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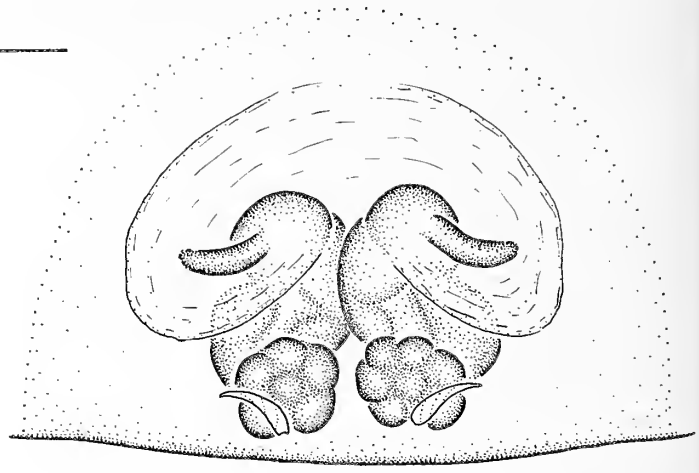

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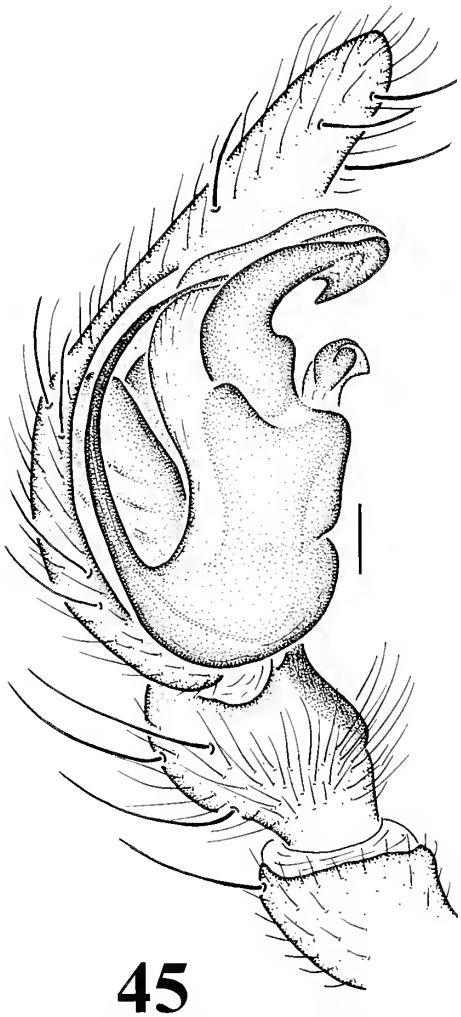

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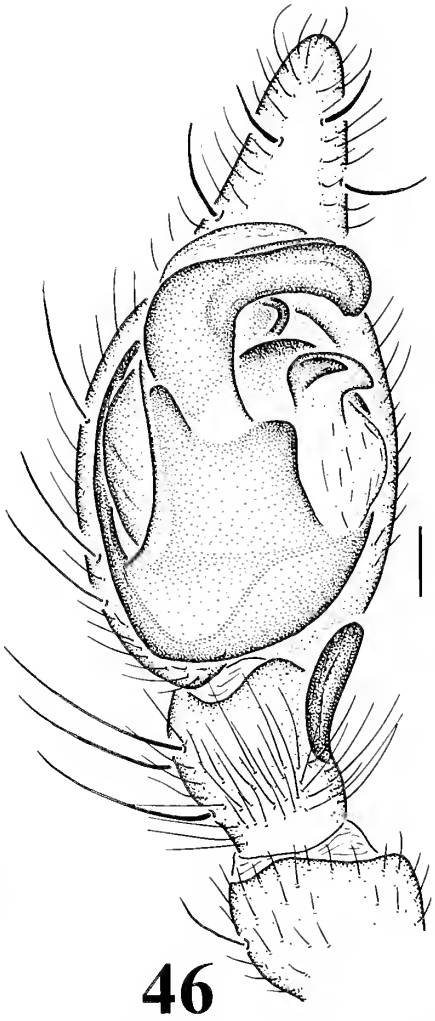

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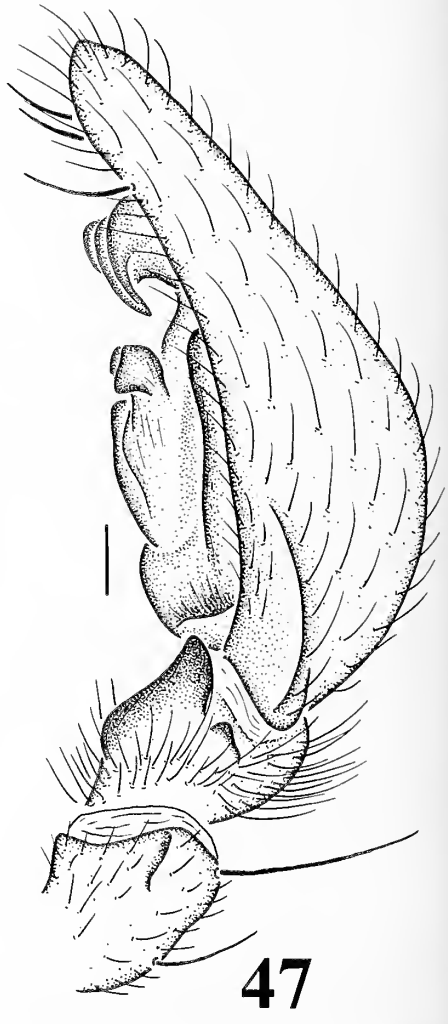

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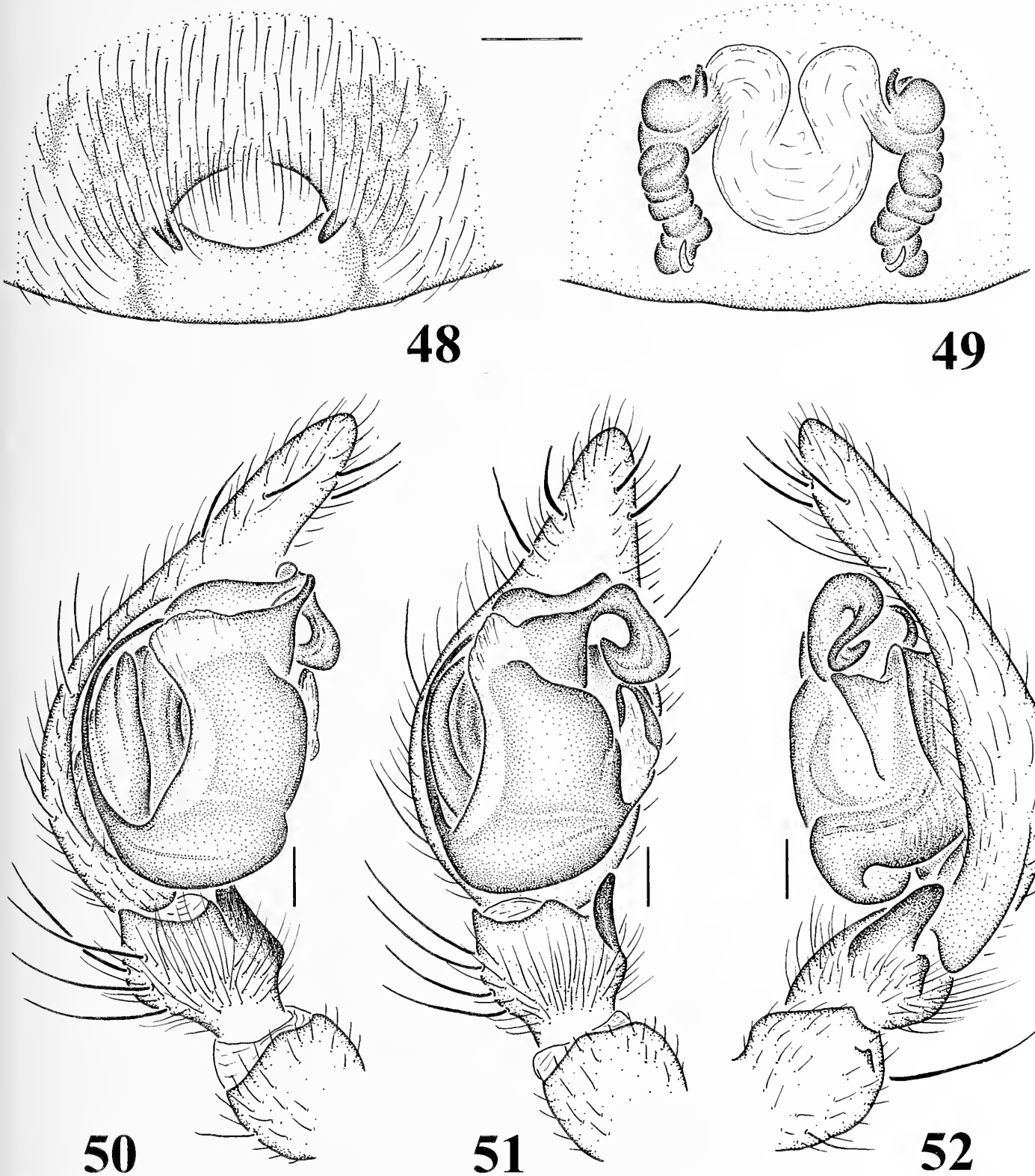
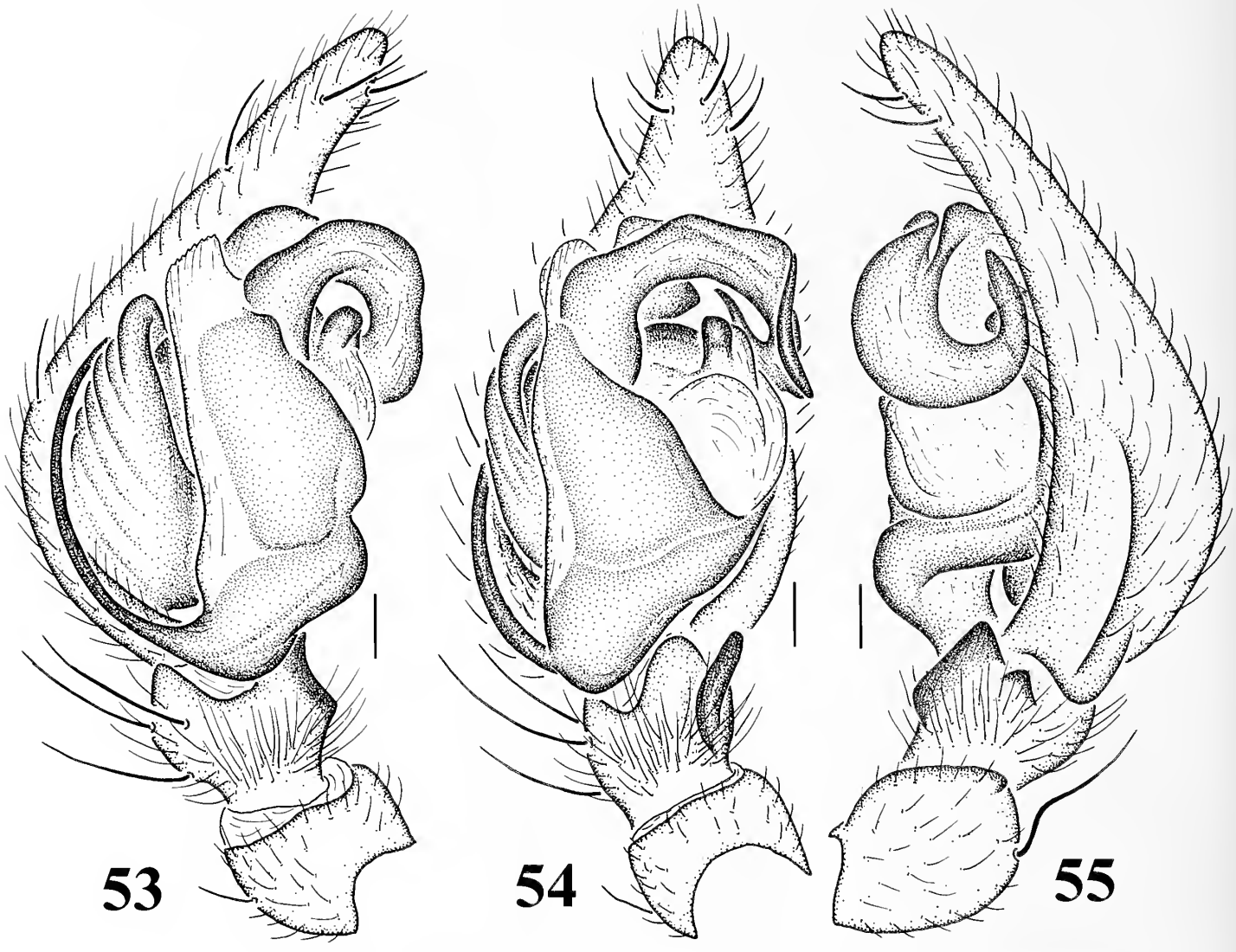
Figures 43–47.—*Eurocoelotes kulczynski* (Drensky). 43, 44. Female epigynum, ventral and dorsal view. 45–47. Male palp, prolateral, ventral and retrolateral view.



Figures 48–52.—*Eurocoelotes microlepidus* (de Blauwe). 48, 49. Female epigynum, ventral and dorsal view. 50–52. Male palp, prolateral, ventral and retrolateral view.



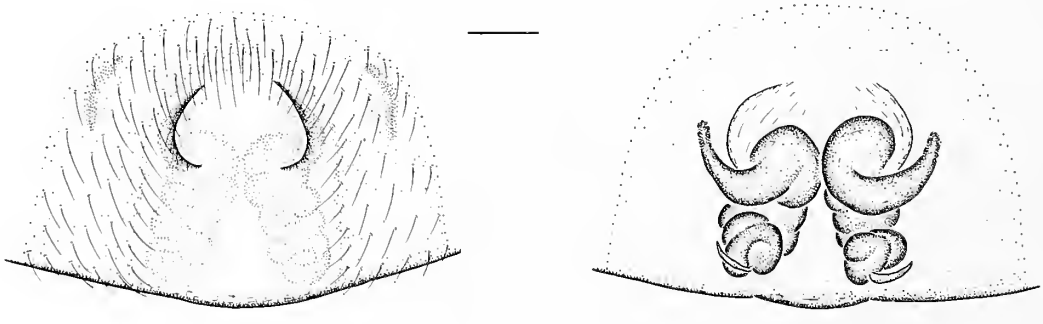
Figures 53–55.—*Eurocoelotes paramicrolepidus* new species, male palp, prolateral, ventral and retrolateral view.

Distribution.—Greece.

Eurocoelotes xinpingwangi Deltshev 2009
(Figs. 56, 57)

Eurocoelotes xinpingwangi Deltshev 2009:293, figs. 1–2 (male holotype, male and female paratypes from Rila Mountains, Bulgaria, deposited in IZS, not examined; paratypes from Sitnjakow, Bulgaria, deposited in ZMB, examined).

Material examined.—BULGARIA: Sitnjakowo, 1750 m, May 1916, 1♀ paratype (v. Boebbicher, ZMB, J. N. 478/16, E. N. K.); Sitnjakowo,, 1750 m, May 1916, 3♀ paratypes (Boebbicher, ZMB).



Figures 56, 57.—*Eurocoelotes xinpingwangi* Deltshev, female epigynum, ventral and dorsal view.

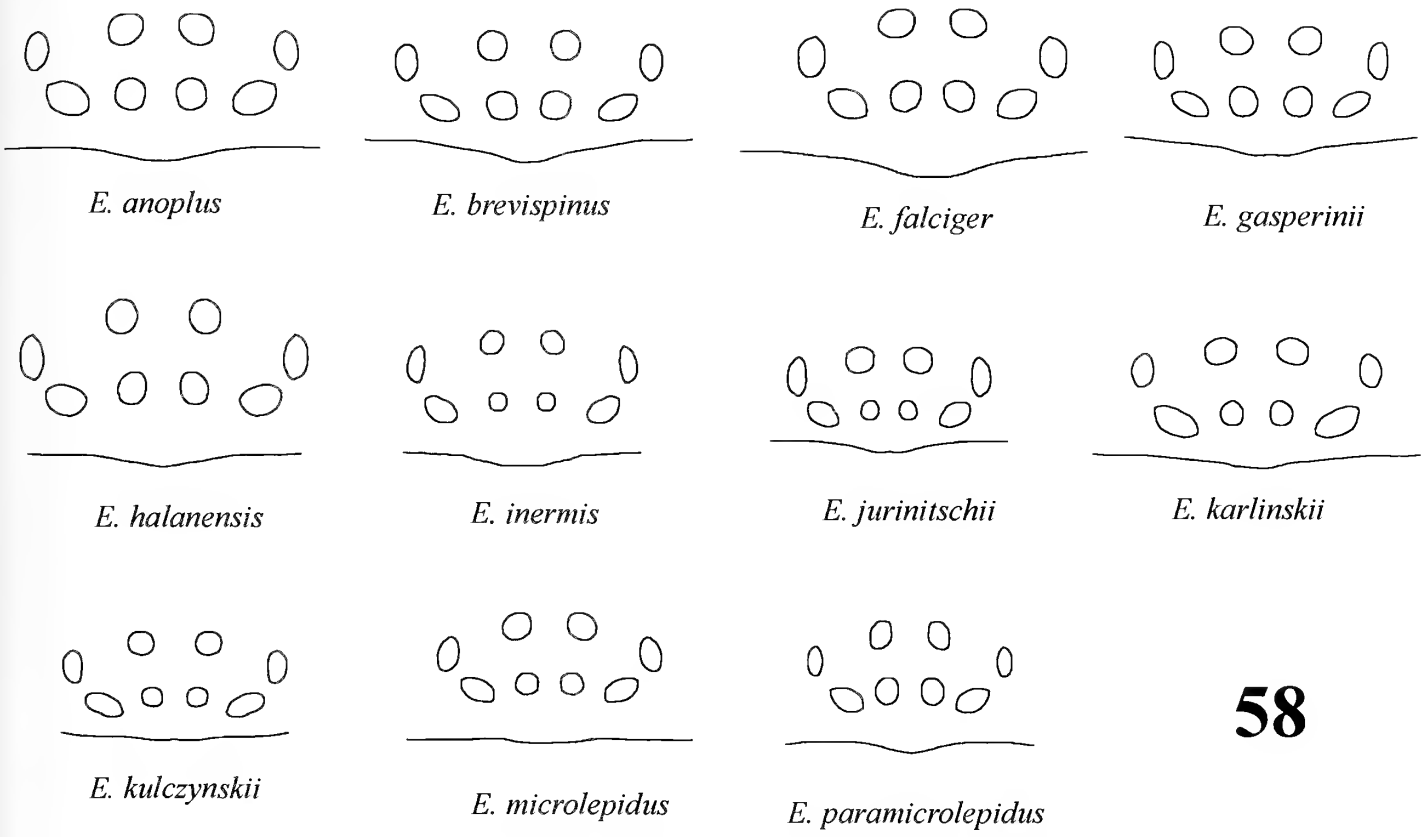


Figure 58.—Eyes of eleven *Eurocoelotes* species, view between dorsal and front.

Diagnosis.—The female can be easily recognized by the absence of epigynal teeth (Figs. 56, 57). The male resembles *E. kulczynskii* in having a small median apophysis, but can be distinguished by the slender conductor (Deltshev 2009: Figs. 1a–c).

Description.—See Deltshev (2009).

Female: Without epigynal teeth; atrium with anterior origin, large, length and width subequal, separated from epigastric furrow by approximately its length or slightly more; copulatory ducts small, originating anteriorly, slightly extending posteriorly; spermathecal bases small, round, separated by about their width, stalks extending and converging anteriorly, contiguous; spermathecal heads long, extending laterally (Figs. 56, 57).

Male: Patellar apophysis absent; RTA distinctly extending distally; lateral tibial apophysis small; cymbial furrow slightly

Figure 59.—Distribution of *Eurocoelotes* species.

less than half of cymbial length; conductor short, slender, with a broad dorsal apophysis, a small basal lamella; median apophysis small, spoon-shaped, retrolateral and prolateral margins subequal in length; embolus short, filiform, prolateral in origin (Deltshev 2009: Figs. 1a–c).

Distribution.—Bulgaria.

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Silk gene transcripts in the developing tubuliform glands of the Western black widow, *Latrodectus hesperus*

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Abstract. Spiders spin a variety of task-specific silk fibers, each composed of one or more unique proteins synthesized within specialized glands in the spider's abdomen. Tubuliform glands are the source of the large diameter silk fibers used by many species in the construction of egg cases. Unlike other silk glands that synthesize protein throughout a spider's lifetime, the tubuliform glands synthesize silk in association with the maturation of oocytes, culminating in the production of an egg case. In the Western black widow, *Lactrodectus hesperus* Chamberlin & Ivie (1935), egg case fibers are composed of at least three proteins: tubuliform spidroin 1 (TuSp1), egg case protein-1 (ECP-1), and egg case protein-2 (ECP-2). Here, we present the first study to quantify the pattern of transcription for these three genes in a developmental series of tubuliform glands from *L. hesperus*. All three transcripts increase in abundance prior to the production of an egg case, but at different time points. After egg case production, silk transcripts are still detectable in the tubuliform glands. Relative abundance of TuSp1 mRNA is several orders of magnitude higher than that of ECP-1 and ECP-2 at almost every stage. The relative abundance of silk transcripts across the reproductive life history of black widows suggests differential regulation of silk gene transcription within tubuliform glands.

Keywords: Spider silk, egg case, tubuliform spidroin 1, egg case protein-1, egg case protein-2

Orb-weaving spiders and their relatives (Orbiculariae) possess a set of seven distinct gland types that produce silks used for functions such as prey capture and swathing, habitat construction, and reproduction. Each gland-specific fiber is made up of one or more unique structural spider silk proteins (spidroins, contraction of "spider fibroins") (Guerette et al. 1996). Tubuliform spidroin 1 (TuSp1) is the major protein component of the large diameter silks composing the egg case that forms the protective environment for the developing spiderlings (Garb & Hayashi 2005; Tian & Lewis 2005; Hu et al. 2005a; Zhao et al. 2005; Huang et al. 2006). Large diameter silk fibers are the product of tubuliform (cylindrical) glands (Stubbs et al. 1992; Moon 2003; Vasanthavada et al. 2007). Two additional silk associated proteins, egg case proteins-1 and -2 (ECP-1 and ECP-2), have been identified in the tubuliform glands of black widows (Hu et al. 2005a, 2005b).

In contrast to most silk glands that synthesize protein throughout the lifetime of a spider, tubuliform glands are found only in mature females and their development parallels the maturation of the ovaries such that the glands reach a maximal size in gravid spiders (Kovoor 1990; Moon 2003). This unique feature of egg case silk synthesis makes the tubuliform gland a potential model system for investigating the regulatory mechanisms responsible for dramatically increasing spider silk gene expression. Histochemical and morphological observations of tubuliform glands indicate that protein synthesis peaks just prior to the construction of an egg case (Candelas et al. 1986; Moon 2003; Huang et al. 2006). However, the signaling pathways and regulatory molecules used to influence egg case silk protein production are unknown. With the advent of highly sensitive nucleic acid techniques such as quantitative PCR, we can begin to address questions about the transcriptional regulation of spider silk.

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Here, we use quantitative PCR to examine the transcript levels for the three tubuliform gland-specific silk genes over a developmental time series for the Western black widow, *Latrodectus hesperus* (Araneae: Theridiidae). Our goal is to correlate vitellogenesis with the development of tubuliform glands and the relative levels of egg case-specific gene transcripts. Our results provide a foundation for gene expression studies of spider silk glands both within and across gland types and species. More broadly, we show the utility of black widow tubuliform glands as a model system for the investigation of signal pathways responsible for the transcriptional regulation of silk proteins.

METHODS

Spider collection, dissection, and staging.—Female Western black widow spiders (*L. hesperus*) were collected in Riverside and Fullerton, California (USA). Voucher specimens have been deposited in the Entomology Research Museum at the University of California, Riverside (UCRC ENT 229278). Spiders were housed in individual containers and maintained at an ambient temperature of 27° C with a 14:10 h day:night cycle. We fed the spiders mealworms (juvenile *Tenebrio molitor*) every three weeks. Tubuliform glands and ovaries/eggs were isolated from individual spiders by dissection under 0.15 M sodium chloride, 0.015 M sodium citrate buffer (SSC). We immediately froze the glands in liquid nitrogen and then stored them at −80° C. Individual spiders were assigned to one of seven life history stages based in part on the scheme developed by Trabalon et al. (1992). Virgin spiders (V) were identified as spiders that had undergone at least one molt in captivity and possessed small, undeveloped oocytes. Gravid spiders (G) possessed a loose mass of large yolky eggs within their abdomens. Using the criteria established by Trabalon et al. (1992), black widow spiders were characterized as being in either early (Vitel-E) or late vitellogenesis (Vitel-L) based on measurements of the overall diameter of the oocytes and the

size and distribution of the yolk granules within those oocytes. The final three stages represent time points following the production of an egg case: within 8 h of spinning (D0), 24 h after (D1), or 48h after (D2).

Microscopy.—Ovaries, eggs, or tubuliform glands were prepared for microscopy by an overnight fixation in 2% formaldehyde, 2% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.4) at 4° C. We then rinsed the tissue in 0.1 M sodium phosphate buffer and stored it under buffer at 4° C. Morphological characteristics of ovaries and eggs were visualized using an environmental scanning electron microscope (Hitachi TM1000). We photographed tubuliform glands using a Leica MZ125 stereomicroscope with a digital camera (Spot Insight). We determined the ooctye and gland diameters using the measurement tools available from the image-capture software of each respective microscope. We calculated mean gland diameter from measurements of multiple tubuliform glands from three individuals at each life history stage. In order to test the categorization of oocytes and mean gland diameter across various reproductive life history stages, we used one-way ANOVA assuming unequal variance. We used SPSS software (SPSS Inc., Chicago, Illinois) to conduct the statistical analysis.

Total RNA isolation and cDNA synthesis.—Total RNA was extracted from tubuliform gland tissue by homogenization in TRIzol® Reagent (Invitrogen). We processed glands from individual spiders separately with the exception of the virgin spiders in which glands from multiple individuals were pooled in order to obtain sufficient quantities of total RNA for cDNA synthesis. Genomic DNA carryover was eliminated with TURBO^{TM}-DNase (Ambion). The absence of any product in standard PCR amplification of every total RNA sample used in this study with the TuSp1 primer set (see below) supports the conclusion that the total RNA samples were free of genomic DNA contamination. We synthesized single-stranded cDNA from 1 µg of total RNA with Superscript® III reverse transcriptase (Invitrogen) primed from an anchored oligo(dT) primer.

Real-time quantitative PCR.—Tubuliform silk gland cDNA formed the template for real-time quantitative PCR (RT-qPCR) amplification. We designed primers to amplify the C-terminal encoding region of *L. hesperus TuSp1* (forward primer LhTuSp1_3733F: CCTGGTTTGATTGTAGGACCCTC; reverse primer LhTuSp1_3993R: GGATTTCCGCTTTGAA TGGATG). Primers used for the amplification of transcript for the egg case proteins ECP-1 and ECP-2 in *L. hesperus* were based on those of Hu et al. (2006). Calreticulin, a calcium-binding protein of the endoplasmic reticulum, was used as the housekeeping reference gene for the RT-qPCR reactions. We designed primers for calreticulin from the *L. hesperus* calreticulin sequence (GenBank accession number GQ402146; forward primer Cal Lh620F, AGAAAATGAAAGATCCCGAGGC; reverse primer Cal Lh801R, AATTTGAGGTGGTTCCC ACTCTC). We confirmed specificity of the primer sets through sequencing of the respective PCR products at every developmental stage.

RT-qPCR was completed using the MyIQ5 thermocycler (BioRad) and associated software (Version 2.0). Each 20 µl reaction volume contained 0.1 µg of template cDNA and 200 nM of each primer in SYBR Green Supermix (BioRad). The reaction profile included 40-cycles of 15 s denaturation at 95° C, 40 s annealing at 60° C, and 45 s extension at 72° C followed by a 56° C to 94° C melt curve analysis. Reactions using cDNAs from individual spiders representing each of the reproductive life history stages (or pooled cDNAs in the case of virgin spiders) were done in triplicate with four independent trials for each stage. Efficiency of each primer set was determined using a ten-fold serial dilution of G stage cDNA. The reference gene, calreticulin, was found to have an efficiency of 90% while each of the silk primer sets had an efficiency of greater than 100%. We confirmed the absence of primer:dimer formation during PCR by using a melt curve analysis. Abundance of transcript levels was calculated relative to the reference gene (calreticulin) and a control condition (D0 stage) using the method developed by Pfaffl (2001). We defined the D0 stage as the control eondition based on the finding that the cycle threshold (C_t) values at whieh the target-specific signal exeeeds background for all transcripts tested supported the assumption that mRNA levels were lowest immediately following egg case production.

RESULTS AND DISCUSSION

Oocyte and gland morphology change relative to vitellogenesis.—The process of vitellogenesis alters the size and surface morphology of the developing oocytes as visualized by scanning electron microscopy (Fig. 1). Stages of oocyte development similar to those noted by Trabalon et al. (1992) for two agelenid species were identified in the black widow. We found each of the stages to be distinet based on measurements of oocyte diameter [$F_{(7,454)} = 1403.6$, $P < 0.001$]. Oocytes increase in mean diameter from 70 ± 1 µm (S.E.) in virgin spiders (Fig. 1A) to 183 ± 5 µm in early vitellogenic (Fig. 1C) and 295 ± 4 µm late vitellogenic (Fig. 1E) spiders. These developing ooeytes attach to the ovarian wall by means of a pedicle (Figs. 1C & E), but are released into the abdomen of the gravid spider (Fig. 1G). The oocytes in the gravid female have a mean diameter of 574 ± 17 µm. In addition to size, the accumulation and organization of yolk granules within the oocytes are distinguishing characteristics for the stages of vitellogenesis (Figs. 1A, C, E and G).

Similarly, we also found the appearanee of the tubuliform glands to differ significantly with the reproductive stage of the spider [$F_{(6,160)} = 59.82$, $P < 0.001$] (Fig. 1). The tubuliform glands of virgin spiders (Fig. 1B) had the smallest mean diameter (125 ± 2 µm) relative to the glands from any of the other stages. Tubuliform gland diameter increased from early (Fig. 1D) to late vitellogenesis (Fig. 1F), reaching a maximum mean diameter of 340 ± 8 µm in gravid spiders (Fig. 1H). Following the production of an egg case, the tubuliform glands had a flattened appearance and returned to a mean diameter of 157 ± 7 µm (data not shown).

Silk gene transcript levels in tubuliform glands vary with reproductive life history stage.—RT-qPCR detected TuSp1, ECP-1 and ECP-2 silk transcripts within the tubuliform glands at all reproductive life history stages examined, including virgin spiders (Fig. 2). The relative quantities of all three silk transcripts (TuSp1, ECP-1, and ECP-2) reached their highest level during late vitellogenesis (Vitel-L). TuSp1 and ECP-2 mRNA levels increased during early vitellogenesis while

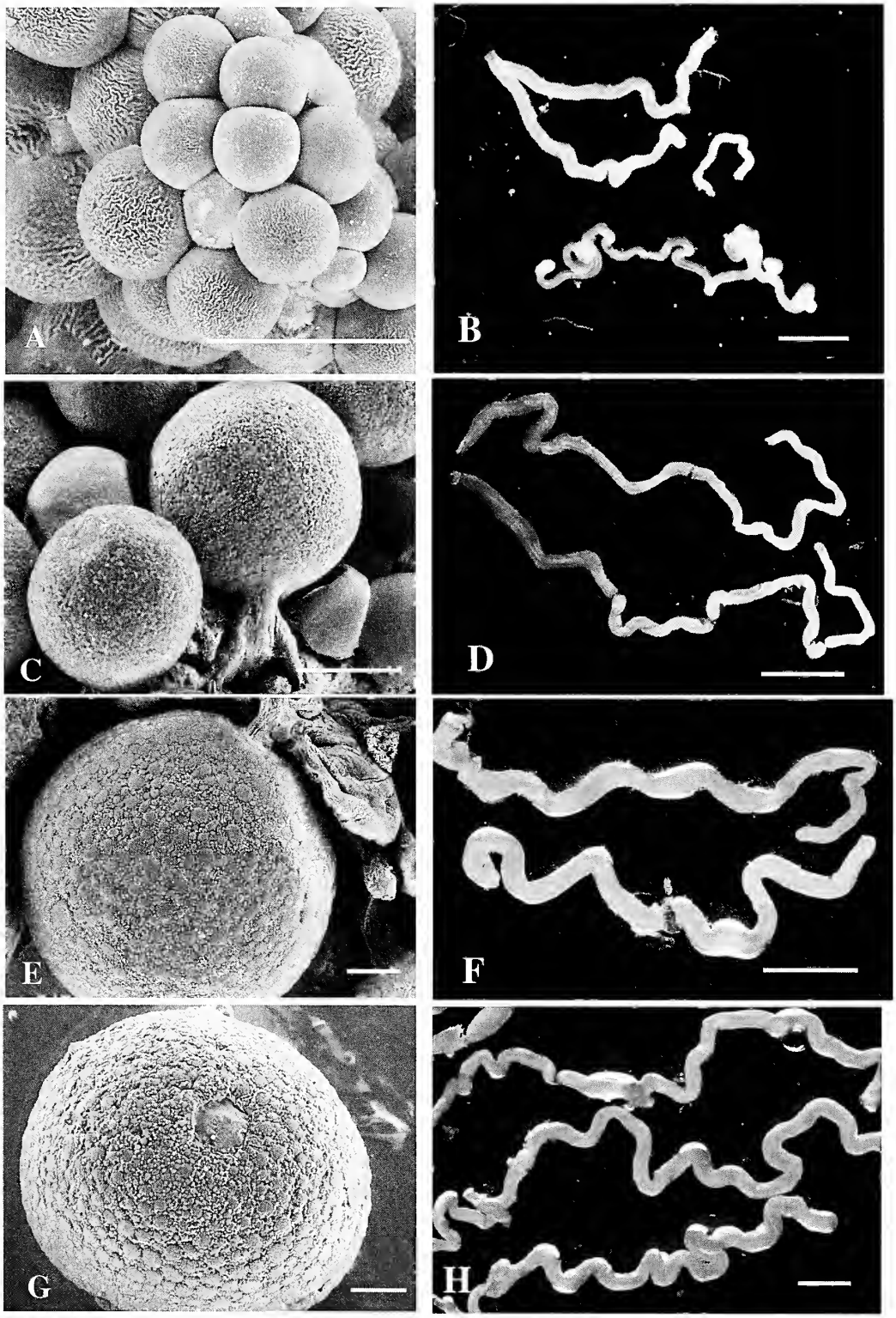

Figure 1.—Tubuliform gland morphology changes coincident with vitellogenesis in *L. hesperus*. (A & B) Oocytes and tubuliform glands in virgin spiders are small (mean diameters; 70 ± 1 μm S.E. and 125 ± 2 μm S.E., respectively). (C & D) During early vitellogenesis, the oocytes and glands begin to increase in size (mean diameters; 183 ± 5 μm S.E. and 174 ± 8 μm, respectively). (E & F) Growth of oocytes and tubuliform glands continue into late vitellogenesis (mean diameters; 295 ± 4 μm S.E. and 189 ± 8 μm, respectively) reaching a maximum (mean diameters; 574 ± 17 μm S.E. and 340 ± 8 μm, respectively) in gravid spiders (G & H). Scale bars for all oocytes images = 100 μm. Scale bars for all gland images = 1mm.

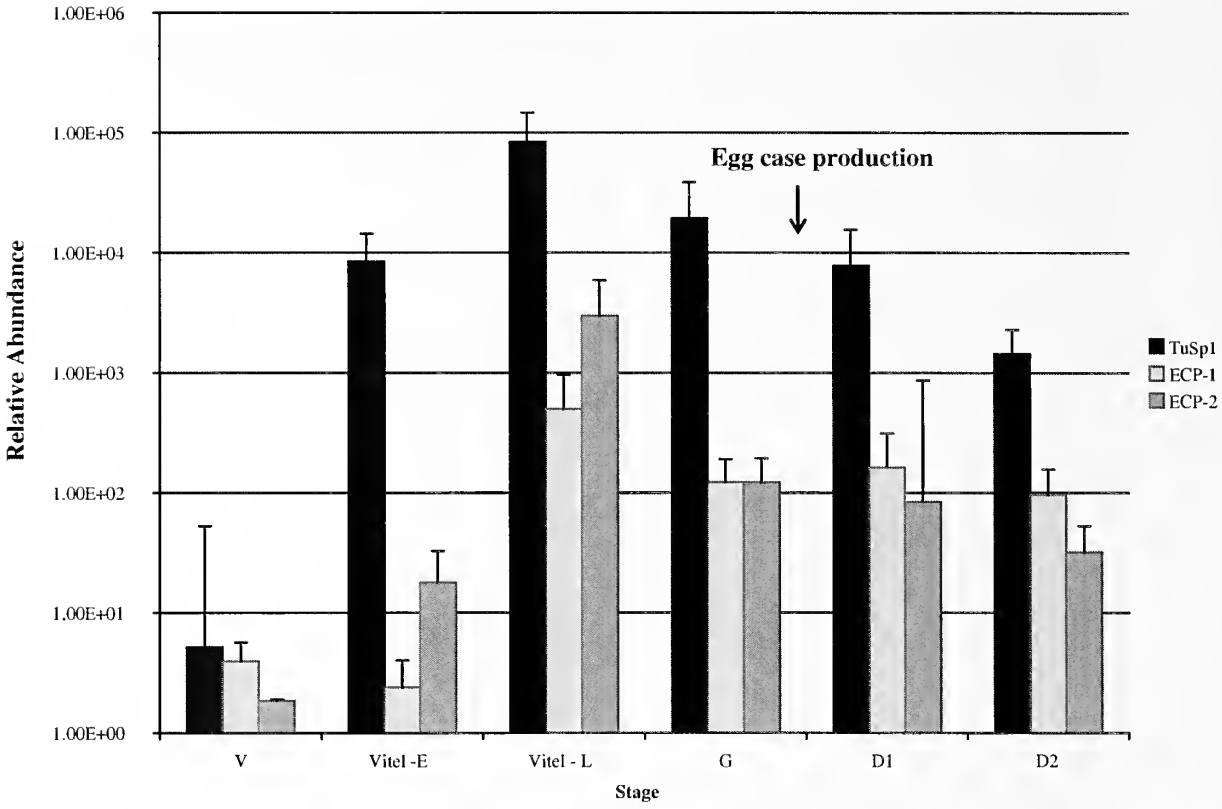

Figure 2.—Silk gene transcript levels vary with reproductive life history stage for the tubuliform gland of *L. hesperus*. The relative abundance of the tubuliform silk mRNAs, TuSp1, ECP-1, and ECP-2 was determined by RT-qPCR. Transcripts for all three silks were detected in tubuliform glands from virgin spiders. The quantity of silk mRNAs present in the gland increases through vitellogenesis (Vitel-E and Vitel-L), and is maintained following the production of an egg case. Levels of TuSp1 mRNA exceed those of the ECPs at all stages from early vitellogenesis on. All samples were tested in triplicate and normalized to the housekeeping gene, calreticulin. The D0 stage was used as the control condition. Results presented here are the average of four independent trials. Error bars indicate SEM.

ECP-1 mRNA levels did not increase until late vitellogenesis. The amount of each of the silk transcripts present in the glands at 48 h post egg case production was similar to that in gravid spiders. It has yet to be determined whether the accumulation of silk mRNAs over these developmental stages is due to increased initiation of transcription, changes in mRNA stability, or some combination of the two.

Given the specific function of the tubuliform gland in spider reproduction, it has been proposed that egg case silks are likely under hormonal control (Kovoor 1990). Hormonal regulation of silk production has been demonstrated in the silkworm moth, *Bombyx mori* (Fukuta et al. 1993; Xu et al. 1994; Tang et al. 2007). Juvenile hormone and ecdysterol have been shown to influence the expression of the lepidopteran silk protein, seroin (Žurovec et al. 1998), and it is known that these same hormones influence the process of vitellogenesis in spiders (Trabalon 1992; Pourie & Trabalon 2003).

While a hormone might initiate a signaling pathway leading to silk gene expression, a transcription factor is ultimately required to promote the binding of RNA polymerase to the promoter region of the silk gene. One candidate protein has been identified in the black widow spider (Kohler et al. 2005). Silk gland subset factor (SGSF) is a class II basic helix-loop-helix (bHLH) transcription factor expressed primarily in silk glands. The expression level of SGSF is reported to be elevated in late vitellogenesis, coincident with the peak of silk transcription demonstrated here. Kohler et al. (2005) have proposed that SGSF may function in concert with other regulatory proteins to trigger the differentiation of the tubuliform gland itself, as well as to control the expression of the egg case silk genes.

The relative amount of TuSp1 mRNA present in black widow tubuliform glands was, with the exception of virgin spiders, consistently greater than that of ECP-1 or ECP-2 (Fig. 2). The most dramatic difference in relative abundance occurred during early vitellogenesis when TuSp1 levels were ~3500 fold greater than ECP-1 and ~470 fold greater than ECP-2. At this same stage, ECP-2 was 7.5 fold more abundant than ECP-1. Following the production of an egg case, the differences between the amounts of TuSp1 and ECP message were less extreme. This co-expression of all three genes is consistent with the observation that the large diameter silk fibers of the black widow egg case are a trimeric complex of the three proteins (Hu et al. 2006). Our data are consistent with and expand upon earlier findings that TuSp1 is the major protein product of the black widow tubuliform gland and that the abundance of TuSp1 mRNA exceeds that of the ECPs at all stages (Garb & Hayashi 2005; Hu et al. 2006). It has been proposed that ECP-1 and -2 function as intermolecular linkers within the tubuliform silk fiber through the formation of disulfide bridges (Hu et al. 2006). The difference in transcript levels of ECP-2 compared to ECP-1, however, has implications for the putative role of these proteins in fiber assembly. ECP-2, with its potential for disulfide bond formation at its N-

terminus and beta-pleated sheet formation at its C-terminus, appears to be required in greater abundance and at an earlier stage in silk protein synthesis than ECP-1.

The D0 stage (within 24 h following egg case production) is not represented in the profile (Fig. 2) since the cycle threshold (C_t) values from this population of spiders were used in the calculation of relative abundance (Pfaffl 2001). D0 C_t values (number of thermocycles required to detect PCR product above background) were consistently higher than any other stage for all transcripts, supporting the assumption that mRNA levels are lowest at this stage. It should be noted, therefore, that the relative abundance of mRNA observed at D1 represents a marked increase from the reduced transcript levels found at D0. The levels of TuSp1 and ECP mRNAs detected in the D1 and D2 tubuliform glands are comparable to the abundance found in late vitellogenesis. Further research is required to determine the protein synthetic activity of these post-egg case glands.

Analysis of silk gene transcripts in the tubuliform glands of black widows has revealed that transcript levels are associated with vitellogenesis, but each gene displays a distinct pattern. Given the relative amounts and profile of each silk transcript described in this paper, it seems unlikely that a single transcriptional regulatory mechanism could account for the expression of all three genes. These results support the use of *Latrodectus* tubuliform glands as a powerful model system for further investigation into the details of the regulatory mechanisms of spider silk gene expression. Future comparative studies of silk gene expression across species and gland types will add new dimensions to our understanding of both the mechanistic and evolutionary elements of silk synthesis in the impressive diversity of silk glands in spiders.

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Agnostopelma: a new genus of tarantula without a scopula on leg IV (Araneae: Theraphosidae: Theraphosinae)

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Abstract. The new genus *Agnostopelma* Pérez-Miles & Weinmann is proposed for the type species *A. tota* n. sp. and *A. gardel* n. sp. from Boyacá, Colombia. *Agnostopelma* build shelters under stones at high elevation. The new genus is unusual in lacking tarsal scopulae on its posterior legs and in having few labial cuspules and short leg tarsi in females.

Keywords: Colombia, spider legs, spider systematics

Taxonomists have considered the presence of scopulae on the metatarsi and tarsi of legs I–IV an important synapomorphy of the Theraphosidae, with a parallelism in the Barychelidae (Raven 1985, 1994; Pérez-Miles et al. 1996). They interpreted the presence of dense tarsal scopulae as synapomorphic for most subfamilies of Theraphosidae, with exception of Ischnocolinae (Raven 1985). Goloboff's cladogram (1993:fig. 27, table 4) indicated that the presence of dense tarsal scopulae on the anterior legs is a synapomorphy of Theraphosinae + *Ischnocolus*.

The condition of the tarsal scopulae has been an important taxonomic tool within Theraphosidae, intensively studied for more than a century (Simón 1892; Pocock 1897; Gerschman de Pikelin & Schiapelli 1973; Raven 1985; Pérez-Miles 1994; Guadanucci 2005). In several groups, the condition varies over ontogenetic development, being divided in juveniles and becoming entire in adults (Gerschman de Pikelin & Schiapelli 1973; Pérez-Miles 1994). The characteristics of the scopulae (entire or divided) were used to diagnose subfamilies and genera, but Pérez-Miles (1994) found that divided scopulae were usually present in small theraphosids, while the entire form was present in large tarantulas, bringing its phylogenetic value into question. Guadanucci (2005) did not confirm this trend in the Ischnocolinae, where scopula condition could contribute valuable phylogenetic information. Previously unpublished data also showed some exceptions to Pérez-Miles' (1994) results in the Theraphosinae. For these reasons, tarsal scopula condition seems an important characteristic to be considered in phylogenetic studies of Theraphosidae, followed in the methodology of Guadanucci (2005).

Examining spiders collected by collaborators in the Departmento de Boyacá, Colombia and by one of us (DW), we noted that some specimens of Theraphosidae lack the usual tarsal scopulae on legs IV (Figs. 1, 2). Ventral faces of tarsi IV are mainly covered by conical setae that are not orthogonal to the cuticle surface; intercalated to these, there are few setae with curved apices similar to scopulae setae but not erected. This could be an extreme case of anterior-posterior gradation as indicated by Raven (1985) for some leg characters in the Mygalomorphae.

These specimens share the main synapomorphies of the Theraphosinae: extended subtegulum, keels on palpal organ, and Theraphosinae types of urticating hairs, which suggest that these spiders are a new genus placed in this subfamily. Male and female of the type species and a second species of this new genus are here described.

METHODS

Abbreviations.—AME = anterior median eyes, ALE = anterior lateral eyes, PME = posterior median eyes, PLE = posterior lateral eyes, OQ = ocular quadrangle (including lateral eyes), d = dorsal, p = prolateral, r = retrolateral, v = ventral, FCE = arachnological collection of the Facultad de Ciencias, Montevideo, Uruguay. All measurements are in millimeters, geographical coordinates in parentheses are approximate, and not taken by GPS. Spination description follows Pérez-Miles (1998) and Pérez Miles & Locht (2003). Drawings were made with a camera lucida.

SYSTEMATICS

Family Theraphosidae Thorell 1869

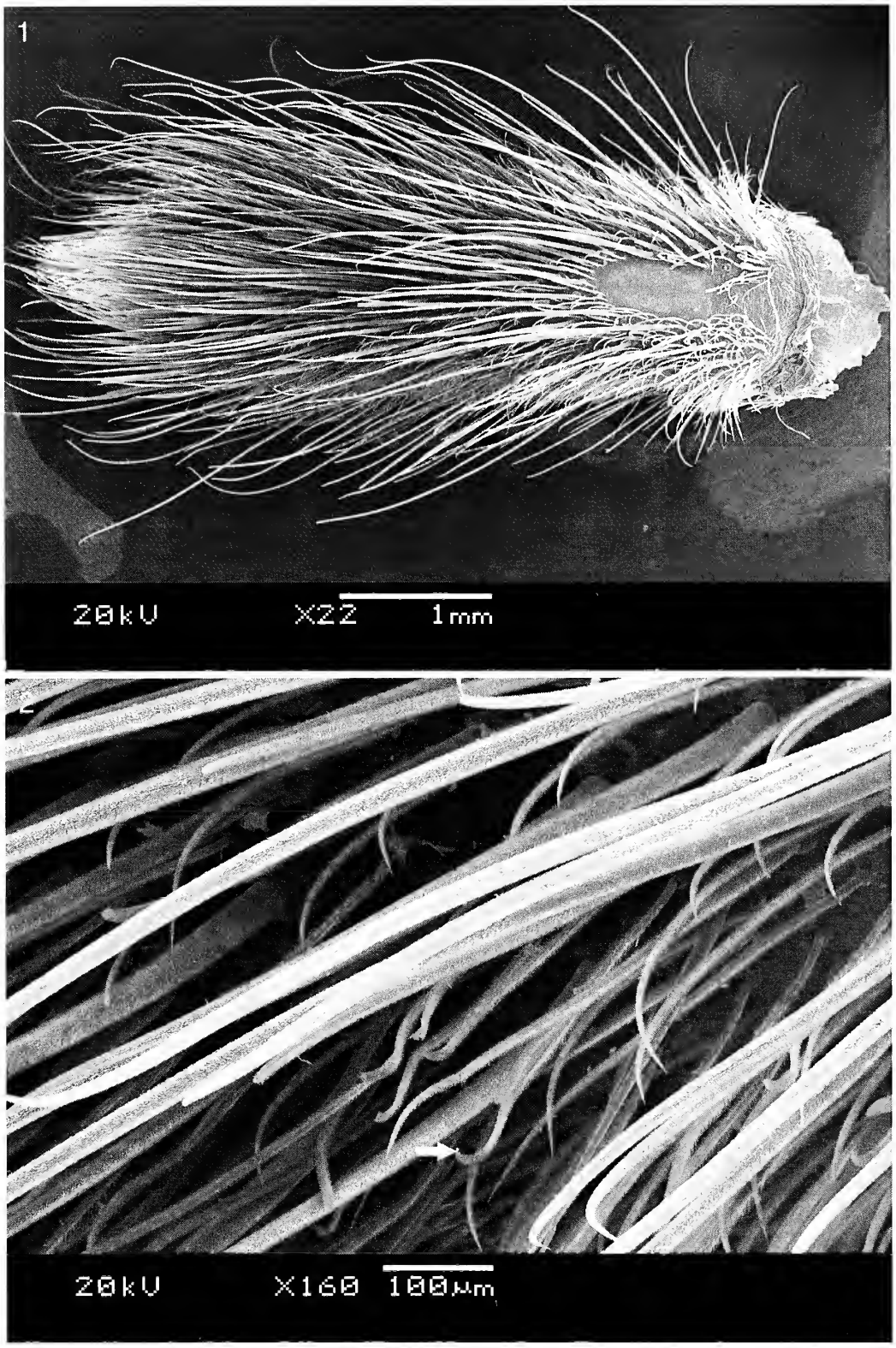
Agnostopelma Pérez-Miles & Weinmann new genus

Type species.—*Agnostopelma tota* Pérez-Miles & Weinmann new species

Etymology.—*Agnostopelma* (neuter) is a composition of two Greek words: *Agnostos*, which means "unknown" and *pelma*, which means "sole of the foot." The name makes reference to the absence of scopulae on leg IV of this tarantula, which is unusual in Theraphosidae.

Diagnosis.—Differs from other known genera of Theraphosidae except the Ischnocolinae genus *Acanthopelma* in the absence of tarsal scopulae on legs IV; from *Acanthopelma* in the presence of abdominal urticating setae, keels on palpal organ and the absence of rigid spines on ventral tarsi; and from most genera of Theraphosinae in the reduced number of labial cuspules. Additionally, females differ from most genera except *Magulla* Simon 1892 in their very short tarsi. Also differs radically from *Magulla* in the absence of male tibial apophysis and in the morphology of palpal organ and spermathecae.

Description.—Medium-sized spiders. Carapace oval to subcircular, slightly hirsute, with longer marginal hairs. Eye tubercle distinctly sub-rectangular, clypeus narrow, anterior row of eyes procurved, posterior recurved, a group of strong setae present on the median anterior margin of the tubercle. Eye tubercle oval, wider than long (Fig. 3). Fovea transverse, straight. Chelicerae without rastellum, strong, with teeth on



Figures 1, 2.— *Agnostopelma tota* female. 1. (Top) Tarsus of right leg IV, ventral view; 2. (Bottom) Close-up of setae (arrow shows curved hairs).

the promargin and smaller teeth in the retromargin (except female *A. tota*), intercheliceral tumescence absent in males. Labium wider than long, with 1–6 cuspules on the subapical margin (Fig. 4). Labiosternal groove narrow, homogeneous. Maxillae with the prolateral distal angle very pronounced, 46–121 cuspules present on prolateral proximal angle. Sternum oval, elongated in both sexes (Fig. 5); six oval sternal sigilla, posterior separated from the margin by almost one length. Stridulatory apparatus absent. Spination as in species description. Male without tibial apophysis. Female with leg

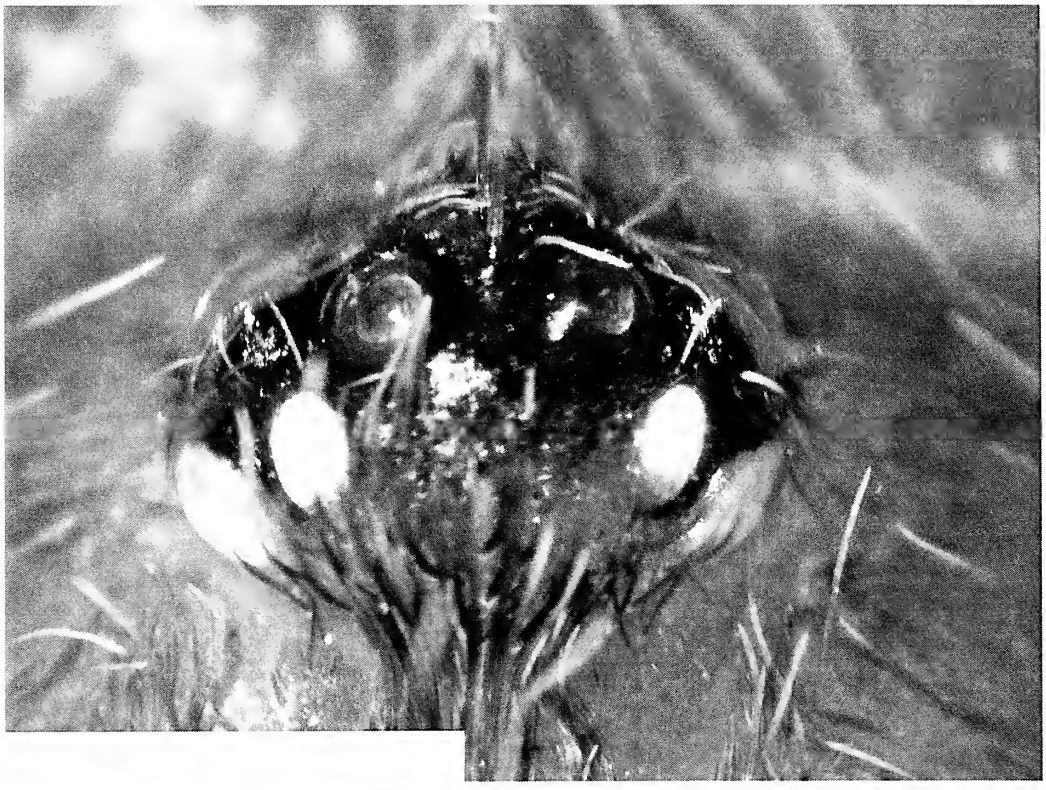


Figure 3.—*A. tota* male, ocular tubercle (scale = 1 mm).

Figure 4.—*A. tota* male, labium and maxillae (scale = 1 mm).

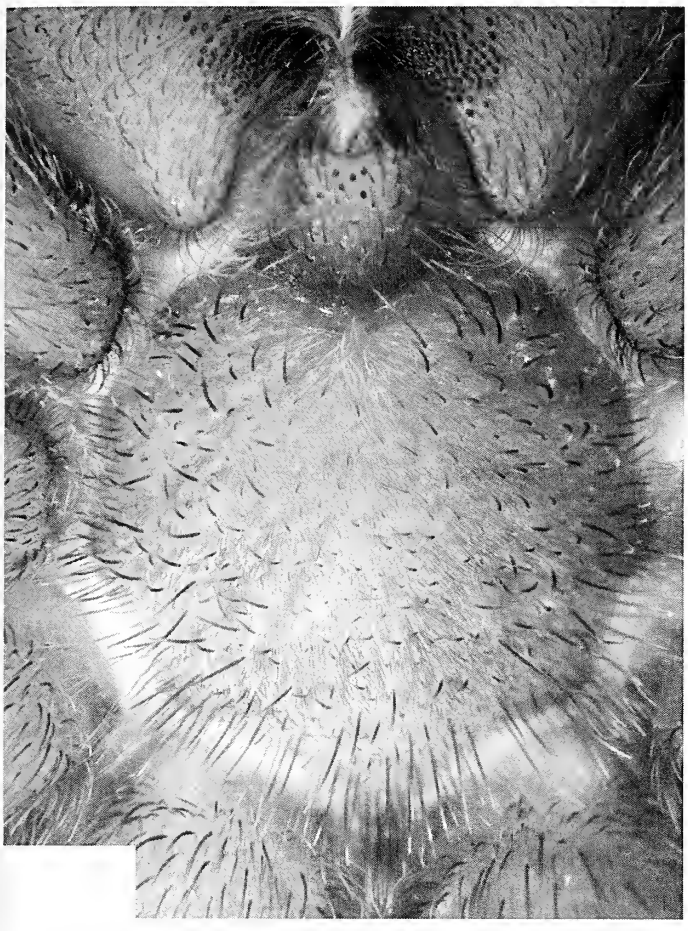


Figure 5.—*A. tota* male, sternum (scale = 1 mm).

tarsi shorter than patellae. Scopulae retrolateral, absent on femur IV. Dense scopulae on tarsi I, II; slight scopulae on tarsi III; tarsi IV without scopulae, with long, conical setae and few shorter, curved setae (Figs. 1, 2). Metatarsi I and II scopulate on their distal portion, III and IV ascopulate. Tricobothria of three types on tarsi, clavate short, filiform long, and fusiform medium sized in a disordered dorsal longitudinal pattern. Metatarsi and tibiae with only filiform tricobothria in a median, longitudinal, dorsal stripe. Third claw absent on all tarsi. Two tarsal claws with 3 teeth in a median ventral line. Claw tufts dense, bilobate, present on all tarsi. Four spinnerets: PLS with three segments, apical digitiform, PMS mono-segmentated.

Distribution.—Colombia: Boyacá: Mongui, Laguna de Tota, Belén.

Agnostopelma tota Pérez-Miles & Weinmann new species
Figs. 1, 2, 3–10, Tables 1, 2

Types.—COLOMBIA: Holotype male, *Dep. Boyacá*: Mongui (5°43′11″N, 72°50′0″W), 2 August 1997, D. Weinmann & F. Pribik. Paratypes: 1 female and 2 males from the same locality as the holotype and a female from *Dep. Boyacá*: Laguna de Tota (5°36′34″N, 72°53′56″W), 6 March 1994, D. Weinmann. All specimens deposited in FCE.

Etymology.—The specific epithet is a noun in apposition, which refers to the name of a lake sacred to the Muisca indigenous people (of the linguistic family of the Chibchas) and the place where one of the paratypes was captured.

Diagnosis.—Males differ from *A. gardel* (see below) in the absence of clear bands in leg articulations and by the greater number of maxillary cuspules (108–121); females differ in the spermathecal receptacles fused at their basis (Fig. 10).

Description.—*Holotype male* (Fig. 6): Total length, not including chelicerae or spinnerets, 17.9. Carapace length 8.5,

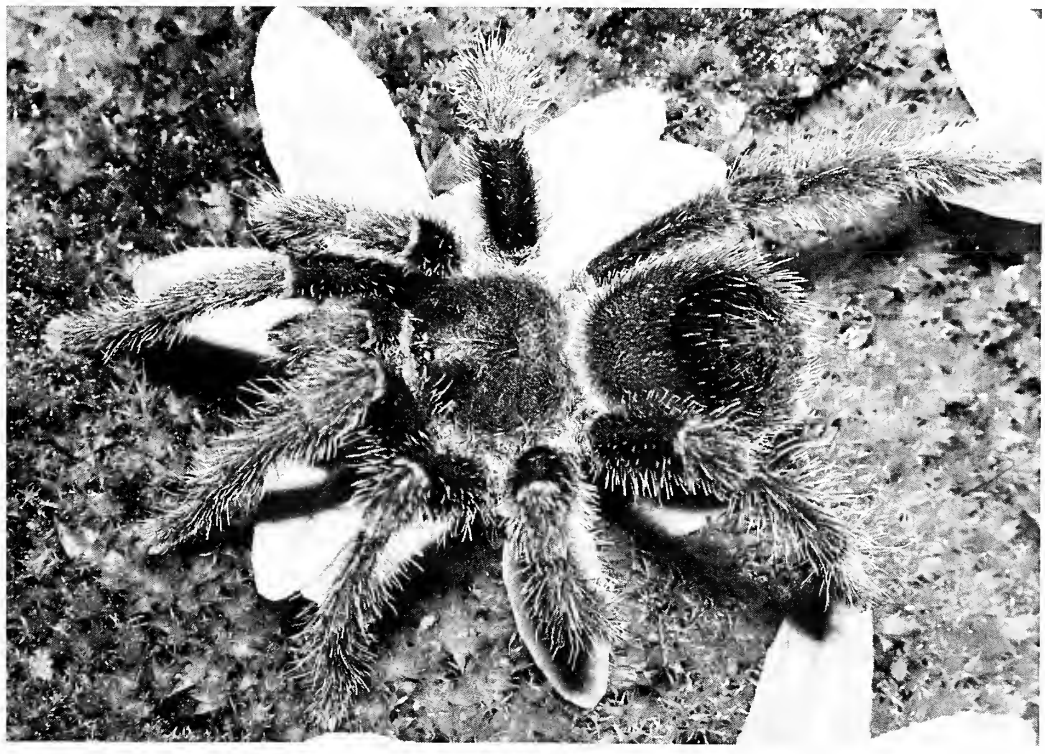
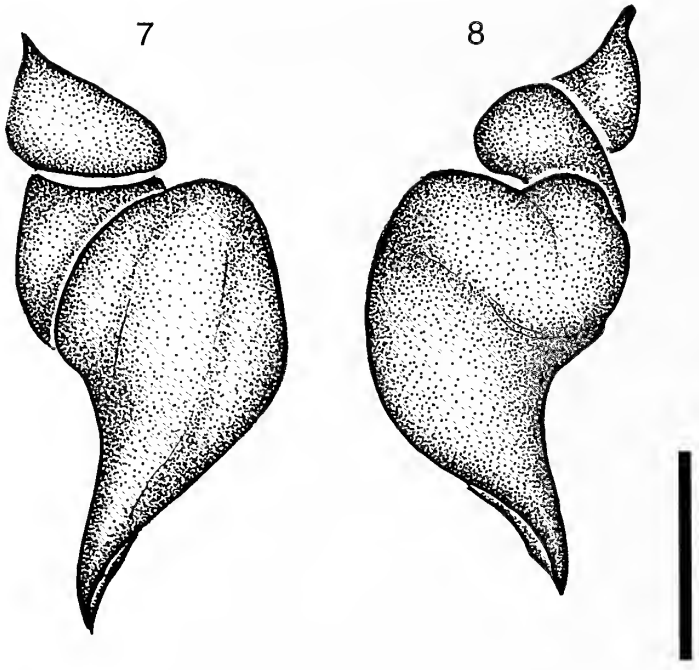


Figure 6.—*A. tota* male, habitus.



Figures 7, 8.—Right palpal organ of male *A. tota*. 7. (Left) Prolateral view; 8. (Right) Retrolateral view (scale = 1 mm).

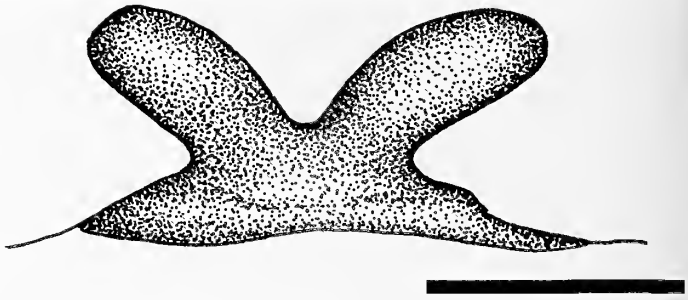


Figure 10.—Spermathecae of female *A. tota*, ventral view (scale = 1 mm).

width 8.3. Anterior eye row slightly procurved, posterior recurved. Eyes sizes and interdistances: AME 0.30, ALE 0.56, PME 0.30, PLE 0.50, AME–AME 0.30, AME–ALE 0.08, PME–PME 0.72, PME–PLE 0.04, ALE–PLE 0.14, OQ length 0.86, width 1.56, clypeus 0.20. Fovea transverse straight, width 1.40. Labium sub-semicircular, anterior edge curved, length 1.30, width 1.40, with 6 cuspules in a group in the center of the anterior half. Maxillae sub-rectangular, anterior prolateral and posterior retrolateral angles produced, with 121/108 cuspules in a group on the proximal prolateral angle. Sternum length 4.4, subcircular, posterior sigilla oval, narrow, submarginal, long setae on margins. Chelicerae with 11 teeth on basal promargin, 5–7 basal retrolateral teeth smaller, intercheliceral tumescence absent. Scopulae: tarsi I–II densely scopulated; I distally divided with longer conical setae; II divided by wide band of such setae; III slightly scopulate, divided by longer conical setae; and IV without scopulae, with long conical setae and lower layer of few curved setae (as in female, Figs. 1, 2). Metatarsi I and II scopulate on their apical half, III and IV not scopulate. Tibia I without apophysis. Palpal organ

Figure 9.—Female *A. tota*, habitus.

Table 1.—*Agnostopelma tota* sp. n., male holotype, length of legs and palpal segments.

	I	II	III	IV	Palp
Femur	9.1	7.7	7.4	8.1	5.3
Patella	3.7	3.7	3.3	4.1	2.8
Tibia	7.5	5.8	5.2	6.5	3.0
Metatarsus	4.5	4.4	7.0	9.3	—
Tarsus	4.1	3.9	3.7	4.3	1.7

Table 2.—*Agnostopelma tota* sp. n., female paratype, length of legs and palpal segments.

	I	II	III	IV	Palp
Femur	7.5	6.5	5.9	6.9	6.0
Patella	4.3	4.3	4.1	4.2	3.7
Tibia	5.2	4.2	3.5	4.6	4.0
Metatarsus	3.0	3.7	4.7	6.6	—
Tarsus	2.2	2.0	2.3	2.8	3.5

piriform, with prolateral superior and inferior keels (Figs. 7, 8). Length of leg and palpal segments in Table 1, femora III slightly incrassate. Spination: femora I–IV and palp 0. Patellae I–IV and palp 0. Tibia I, 5V 1D; II, 4V 1R; III, 5V 6P 2R; IV, 7V 5P 5R; palp, 1P. Metatarsi I, 5V 2R; II, 5V 2P 1R 2D; III, 12 V 6P 3R 3D; IV, 20V 10P 4R. Tarsi I–IV and palp 0. Color: Cephalothorax, legs, and abdomen dorsally dark brown, ventrally lighter; longer hairs with lighter tips all over abdomen and legs. Types III and IV, urticating hairs present; urticating hairs of intermediate length and morphology between III and IV present.

Female (Fig. 9): Total length, excluding chelicerae and spinnerets, 24.1. Carapace length 11.1, width 11.0. Anterior eye row slightly procurved, posterior recurved. Eye sizes and interdistances: AME 0.28, ALE 0.44, PME 0.30, PLE 0.54, AME–AME 0.34, AME–ALE 0.20, PME–PME 0.96, PME–PLE 0.12, ALE–PLE 0.24, OQ length 1.06, width 1.88, clypeus 0.10 wide. Fovea transverse, straight, width 2.80. Labium subsemicircular, anterior edge curved, length 1.80, width 2.0, with 6 cuspules in a group in the center of the anterior half. Maxillae subrectangular, anterior prolateral and posterior retrolateral angles with 97/101 cuspules in group on the proximal prolateral angle. Sternum length 5.0, subcircular, posterior sigilla oval, narrow, submarginal, long setae on the periphery. Chelicerae with 11 teeth on the promargin, basal teeth absent, intercheliceral tumescence absent. Scopula: tarsi I–II densely scopulated; I distally divided with longer conical setae, II divided by wide band of such setae; III slightly scopulated, divided with longer conical setae; and IV without scopula but with long, conical setae and a lower layer of few curved setae (Figs. 1, 2). Metatarsi I–IV not scopulate. Length of leg and palpal segments in Table 2. Femur III not incrassate. Spination: femora I–IV and palp 0. Patellae I–IV and palp 0.Tibia I, 0; II, 0; III, 1V 2P 1R; IV, 1V 2P, palp 2V. Metatarsi I, 2V; II, 3V 4P; III, 8V 6P 7R; IV, 10V 9P 4R. Tarsi I–IV and palp 0. Two tubular spermathecal receptacles fused at their bases (Fig. 10). Color: cephalothorax, legs, and abdomen dorsally dark brown, ventrally lighter; longer hairs with lighter tips all over abdomen and legs. Types III and IV, urticating hairs present; urticating hairs of intermediate length and morphology between III and IV, present.

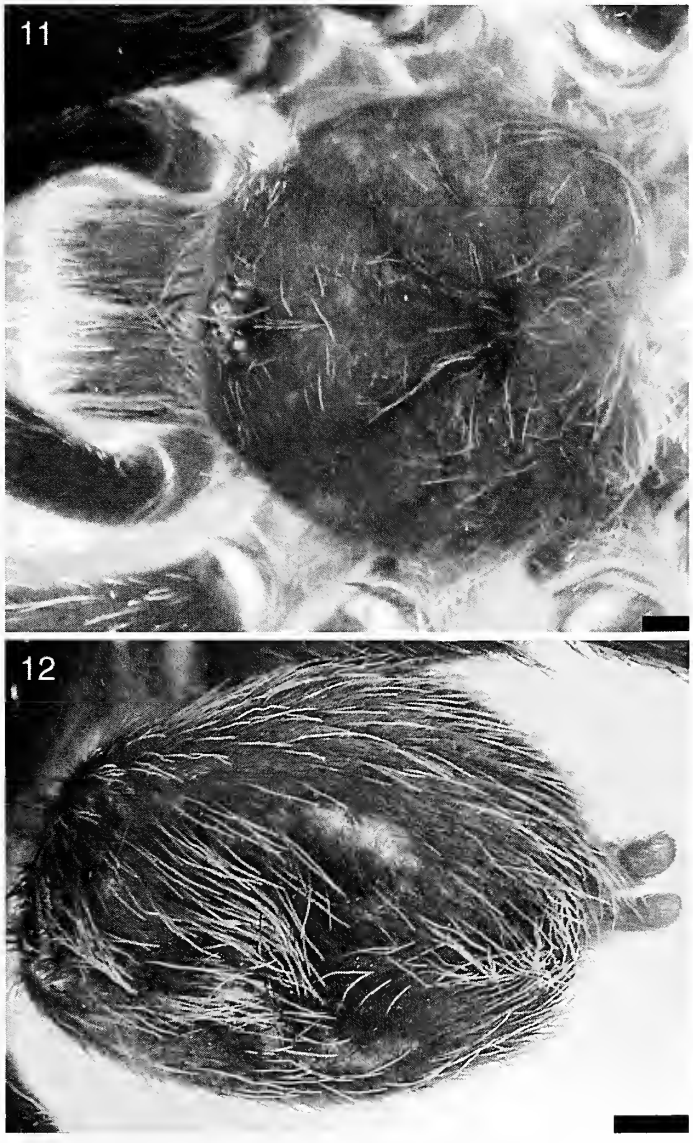
Agnostopelma gardel Pérez-Miles & Weinmann new species
Figs. 11–17, Tables 3, 4

Types.—COLOMBIA, holotype male, *Dep. Boyacá*: Belen (road to Soata) (6°19′60″N, 72°42′0″W), 3 August 1997, D. Weinmann & F. Pribik. Paratypes: two females from same locality. All specimens deposited in FCE.

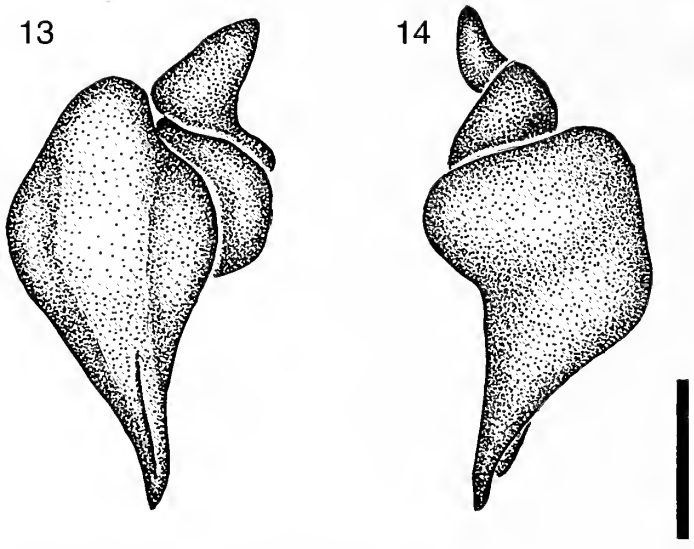
Etymology.—The specific epithet is a noun in apposition, which refers to the most famous Uruguayan tango singer, Carlos Gardel, born in Tacuarembó (1887) and died in Medellín, Colombia (1935).

Diagnosis.—Males differ from those of *A. tota* in presence of clear bands on leg articulations (patellae, tibiae, metatarsi) and in smaller number of maxillary cuspules (46–84). Females differ in separated spermathecal receptacles (Fig. 17) and in the presence of basal teeth on chelicerae.

Description.—*Holotype male* (Figs. 11, 12): Total length, not including chelicerae or spinnerets, 17.0. Carapace length



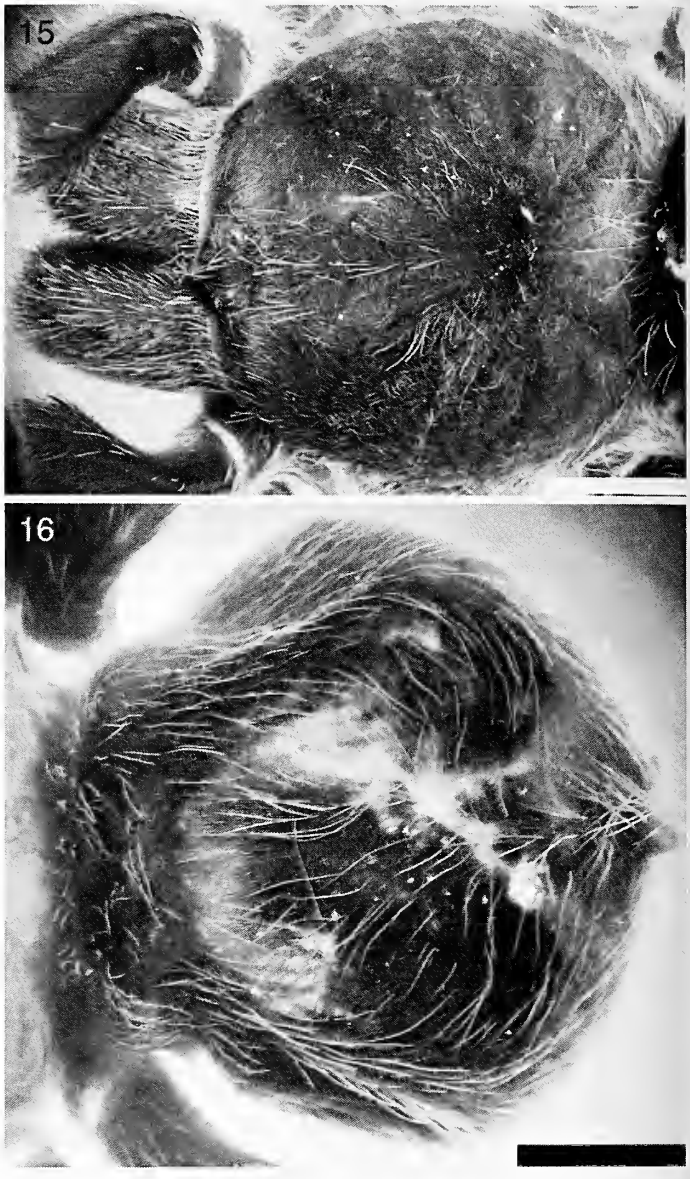
Figures 11, 12.— *Agnostopelma gardel* male, dorsal view. 11. (Top) Carapace; 12. (Bottom) Abdomen. (scales = 1 mm).



Figures 13, 14.— Left palpal organ of male *A. gardel*. 13. (Left) Prolateral view; 14. (Right) Retrolateral view (scale = 1 mm).

8.7, width 8.5. Anterior eye row slightly procurved, posterior recurved. Eye sizes and interdistances: AME 0.26, ALE 0.44, PME 0.20, PLE 0.30, AME–AME 0.22, AME–ALE 0.08, PME–PME 0.68, PME–PLE 0.06, ALE–PLE 0.14, OQ length 0.70, width 1.36, clypeus 0.12. Fovea transverse straight, width 2.30. Labium sub-semicircular, anterior edge notched, length 1.40, width 1.50, with 1 cuspule near anterior edge, maxillae subrectangular, anterior prolateral and posterior retrolateral angles produced, with 84/77 cuspules in a group in the proximal prolateral angle. Sternum length 4.0, subcircular, posterior sigilla, oval, submarginal long setae all over the surface. Chelicerae with 11 teeth on the promargin, plus 6 smaller teeth on the retromargin; intercheliceral tumescence absent. Scopulae: tarsi I–II densely scopulated; I distally divided with longer conical setae, II divided by wide band of such setae; III slightly scopulated, divided with longer conical setae; and IV without scopulae but long conical setae with a sparse lower layer of curved setae (as in female *A. tota*: Figs. 1, 2). Metatarsi I and II scopulate on their apical quarter, III and IV ascopulate. Tibia I without apophysis. Palpal organ piriform, with prolateral superior and inferior keels (Figs. 13, 14). Length of leg and palpal segments in Table 3, femora III incrassate. Spination: femora I–IV and palp 0. Patellae I–IV and palp 0. Tibia I, 4V 2P 1R; II, 3V 2P 1R 2D; III, 5V 2P 1R, 2D; IV, 6V 4P 2R; palp 0. Metatarsi I, 6V 2P 2R; II, 7V 4P 2R; III, 8V 7P 3R; IV, 12V 7P 8R. Tarsi I–IV and palp 0. Color: cephalothorax, legs, and abdomen dorsally dark brown, ventrally lighter, clearer bands on leg articulations (femur, patellae, tibia metatarsi), longer hair with lighter tips all over abdomen and legs.

Female (Figs. 15–17): Total length, excluding chelicerae and spinnerets 28.0. Carapace length 12.5, width 12.5. Anterior eye row slightly procurved, posterior recurved. Eyes sizes and interdistances: AME 0.28, ALE 0.40, PME 0.24, PLE 0.64, AME–AME 0.52, AME–ALE 0.08, PME–PME 1.20, PME–PLE 0.14, ALE–PLE 0.20, OQ length 0.96, width 1.96, clypeus 0.16. Fovea transverse, straight, width 3.50. Labium sub-semicircular, anterior edge slightly notched, length 2.50, width 2.5, with 3 cuspules near the anterior edge, maxillae sub-



Figures 15, 16.—*Agnostopelma gardel* female, dorsal view. 15. (Top) Carapace; 16. (Bottom) Abdomen (scales = 5 mm).

rectangular, anterior prolateral and posterior retrolateral angles produced, with 63/56 cuspules in a group on the proximal prolateral angles. Sternum length 5.3, sub-circular, posterior sigilla oval, narrow, submarginal, long setae all over

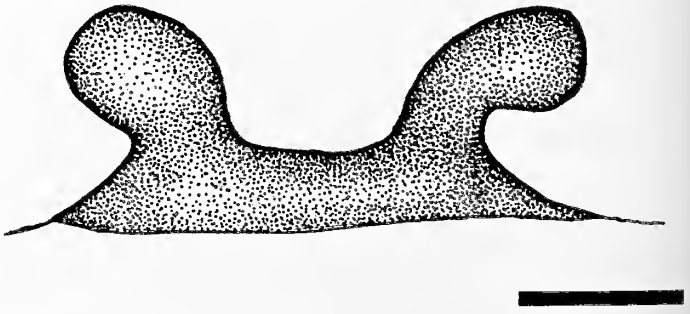


Figure 17.—Spermathecae of female *A. gardel*, ventral view (scale = 1 mm).

Table 3.—*Agnostopelma gardel* sp. n., male holotype, length of legs and palpal segments.

	I	II	III	IV	Palp
Femur	8.8	8.5	8.1	8.8	5.6
Patella	4.2	4.2	3.8	4.4	3.5
Tibia	7.5	6.4	5.5	7.5	3.9
Metatarsus	5.3	5.5	7.4	10.5	—
Tarsus	3.5	3.6	3.7	4.3	2.3

Table 4.—*Agnostopelma gardel* sp. n., female paratype, length of legs and palpal segments.

	I	II	III	IV	Palp
Femur	9.5	8.8	7.5	9.5	7.6
Patella	5.1	4.6	4.3	5.2	4.6
Tibia	7.2	5.3	4.7	6.3	5.5
Metatarsus	3.3	3.6	5.4	8.1	—
Tarsus	2.5	2.3	2.3	3.4	3.8

the surface. Chelicerae with 11 teeth on the promargin, 6 smaller teeth on the retromargin. Scopulae: tarsi I–II densely scopulated; I distally divided with longer conical setae, II divided by wide band of such setae; III slightly scopulated, divided with longer conical setae; and IV without scopulae but with long conical setae and lower layer of a few curved setae (as in *A. tota*, Figs. 1, 2). Metatarsi I–IV ascopulate. Length of leg and palpal segments in Table 4. Femur III not incrassate. Spination: femora I–IV and palp 0. Patellae I–IV and palp 0.Tibia I, 3V; II, 3V 1D; III, 2V 2P 1R 1D; IV, 7V 3P 2R; palp 2V. Metatarsi I, 2V; II, 3V 2P; III 6V 5P 2R 4D; IV, 6V 3P 6R 4D. Tarsi I–IV and palp 0. Two tubular spermathecal receptacles not fused at their bases (Fig. 17). Color: cephalothorax, legs and abdomen dorsally dark brown, ventrally lighter, clear bands on leg articulations (femur, patella, tibia, metatarsus); longer hairs with lighter tips all over the abdomen and legs.

DISCUSSION

Agnostopelma has an unusual tarsal scopula condition, unknown up to now within the Theraphosinae and here considered as a generic apomorphy. Theraphosid tarsal scopulae can be entire, with homogeneous spatulate setae, or divided by a longitudinal stripe of conical longer setae; this division is related to spider size in most Theraphosinae (Pérez-Miles, 1994), but not in the Ischnocolinae (Guadanucci, 2005). The Ischnocolinae genus *Acanthopelma* F.O. Pickard-Cambridge 1897 has different tarsal scopulae, as indicated, but Raven (1985) divided them by rigid spiniform bristles, although they obviously lack the apomorphies of Theraphosinae (urticating setae, keels on palpal organ, subtegulum extended). Ontogenetic changes have been reported for some theraphosids that have divided scopulae as juveniles and entire ones as adults (Gerschman de Pikelin & Schiapelli 1973; Pérez-Miles 1994). Anterior-posterior gradations were also described in the condition of the tarsal scopulae (ranging from entire in forelegs to divided in hind legs) Raven (1985), which suggests the importance of examining each tarsus, as Guadanucci (2005) did with Ischnocolinae.

Agnostopelma scopulae exhibit anterior-posterior gradations with an increased division from leg I to III and absence on leg IV, along with dominant conical setae. These conical setae are similar to those of the medial stripe found in divided scopulae (called *type b* hairs in lycosids by Rovner 1978) and were stated to have more of a traction function than an adhesive one by Pérez-Miles (1994). This characteristic suggests walking rather than climbing habits for *Agnostopelma* species. Recently Gorb and coworkers (2006) described tarsal silk-like secretions with possible adhesive functions for the tarantula *Aphonopelma seemanni*. If this secretion were confirmed in other theraphosids, *type b* hairs could help to lift the tarsi.

Other uncommon characters are present in this genus, such as a reduced number of labial cuspules, also found in *Tmesiphantes* Simon 1892, *Hapalotremus* Simon 1903, *Mello-leitaoina* Gerschman & Schiapelli 1960, and some species of *Paraphysa* Simon 1892. Another uncommon character shared with *Magulla* is the presence of very short tarsi in females. The morphology of the spermathecae is very different from all known theraphosid genera, with two divergent digitiform receptacles fused at their base. By the presence of type IV urticating hairs, *Agnostopelma* could probably be placed in the basal group of the Theraphosinae (Pérez-Miles 2000), which also includes *Grammostola* Simon 1892, *Paraphysa*, *Homoeomma* Ausserer 1871, *Plesiopelma* Pocock 1901, and *Maraca* Pérez-Miles 2006 (previously *Iracema*). *Cyriocosmus* is now not considered to belong in this group because it lacks type IV urticating hairs (Fukushima et al. 2005). With a palpal bulb morphology consisting of only one keel, *Agnostopelma* seems to be more similar to *Maraca* than to other genera in this group.

The capture sites are about 3000 m above sea level and have an annual mean temperature of 11° C. All specimens were found in shelters under stones.

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A new orophilous species of the genus *Dasylobus* (Opiliones: Phalangiidae) from Sierra Nevada, Spain

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Abstract. A new species, *Dasylobus nevadensis*, is described; it inhabits high areas of the Sierra Nevada, the highest mountain chain in Spanish mainland. The new species is smaller than all other Iberian *Dasylobus* species and has short, annulated legs, light silver coloration, and is juvenile-like. Together with *Roeweritta carpentieri* (Roewer 1953), this is an orophilous endemic species from Sierra Nevada.

Keywords: Iberian Peninsula, Phalangiinae, new species, taxonomy, Europe

The subfamily Phalangiinae comprises around 25 genera within the Holarctic region, with high representation in tropical Africa (Martens 1978; Staręga 1984; Crawford 1992). In the western Mediterranean area, the subfamily is represented by only three genera (Staręga 1984), *Phalangium* Linnaeus 1758, *Metaphalangium* Roewer 1911, and *Dasylobus* Simon 1878, as well as four endemic Macaronesian genera, *Bunochelis* Roewer 1923, *Metadasylobus* Roewer 1911, and *Parascleropilio* Rambla 1975 from the Canary Islands, and *Ramblinus* Staręga 1984 from Madeira Island.

The genus *Dasylobus* is characterized by a unidentate supracheliceral lamina, a basichelicerite with a dorsal granulated mound, a palpal patella with a mesal, conical/rounded, hairy apophysis, medium to long legs with an incrassate leg I, and a penis with a broad basis, slender trunk and wedge-shaped glans, and sometimes with dorsodistal spoon (Staręga 1976; Martens 1978). Additionally, it has a denticulate carapace, frontal corners and, sometimes, a saddle with a median lighter broad band.

In the Iberian Peninsula the genus is represented by several species, although the status of some of them is not very clear. *Dasylobus echinifrons* Simon 1879 was described from Narbonne, La Clape and Le Vernet (France) although Simon (1879) also reported it from Aranjuez and Sierra Morena (Spain). The known range of *D. graniferus* (Canestrini 1871), extends from Yugoslavia, Austria, Switzerland, Italy and France to Spain (Chemini 1989), where it was reported as *Eudasylobus nicaeensis* (Thorell 1876) from Toledo (Roewer 1923), a synonym of *D. graniferus* according to Chemini (1986), and several sites from Sierra de Guadarrama (Simon 1879; Rambla 1967; Martens 1978). *Dentizacheus ibericus* Rambla 1968 was described from Torre de Moncorvo (Portugal) and transferred to *Dasylobus* by Prieto (2003).

Three other nominal species have been removed from *Dasylobus*: *D. lusitanicus* Roewer 1956, which was described from Coimbra (Portugal) but Staręga (2004) redescribed it as *Metaphalangium lusitanicum*, and *Eudasylobus rondaensis* Kraus 1959, described from Sierra del Oreganal near Ronda (Málaga) and localities in Tarragona (Sierra de Monsech) and Murcia (Sierra Espuña), which was transferred to *Dasylobus* by Prieto (2003) and synonymized with *M. lusitanicum* by Staręga (2004). The third nominal species, *De. zuluetai* Rambla 1959, described from El Escorial (Madrid), was synonymized with *Da. echinifrons* by Staręga (1973) who later (Staręga 2004) placed it in the synonymy of *M. lusitanicum*.

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The Sierra Nevada (Fig. 1), located in Andalusia between Granada and Almería provinces, is the highest mountain range on the Iberian Peninsula, reaching 3,478 m in the Mulhacen and 3,395 m in the Veleta. The chain was formed during the Alpine Orogeny, following the collision between African and European plates, and the central massif is mainly composed of heavily deformed metamorphic rocks, mainly mica schists, locally with gneiss, quartzite and amphibolite (López-Bermúdez et al. 1989). The highest part of the range is included in the Sierra Nevada National Park.

Opilionological knowledge about the Sierra Nevada is very scarce because only two species have been previously recorded: *Homalenotus coriaceus* (Simon 1879) recorded by Kraus (1961) from Río Monachil (30SVG60, 2,300 m) and *Roeweritta carpentieri* (Roewer 1953), an endemic monotypic genus recorded from six localities between 2,000 and 3,000 m (Rambla 1960; Marcellino 1967; Barea 2008). During a trip to the region in November 1982, we found many small harvestmen under schistose stones. We routinely considered these specimens as juveniles during sorting, but on close inspection determined that they are adults belonging to an undescribed species, which is described here.

METHODS

Taxonomic methods follow outlines proposed by Pinto-da-Rocha et al. (2007). Body (carapace) width was measured between the incisions of coxae II and III. BLI index is the relation of the femur I length to the carapace width. All measurements are in mm.

Specimens were studied, photographed and drawn with a Nikon SMZ-1500 stereomicroscope provided with a drawing tube and a digital camera. The penis and spermathecae were drawn with a Nikon Optiphot. Photo stacks were combined with the software Helicon Focus, and backgrounds were cleaned with Photoshop.

The specimens studied in this contribution are lodged in the Museo Nacional de Ciencias Naturales, Madrid (MNCN) (holotype and female paratype) and the Departmento de Zoología y Biología Celular, Universidad del País Vasco, Bilbao (ZUPV) (remaining paratypes).

TAXONOMY

Family Phalangiidae Latreille 1802

Genus *Dasylobus* Simon 1878

Dasylobus Simon 1878:ccxviii (footnote).

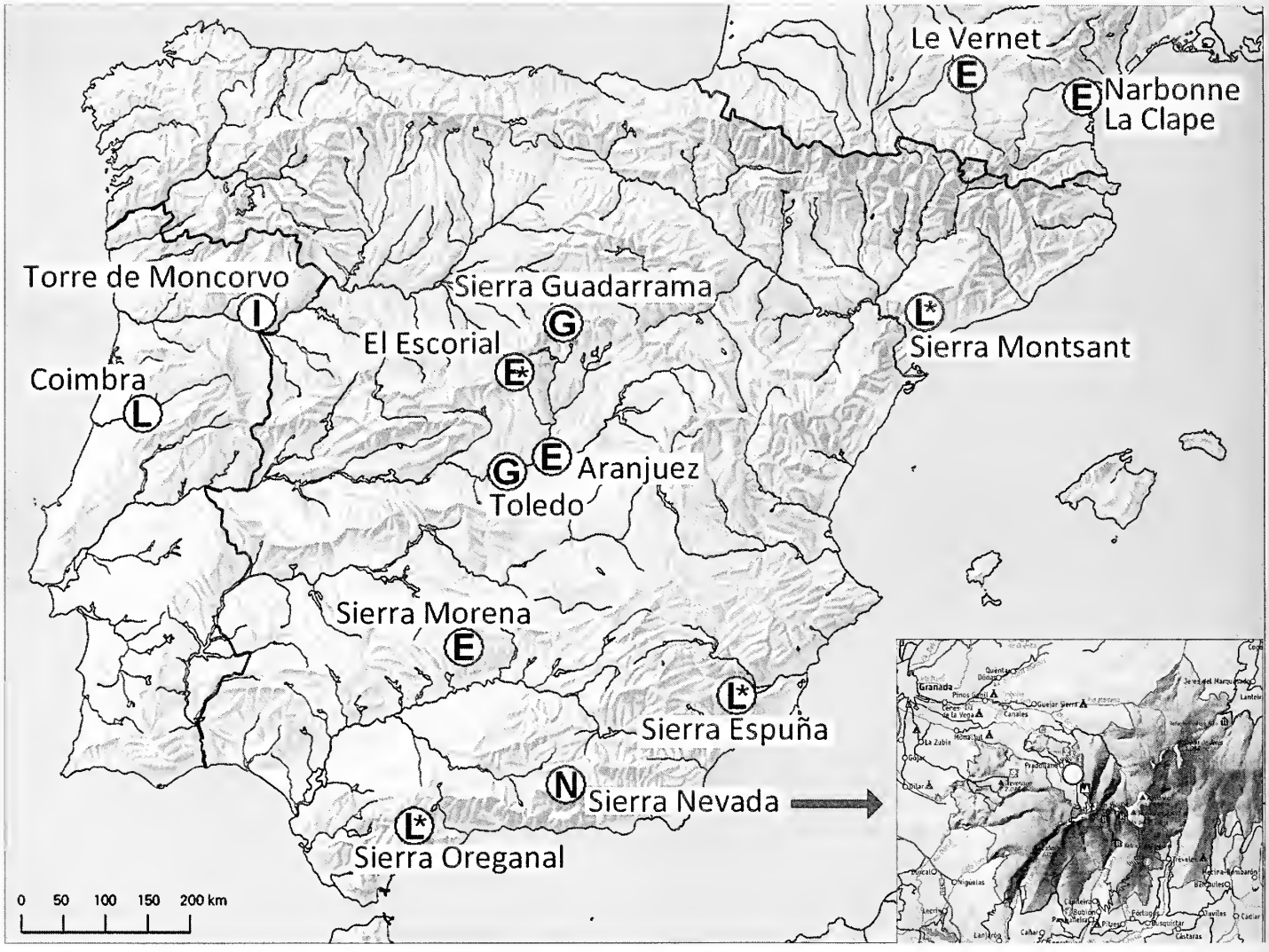


Figure 1.—Known localities of Iberian *Dasylobus* species and *Metaphalangium lusitanicum*. E, *D. echinifrons* (*, locality of the synonym *Dentizacheus zuluetai*); G, *D. graniferus*; I, *D. ibericus*; N, *D. nevadensis* new species; L, *M. lusitanicum* (*, localities of the synonym *Eudasylobus rondaensis*). Right bottom inset, western part of Sierra Nevada National Park with the type locality of *D. nevadensis* new species (white dot).

Eudasylobus Roewer 1911:53. Synonymized by Chemini (1989).

Euplatybuuus Roewer 1912:252. Synonymized with *Eudasylobus* by Staręga (1984).

Parazacheus Lerma 1952:7. Implicitely synonymized with *Dasylobus* by Chemini (1989).

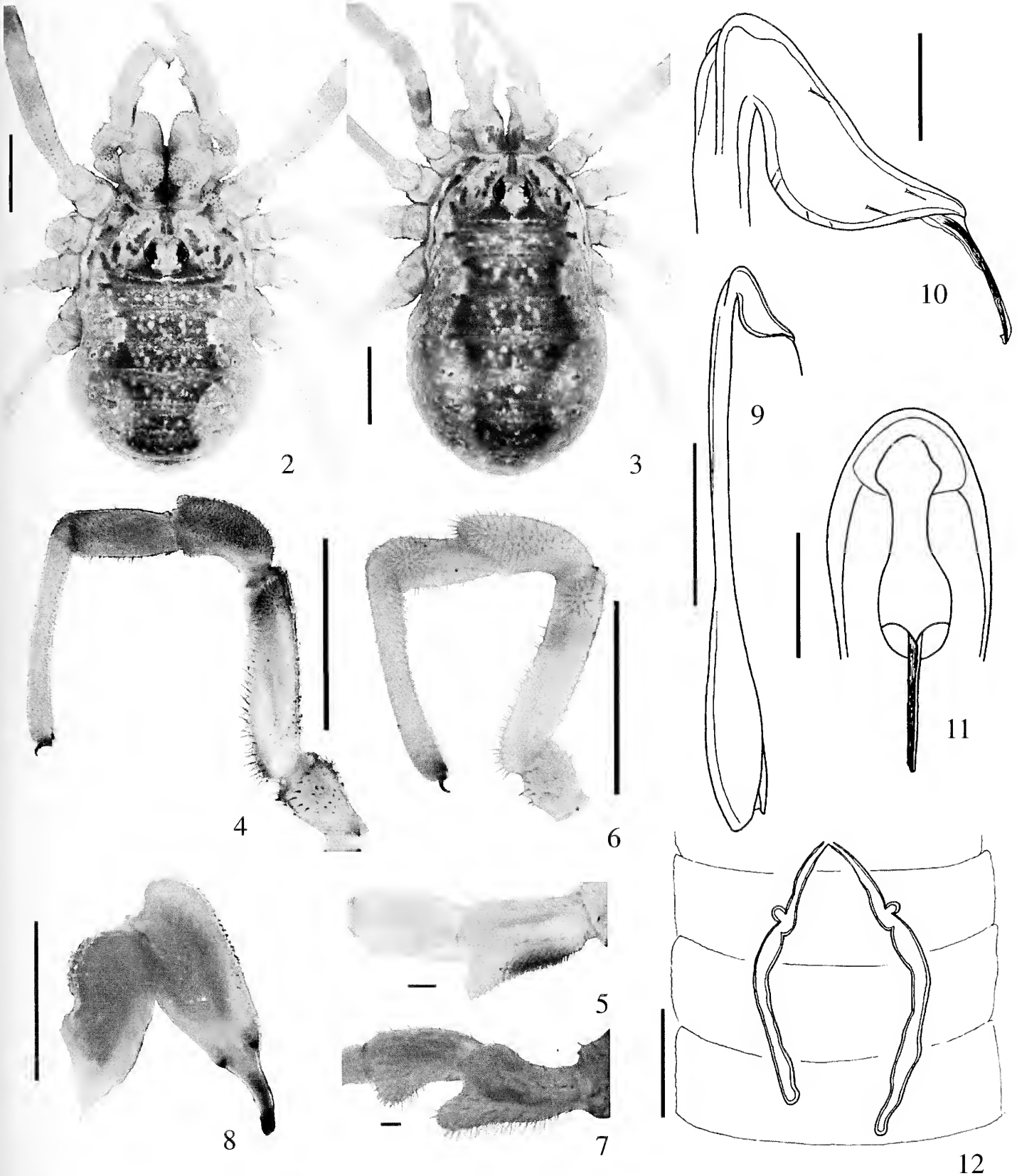
Type species.—*Opilio argentatus* Canestrini 1871 by original designation.

Diagnosis.—Penis shaft continuously narrowed from the basal to the middle part, thence distad almost parallel or little divergent edges, and moderately compressed dorsoventrally. Glans strongly compressed, swollen underneath and plain above. Palp femur smooth, patella with a mesodistal, short, blunt conical apophysis, tibia and tarsus smooth and hairy. Ocularium small, separated by half of its length from the frontal border. Supracheliceral laminae with a pointed granule on each (Martens 1978:290–291 for *Eudasylobus*).

Remarks.—Chemini (1989) reviewed the Italian taxa, concluding that only four species occur in Italy, but the remaining species are poorly known. According to Hallan & Kury (2009), the genus comprises about 20 species distributed within the Mediterranean region, from Asia Minor and Lebanon to Algeria and the Iberian Peninsula, including the larger islands (Cyprus, Crete, Sardinia, Corsica, Balearic), but some entries are doubtful or have been removed recently to synonymy. The Spanish fauna is currently composed of four taxa (Staręga 2004; Prieto 2008); *D. echinifrons* (Simon 1879), *D. graniferus* (Canestrini 1871) and *D. ibericus* (Rambla 1968) from the Spanish mainland, and *D. ferrugineus* (Thorell 1876) from Balearic Islands.

Dasylobus nevadensis new species
(Figs. 2–12)

Type material.—SPAIN: *Granada*: holotype male, Sierra Nevada: road to the Veleta peak (UTM [WGS84]: 30SVG658050), 2,560 m, under stones in a stony slope, 2 November 1982, C.E. Prieto and A. Prieto (MNCN 20.02/17103). Paratypes: 9 males, 11 females, 1 juvenile, same data as holotype (ZUPV/0258bis); 1 female, same data as holotype (MNCN 20.02/17104).



Figures 2–12.—*Dasylobus nevadensis* new species: 2. Male holotype, dorsal view; 3. Female, dorsal view; 4. Male palp, internal view; 5. Male palp tibia and patella, dorsal view; 6. Female palp, internal view; 7. Female palp tibia and patella, dorsal view; 8. Male chelicerae, lateral view; 9. Penis, lateral view; 10. Glans, lateral view; 11. Glans, frontal view; 12. Spermathecae in female ovipositor. Scale bars = 1 mm (Figs. 2–4, 6, 8), 0.5 mm (Fig. 9), 0.1 mm (Figs. 5, 7, 10–12). Figs. 3–12 from male and female paratypes.

Table 1.—Leg measurements and tarsal counts of the male holotype and a female paratype of *Dasylobus nevadensis.*

		Trochanter	Femur	Patella	Tibia	Metatarsus	Tarsus	Total leg	No.tarsomeres
Male	Leg 1	0.32	2.04	0.78	1.90	2.20	3.80	11.04	26
	Leg 11	0.38	3.57	1.00	3.15	3.29	7.03	18.42	45
	Leg 111	0.27	2.13	0.80	1.82	2.63	4.16	11.81	29
	Leg 1V	0.37	2.92	0.83	2.38	3.68	5.52	15.70	32
Female	Leg 1	0.29	1.65	0.70	1.50	1.75	3.01	8.9	27
	Leg 11	0.37	3.14	0.92	2.77	2.85	5.86	15.91	43
	Leg 111	0.29	1.79	0.66	1.55	2.05	3.36	9.70	27
	Leg 1V	0.32	2.61	0.74	2.05	3.05	4.39	13.16	32

Etymology.—The specific epithet refers to the Sierra Nevada, the highest mountain chain on the Iberian Peninsula, which this species inhabits.

Diagnosis.—Belonging to the genus *Dasylobus*, this species can be recognized by its juvenile appearance, small size (3.0–3.9 mm in males and 3.5–4.5 mm in females), short (BLI index, 0.87–1.01 in males and 0.69–0.81 in females), clearly annulated legs, and palpal patella with a conical apophysis.

Description.—*Male holotype:* body length 3.76 mm, carapace width 2.26 mm, femur I 2.04 mm, BLI 0.90.

Carapace (Fig. 2)*:* dorsum smooth except for some scattered denticles, mainly concentrated between ocularium and ozopores. Supracheliceral laminae with one denticle on each lamina. Frontal edge regularly concave, completely unarmed in the center and with tufts of strong denticles on each anterior corner. Ozopores visible from above and with 1–3 denticles near the anterior and posterior corners. Ocularium silver in color, except for dark ocular rings; medium-sized (1/4 of width and 1/3 of length of carapace) with a medial groove and a row of 7–8 denticles in each side around the eyes. Carapace divided by a ledge, parallel to lateral borders, in a central area and two lateral ones; lateral areas with a marginal silvery stripe and four brown patches; central area with two brown close parallel stripes on the preocular region, lateral sides with brown patches separated by silvery color and two triangular patches behind the ocularium. First and second thoracic tergites each with a row of small denticles.

Abdomen: cream ground color. Abdominal scutum smooth, but with several brown sclerotization spots. Dorsal saddle wide, brownish but spotted in white, extending from first thoracic tergite to area V, narrowed in areas I and IV, with area I lined with silvery stripes. Remainder of abdominal surface with brownish dots and whitish halos. Silvery anal operculum. Ventral side cream-colored. Leg coxae smooth and covered with numerous short setae, and fields of brown spots on posterior faces. Genital operculum with irregularly arranged short setae and 2 pairs of close brown spots basally. Posterior border of genital operculum indicated by a brownish 'M'-shaped patch. Abdominal sternites smooth, with scattered setae and rows of brown patches on anterior edges.

Chelicerae (Fig. 8)*:* first segment short, thickened, and with a large dorsal protuberance with irregular surface, more elevated on internal side and covered by granules and scattered setae. Second segment with a dorsal domed protuberance with frontal side covered by granules and setae.

Palps (Figs. 4, 5)*:* trochanter with some dorsal and ventral granules distally. Femur with a ventral field of setae and scarce granules, a basal mesodorsal granular field, a dorsal row of setae and few denticles, and a small distomesal thickening covered by setae. Patella with a distomesal conical apophysis, mesal side densely covered by a setose field, which continues to the apophysis. Tibia with a very small setose distomesal swelling. Tarsus densely covered by setae, mesal side with two inconspicuous rows of microgranules. Femur to tibia each with a dorsal light brown stripe.

Legs: relatively short (BLI = 0.90). Femur, patella, and tibia of leg I thickened. Femur with a whitish basal ring (suture line for autotomy), rounded in transversal section and with five rows of setose granules (13–17 in femur I). Patella with rows of setae and 3 denticles on distodorsal edge. Tibia with 3 dorsal rows of setae and 2 ventral rows of sharp granules, and a small basodorsal denticle; spiracle located retrobasally. Metatarsus with some granules on ventral side and a pseudoarticulation at the distal third. Tarsus with many tarsomeres (26, 45, 28–29, 31–32 in legs I–IV, respectively). Legs brownish, with white annulations located as follows: femur with basal, central and apical rings; apical on patella; central and apical on tibia; basal and surrounding the pseudoarticulation on tarsus.

Penis (Figs. 9–11)*:* 1.75 mm long. Shaft widened basally (0.3 mm), strongly tapering until half of its length (0.1 mm wide), then very gradually widened towards distal end (0.15 mm wide). End of shaft without dorsal excavation. Glans with triangular profile, and apically dilated in frontal view; stylus with a secondary internal point.

Female (Figs. 3, 6, 7)*:* similar to male but abdomen wider and longer, and saddle less profiled. Chelicera normally developed. Palpal patella (Fig. 7) with a more developed apophysis and mesal side covered by longer setae; distomesal apophysis of the tibia more developed and covered by longer setae.

Spermathecae (Fig. 12)*:* long and slender, bent toward the ovipositor axis and extended until the third complete ring, with a small secondary pouch on the first third of its length.

Measurements: see Tables 1 and 2.

Remarks.—The new species has male chelicerae with a large dorsal protuberance on the basal article, palp with a conspicuous distomesal apophysis on the patella and another apophysis on the tibia (much smaller in the male), femur I thickened, anterior corners of the carapace with tufts of strong denticles, and supracheliceral laminae with a denticle on each. These features indicate that the species belongs to the genus *Dasylobus.*

Dasylobus nevadensis is easily distinguished from the other Iberian species. *Dasylobus ibericus* and *D. echinifrons* are bigger (body size 6 mm or larger) and robust, with longer and

Table 2.—Average range size and standard deviation of body, legs, and BLI index of male and females adults of *Dasylobus nevadensis*. Width is the distance between the incisions of coxae II and III. BLI index is the relation of the femur I length to the carapace width.

	Length	Width	Femur I	Leg I	Leg II	Leg III	Leg IV	BLI
Males (n = 9)								
Average	3.49	2.04	1.89	10.18	17.48	11.16	14.61	0.92
Minimum	3.06	1.85	1.62	8.73	14.43	9.24	12.47	0.87
Maximum	3.90	2.26	2.09	11.19	18.55	12.68	15.7	1.01
Standard dev.	0.29	0.14	0.15	0.75	1.59	1.15	1.05	0.04
Females (n = 12)								
Average	4.10	2.15	1.60	8.98	15.74	9.72	13.00	0.75
Minimum	3.50	2.00	1.50	8.52	17.01	9.13	12.44	0.69
Maximum	4.52	2.24	1.67	9.51	9.65	10.67	13.89	0.82
Standard dev.	0.30	0.09	0.05	0.28	0.59	0.41	0.51	0.03

non-annulated legs (Simon 1879; Rambla 1968). *Dasylobus graniferus* is distinguished by the presence of a conspicuous protuberance above the articulation of cheliceral fingers on the second cheliceral segment of the male (Rambla 1967; Martens 1978; Chemini 1989).

Metaphalangium lusitanicum, formerly in *Dasylobus*, has some features in common with *Dasylobus*, but has a short and wide distal apophysis on the palpal patella, longer legs, denticle rows on the ocularium with only 4–5 denticles, basal segment of male chelicerae without dorsal denticulate bump, and lacks a distal apophysis on the palpal tibia.

Another piece of data that partly justifies the specific separation is the date of collection of the specimens; adults of *Metaphalangium lusitanicum* were collected in March and April (Kraus 1959, as *Eudasylobus rondaensis*), while the specimens of *Dasylobus nevadensis* were gathered in November, which implies different life cycles for both species.

Curtis & Machado (2007) review the temporal patterns in harvestmen, and Rambla (1985) and Tsurusaki (2003) discuss phenological patterns for several northern or montane species. Most montane and alpine species show hatching and growth in late spring/summer, and maturation and egg-laying in late summer/autumn, and finally hibernating over winter in the egg stage. *Dasylobus nevadensis* may match that life cycle; in fact, it matches the cycle of *Harmanda nigrolineata* Martens 1987, which occurs in the Himalayan Mountains between 2,400 and 3,500 m (Curtis & Machado 2007, based on data from Martens 1984). According to an ombrotermic diagram for an Astronomic Observatory from the Sierra Nevada at 2,507 m and less than a kilometer from the type locality (Rivas-Martínez et al. 1997), there is a long period between November and April when the temperature is below 0 °C and the terrain is usually fully covered with snow that has fallen during autumn and winter. Therefore, as we stated earlier, this species would hatch, grow, and mature between May and October–November.

On the contrary, only a few harvestmen with distributions throughout southern/xeric regions have been studied. *Trachyrhinus marmoratus* Banks 1894 from the arid regions of Texas is a good example, with the adult stage between January and July, with abundance peak in April (Curtis & Machado 2007). Another more relevant example is *D. graniferus* from southern Italy (Chemini 1989); all data for localities in Calabria and Sicily lie between May and early July. *Metaphalangium lusitanicum* matches this southern life cycle, with hatching and growth occurring in winter, and maturation and egg-laying in spring, due to the strong and longer xeric season of summer/autumn, as the phenological profile of *Opilio insulae* Roewer 1956 in lower elevations in Crete (Chatzaki et al. 2009), a species with a high phenological plasticity because it lives also at 2,000 m, where the snow cover lasts generally from November to May, matching the phenological profile of *Dasylobus nevadensis*.

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SHORT COMMUNICATION

Functional diversity of ladder-webs: moth specialization or optimal area use?

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Abstract. Ladder-webs are built by several orb-web spider species and can be divided into two main groups based on the microhabitat in which they are built, either in open spaces (aerial) or against tree trunks (arboricolous). In Australian ladder-web spiders, *Telaprocera*, the elongated webs are a highly plastic behavioral response to building in space-limited conditions against tree trunks, while the aerial ladder-webs of *Scoloderus* are an adaptation for catching moths. However, the relative importance of moth capture in the construction of elongated webs in arboricolous spiders cannot be determined with existing data. We here present observational and experimental data concerning prey capture in the arboricolous spiders *T. maudae* Harmer & Framenau 2008 and *T. joanae* Harmer & Framenau 2008. We found that moths make up only a small fraction (< 4%) of the diet of *Telaprocera* spiders and that the proportions of major prey orders in webs are representative of available prey. Our experiments indicate that these webs do not function well at retaining moths. However, further data are required before more definite conclusions can be drawn regarding whether these webs are more effective at retaining moths than standard orb-webs.

Keywords: *Telaprocera*, orb-web, moth, prey specialization

Some orb-web spiders build webs that are specifically adapted to catch moths (Stowe 1986). Typical orbs are ineffective at retaining these prey because the scales covering a moth's body detach upon contact with a web, allowing it to fall to safety (Eisner et al. 1964). The ladder-webs of the genus *Scoloderus* Simon 1887 are highly effective moth-capturing devices (Eberhard 1975; Stowe 1978). On the other hand, the ladder-webs of another genus, *Telaprocera* Harmer and Framenau 2008, have been shown to be a response to space limitation (Harmer 2009; Harmer and Herberstein 2009). However, the importance of moth capture in the construction of elongated webs in *Telaprocera* has not been explored. In this study we investigate prey capture in *Telaprocera* spiders for the first time.

Moth specialization occurs in several genera and is usually associated with a reduction in the orb-web (reviewed in Stowe 1986). The best-known example of web reduction for moth specialization occurs in bolas spiders (e.g. *Mastophora* Holmberg 1876), which hunt using a single strand of silk with a sticky mass on the end and attract male moths within range by mimicking female pheromones (Eberhard 1977; Yeargan 1994). Extension of the orb-web to target specific prey is much less common than web reduction. The exception is the ladder-web of the genus *Scoloderus*. Eberhard (1975) suggested extreme elongation in ladder-webs assists in retaining moths because as they tumble down the web they lose sufficient scales to become entangled. Stowe (1978) confirmed Eberhard's hypothesis by determining that the diet of *S. cordatus* (Taczanowski 1879) consists of almost 70% moths.

Ladder-webs are built by spiders in three different orb-web families (Araneidae, Nephilidae and Tetragnathidae), yet we know remarkably little about the foraging ecology of these spiders. Based on the microhabitat in which they are built, ladder-webs can be divided into two main groups with potentially different functions. The first group, which we here call aerial ladder-webs, includes the araneid genus *Scoloderus* (Eberhard 1975) and the New Guinean tetragnathid *Tylorida* sp. Simon 1894 (Robinson and Robinson 1972). These webs are built in open spaces among the vegetation. Spiders of these two genera build webs that may be over 1 m long (Robinson and Robinson 1972; Stowe 1978), although placement of the hub differs between the two. The hub of *Scoloderus* webs is at the extreme bottom of the web and the hub of *Tylorida* sp. is at the extreme top. It remains to be seen if *Tylorida* sp. is also a moth specialist, although Robinson and Robinson (1972) suggest the web may target insects with variable flight altitudes.

The ladder-web spiders in the nephilid genera *Herennia* Thorell 1877 (Robinson and Lubin 1979; Kuntner 2005) and *Clitaetra* Simon 1889 (Kuntner 2006; Kuntner and Agnarsson 2009), and the araneids *Cryptaranea atrihastula* (Urquhart 1891) (Forster and Forster 1985) and *Telaprocera* (Harmer 2009) build their webs almost exclusively against tree trunks, hence we refer to them as arboricolous ladder-webs. Web structure varies within the arboricolous ladder-web group. *Herennia* species curve the web around the tree (Robinson and Lubin 1979) while the other species build planar webs slightly offset from the tree surface. The hub position varies from a central position (*C. atrihastula*, *Telaprocera*) to nearer the top (*Herennia*, *Clitaetra*). Evidence indicates that the ladder-webs of *Telaprocera* (Harmer 2009; Harmer and Herberstein 2009), along with those of *C. irenae* (Kuntner et al. 2008), are a response to building webs in space-limited conditions. As these spiders build exclusively against tree trunks, they are limited in horizontal space for web construction. The only way to increase capture area is to elongate the web vertically. Whether or not the other arboricolous ladder-web species also elongate their webs for this reason has yet to be tested.

The differences in fine-scale web structure and web function (moth specialization vs. optimal area use) between aerial and arboricolous ladder-webs suggest they are not convergent structures; however, a moth-capturing function has not been ruled out for arboricolous species. As the highly elongated web structure of *Scoloderus* aids these spiders in catching moths, it is possible that the ladder-web structure of arboricolous species secondarily confers an ability to retain intercepted moths. Among ladder-webs, only the prey of the aerially building *S. cordatus* (moth specialist) and the arboricolous *H. papuana* (generalist) have been surveyed, with moths making up less than 70% and 10% of their diets respectively (Stowe 1978; Robinson and Lubin 1979). Despite strong evidence for *Telaprocera* ladder-webs being the result of space limitation (Harmer 2009; Harmer and Herberstein 2009), we cannot dismiss the possibility of a moth-capturing function in *Telaprocera* ladder-webs without first surveying their prey. In this study we examined whether or not a high proportion of the prey retained by *Telaprocera* webs are moths. We also carried out preliminary experiments to see how long moths are retained and how far they tumble in *Telaprocera* webs. If *Telaprocera*

ladder-webs are adapted for catching moths, we expected that moths would constitute a significant proportion of their natural diet and that a high proportion of moths that contacted the web would actually become ensnared.

***Telaprocera* prey.**—The prey of *T. maudae* Harmer & Framenau 2008 and *T. joanae* Harmer & Framenau 2008 were surveyed in Lamington National Park, southeastern Queensland, Australia, in February and July 2006, and March 2007. During the day, we searched for webs on trees and haphazardly selected approximately 30 webs to be surveyed. Since *Telaprocera* are nocturnal foragers, we placed a small colored marker under each web so we could relocate it at night, but the marker had to be removed following each night's survey. This meant that we may have sampled some webs more than once. However, the mean (± SD) number of prey per web per night was 1.18 ± 0.29, and no web caught more than three prey items in a night. Therefore, it is very unlikely that a single web biased our results. For 6 h beginning at sunset, we inspected each web for prey in a circuit-like fashion, so that after the last web was checked we started back at the first web (~ 1 h per circuit). As has been reported for *Eriophora edax* (Ceballos et al. 2005), preliminary surveys also indicated that few insects are captured between midnight and dawn, so we ceased surveys at midnight. We repeated the survey three times over 13 mo, sampling in both summer and winter. On several occasions, we opportunistically observed prey interception in webs during the day, but spiders did not respond to these prey, so we did not systematically survey diurnal prey capture. We collected prey items directly from webs or feeding spiders with a pair of soft forceps, placed them into a vial of 70% ethanol, and identified them to order and noted whether or not the spider had wrapped the prey. For analysis, we pooled prey from both *T. maudae* and *T. joanae*. We have previously shown (Harmer 2009) that the webs of these species are indistinguishable, and we assumed that the two species exhibit similar prey responses. Voucher specimens are deposited at the Queensland Museum.

To compare the actual prey of *Telaprocera* spiders with prey that was potentially available, we set up sticky traps on trees in similar positions as webs (as recorded by Harmer 2009). On each of the nights we inspected webs for prey, we placed 16 traps out just before sunset and collected them six hours after sunset (i.e., the same period webs were inspected). Sticky traps of approximately the same surface area as an adult *Telaprocera* web (~ 300 cm^2) were made from an A5 sheet of overhead transparency film coated in Tangletrap (The Tangle Foot Company, USA). We pinned these traps to trees with a small piece of wire that held them slightly off the bark, mimicking a *Telaprocera* web. We transferred prey on traps to vials containing 70% ethanol and later identified them to order.

We collected a total of 169 prey items from webs, 107 of which the spider had wrapped, and 273 prey items from traps. Prey belonged to 16 different orders including insects, arachnids and isopods. Twenty-five prey were unidentifiable as they had been partially digested by the spiders. We only included the most common types of prey (frequency > 3) in the analyses due to very low numbers of some orders. Comparisons between web and trap prey are presented in Figure 1. Diptera, Coleoptera and Hymenoptera were the most common types of prey in both webs and traps, with Araneae, Hemiptera, Isopoda and Lepidoptera making up small fractions of the total (Fig. 1). We carried out two separate analyses comparing the proportion of each individual prey order in webs to its proportion in traps using Fisher's exact tests. First, we compared prey in traps to all prey items collected from webs for each order. Significantly more prey were found in webs than traps for the orders Hemiptera ($P < 0.001$), Isopoda ($P = 0.028$) and Lepidoptera ($P < 0.001$). We then compared prey in traps to only those prey in webs that the spider had wrapped. We found a significant difference in the proportion of Diptera ($P < 0.001$), with many more flies in traps than webs.

Moth retention.—In addition to surveying *T. maudae* prey, we also carried out preliminary observations of moth retention in *T. maudae* webs. To do this, we allowed adult female *T. maudae* spiders to build webs in frames made from a 50 cm length of PVC pipe cut in half lengthways. Frames were 9 cm in diameter and lined with mesh for the spiders to walk on (see Harmer and Herberstein 2009). We removed the spider after it had built its web, and we used a new spider for each web tested. To test retention, we anaesthetized individual moths (*Plodia interpunctella*; mean length 6.88 ± 0.77 mm) with CO_2 and placed them directly on the web with the long axis of the body perpendicular to the capture threads (i.e., spanning several spiral turns), as close to the top as possible. We repeated this for five moths in each of five *T. maudae* webs ($n = 25$). Care was taken to ensure a fresh part of the web was used for each moth. Although it is not a very natural situation for moths to "wake up" in a web, we used this method to eliminate any differences in the velocity with which prey struck the web and to standardize moth contact with the sticky spiral. We then timed how long it took the moth to escape, from the time it began struggling until it had either completely left the web or 1 min had elapsed. Afterward, we measured the distance the moth had fallen down the web by measuring the length of the trail of scales it left.

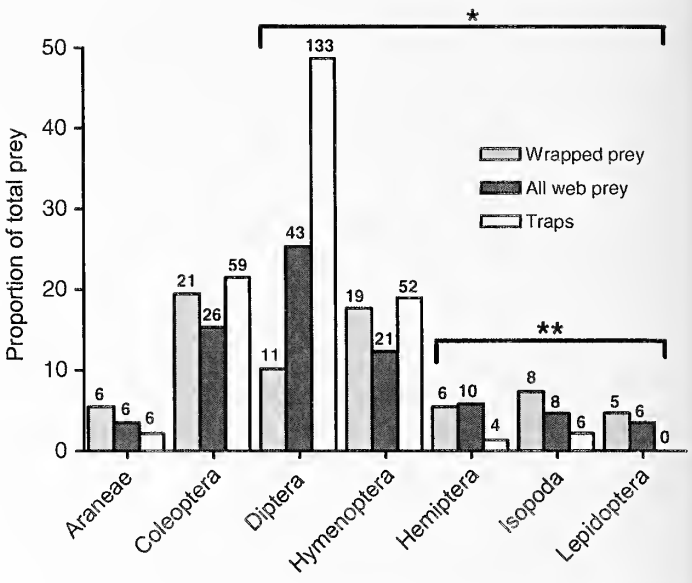

Figure 1.—Proportion of each prey order found in *Telaprocera* webs and in traps. Double asterisks (**) indicate a significant difference in the proportion of prey when comparing all web prey with traps. A single asterisk (*) indicates a significance difference in the proportion of prey when comparing only wrapped prey with traps. Comparisons were made using Fisher's exact tests. Values above each bar are actual counts of each prey order.

The median retention time of moths that escaped *T. maudae* webs was 2 s, range = 1–30 s. Four of the 25 moths did not escape after 1 min. The mean (± SD) distance moths tumbled down the webs was 4.9 ± 2.1 cm. There was no difference in the tumble distances of moths that escaped from those that did not (Mann-Whitney *U*-test: $U = 36.5$, $P = 0.711$, $n_{escaped} = 21$, $n_{retained} = 4$).

Discussion and conclusions.—We found that *Telaprocera* spiders catch a variety of prey orders with Diptera, Hymenoptera and Coleoptera being the most common. We also found very low numbers of moths in both webs and traps and that *Telaprocera* webs did not function well at retaining moths. These results differ greatly from those found for *Scoloderus* (Stowe 1978) and are likely due to differences in web structure, microhabitat and foraging period between these genera. Our results suggest that moth capture has had little role in the evolution of elongated webs in *Telaprocera* and possibly other arboricolous ladder-web species. This is consistent with previous findings that *Telaprocera* ladder-webs are elongated due to space limitation (Harmer 2009; Harmer and Herberstein 2009).

Moths comprise < 4% of the diet of *Telaprocera* spiders in this study, which contrasts sharply with the almost 70% observed for *S. cordatus* (Stowe 1978). Although there were proportionately more moths in webs than traps, it is unlikely that this difference will have biological significance due to the very low total number of moths over the sampling period (six in webs compared to zero in traps in three weeks of sampling). While moths could potentially be more important energetically or nutritionally than other prey, we cannot draw any conclusions on their dietary importance without first investigating the nutritional value of various prey types. The most common prey orders in both webs and traps were Diptera, Coleoptera and Hymenoptera, although Coleoptera and Hymenoptera probably contributed the most biomass. There was no significant difference in the proportion of these orders in webs or traps when all prey were included in the analysis, indicating that *Telaprocera* webs are intercepting what is most commonly available.

Traps have been used extensively to assess the available prey of spiders (reviewed in Eberhard 1990). However, there are drawbacks in using traps to estimate available prey due to different biases between traps and webs in the types and sizes of prey captured (Eberhard 1990). This point is illustrated in this study where we found significantly more dipterans in traps than webs when only comparing prey that had been wrapped by the spiders. This difference is likely due to the majority of unwrapped prey being tiny flies (≤ 1 mm in length). These flies are intercepted in webs, but they are not "available" to the spiders because the vibrations they produce when struggling are likely to be below the spider's response threshold or because they are not energetically worthwhile for a spider to retrieve. However, spiders could still consume these small flies when recycling the web if they do not escape beforehand. Additionally, our rate of web inspection (~ once per hour) could potentially have underestimated the observed numbers of flies if small flies were caught and consumed between inspections. However, it is unlikely that spiders biased the prey survey by consuming large numbers of small flies between inspections because we observed that they rarely attacked these small prey, although we can not completely rule this out. While there are limitations in comparing prey capture in webs and traps, the lack of moths in webs in this study supports our conclusion that *Telaprocera* are not specializing on these insects.

In our experimental tests of moth retention, only four of the 25 moths failed to escape, and almost 60% of moths escaped in 3 seconds or less. While this could still be enough time for spiders to reach prey close to the hub, prey intercepted near the web extremities may escape before spiders can reach them. However, data on spider attack speeds are required before we can draw any conclusions. The mean tumble distance of moths was only ~ 25% of web length, and there was no difference in the tumble distances of caught or escaping moths, indicating that it was not the length of the tumble that actually retained captured moths. The interaction between moth size and web elongation may also play a role in the retention of these prey. Web elongation will have a greater influence on the capture of large moths than small moths as they are heavier, presumably have more scales and so are likely to tumble further. For small moths that tumble only short distances, an elongated web is unlikely to contribute greatly to prey retention. The length of moths in this study averaged 6.88 mm, similar to the body length of female *T. maudae* (5–7 mm: Harmer and Framenau 2008). Further studies of moth retention in ladder-webs would be improved by comparing different sized moths in webs of varying elongation. Future studies should also compare different prey types and the attack speeds of spiders to see if observed retention times are long enough for spiders to reach prey before it escapes. As our data do not compare the retention times and tumble distances of moths of varying sizes, other prey types, or in other web structures, we are limited in the interpretation of our observations. However, the very low retention rate and short tumble distances, in addition to the low moth capture rate in the field, provide at least preliminary evidence that *Telaprocera* ladder-webs are not adapted for moth capture.

The difference in function between *Telaprocera* webs and *Scoloderus* webs is perhaps due to the very different fine-scale architectures between these two ladder-web types. For example, the radials of *Scoloderus* webs are contorted into a parallel arrangement (Eberhard 1975), whereas *Telaprocera* webs have a more typical radial arrangement (Harmer 2009). It is unclear whether this fine-scale difference helps to retain moths; however, web orientation is potentially more important. *Scoloderus* aerial webs are nearly perfectly vertical, causing moths impacting from either direction to tumble down the web rather than falling out (Eberhard 1975). *Telaprocera* webs generally follow the slope of the tree on which they are built, and if the web is slanted a struggling moth will not fall into lower parts of the sticky spiral, but instead fall to safety. A further possibility for the difference in function is that the capture silks of the two genera differ in stickiness, thus resulting in different moth retention rates.

A final clue to the difference in function between aerial and arboricolous ladder-webs is the difference in the frequency of web replacement and foraging period. *Tylorida* sp. and *Scoloderus* webs (aerial ladder-webs) are built at night and always removed the next morning (Robinson and Robinson 1972; Eberhard 1975). On the other hand, *Herennia*, *Clitaetra*, *Cryptaranea atrihastula* and *Telaprocera* webs (arboricolous ladder-webs) are built at night but not replaced for at least several days (Robinson and Lubin 1979; Forster and Forster 1985; Kuntner 2006; Harmer 2009). This means that aerial ladder-webs are restricted to foraging for nocturnal prey such as moths. Arboricolous ladder-webs can intercept prey both day and night (although their responsiveness to prey in the day may vary) and so have access to a greater prey range.

To conclude, the different forms of ladder-webs, both aerial and arboricolous, all share the common feature of vertical elongation, yet they appear to have divergent functions. *Scoloderus* webs are highly effective moth-capturing devices (Stowe 1978), while elongated *Telaprocera* webs are the result of space limitation (Harmer 2009; Harmer and Herberstein 2009). It will be intriguing to see if the functional difference we observed between *Telaprocera* and *Scoloderus* ladder-webs, paralleling their aerial versus arboricolous microhabitats, holds for the other ladder-web building species.

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SHORT COMMUNICATION

Optimal sting use in the feeding behavior of the scorpion *Hadrurus spadix*

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Abstract. Since venom is costly to produce and stinging is not obligatory in prey capture for seorpions, the need to optimize use of resources suggests that venom should be reserved for prey that cannot otherwise be overpowered, (i.e., larger and/or more active prey). In accordance with these predictions, sting use by *Hadrurus spadix* Stahnke 1940 increased with prey size, reaching 100% once prey items were longer than the scorpion's pedipalp patella length, and with prey activity, which we manipulated by varying prey temperature. Surprisingly, the scorpions were slower to capture less active (cooler) prey than those that exhibited higher rates of activity. We suggest this is because prey are located by vibrations in the substrate, with less active prey producing fewer vibrations.

Keywords: Optimal foraging, venom, pectines

Venom is used by scorpions primarily for capture and digestion of prey and secondarily in defense (Lourenço & Cuellar 1995; Yigit et al. 2007). It is not always used in prey capture however; sometimes only the pedipalps are used (Bub & Bowerman 1979; Polis 1990; Rein 2003), and Rein (1993) has suggested that scorpions sting only if prey resist capture.

Scorpion venom is a complex mixture of low molecular-weight proteins, salts, and various other organic compounds, such as oligopeptides and amino acids (Brownell & Polis 2001). Venom is costly to produce; respirometry studies in scorpions show a marked increase in respiration for some time following ejection of venom from the glands (Nisani et al. 2007). The scorpion *Parabuthus transvaalicus* Purcell 1899 manufactures two forms of venom: a clear pre-venom that contains high levels of salt with very few peptides and a primary venom that contains both salts and high levels of metabolically expensive peptides (Inceoglu et al. 2003). Being more costly to produce, the latter should logically be reserved for the capture of large prey.

Since venom is costly to synthesize and stinging is not obligatory in prey capture, optimization models (Caraco & Gillespie 1986) suggest that venom use should be reserved for prey that cannot otherwise be overpowered. Here we investigate sting use in adult *Hadrurus spadix* Stahnke 1940, a desert-dwelling species from North America, and test the hypothesis that venom is only used on large and/or active prey. *Hadrurus spadix* was chosen for study because its large size makes it easy to observe, and because as an 'equilibrium species' (i.e., species that are slow growing, relatively large, have large broods and are generally of low toxicity (Polis 1990)) it may be representative of the majority of non-buthid scorpion species.

Our experiments were conducted using eight male final (adult) instar *H. spadix* individuals obtained from the wild near Cameron, Arizona, USA by a specialist importer of arachnids (voucher specimen deposited in Cole Museum, University of Reading, UK). Specimens were kept alive in a private collection at the conclusion of the experiments. Pedipalp patella length, correlated with overall length, was used as a measure of size following Benton (1991). Individuals were housed singly in clear Perspex terraria measuring 30 × 20 × 15 cm on a 16:8 h light: dark cycle. Light was provided by a single fluorescent tube suspended above the terraria, and temperature was maintained at 27° ± 2° C during the day and 15° ± 2° C at night. Mesh lids allowed air circulation with humidity maintained at ca. 50% relative humidity, measured weekly with a hydrostat. A substrate of sand 5 cm deep lined the bottom of each enclosure to allow burrowing, and a single piece of cork bark was added to each terrarium to provide shelter. Water was provided once every two weeks by misting. The prey species used throughout this study was the brown cricket *Acheta domesticus* Linnaeus 1758. The crickets were raised in enclosures kept under identical temperature, light and humidity conditions as the scorpions. They were fed various vegetable matter.

After introducing the scorpions to the terraria we fed them one large (size 1.5, prey sizes are given here as multiples of pedipalp patella length) *A. domesticus* each and then deprived them of further prey for 14 days prior to initiation of the first experiment, which investigated the effects of feeding prey of six different sizes as shown in Fig. 1. We provided each scorpion with a single prey item at each feeding. Feedings occurred twice a week, two hours into the dark photoperiod under red light (scorpions are insensitive to red spectrum light: Machan 1968). We turned on the red light 1 h before feeding and removed the cork bark hides to ensure the scorpions were visible while feeding. We placed prey in the terrarium in the farthest corner from the scorpion and recorded the scorpion's behavior thereafter. Capture time was operationally defined as the time from the alert stance (Bub & Bowerman 1979) until visible movement of the chelicerae was observed, indicating that the prey item was being devoured. A sting was defined as the successful penetration of the prey's exoskeleton by the aculeus. In order to maintain as constant a level of hunger as possible throughout the trial period, we fed the scorpions reciprocal prey sizes in each week's two feedings. For example, the first feeding was of prey of size 1.5, the next feeding was size 0.4, and the following one 1.2. After each of the eight scorpions had been fed all of the six sizes, the whole process was repeated until each scorpion had eaten each prey size three times.

Experiment 2 investigated responses to prey activity. This experiment was started one week after the last, with no food provided in between. We manipulated prey activity by cooling prey in a domestic refrigerator for five, ten or fifteen minutes prior to feeding: the longer the prey had been in the refrigerator, the lower the level of activity displayed (Mellanby 1939). Prey items of size 0.8 were used throughout. Conditions and recording of behavior were otherwise as in Experiment 1. Statistical testing was carried out using Minitab 15.1 (Minitab Inc., Quality Plaza, 1829 Pine Hall Rd, State College, Pennsylvania 16801-3008, USA), controlling for individual differences by entering scorpion identity as a factor where appropriate.

Sting use by the scorpions in Experiment 1 increased when they were offered large prey (Fig. 1, Ordinal Logistic Regression: Z =

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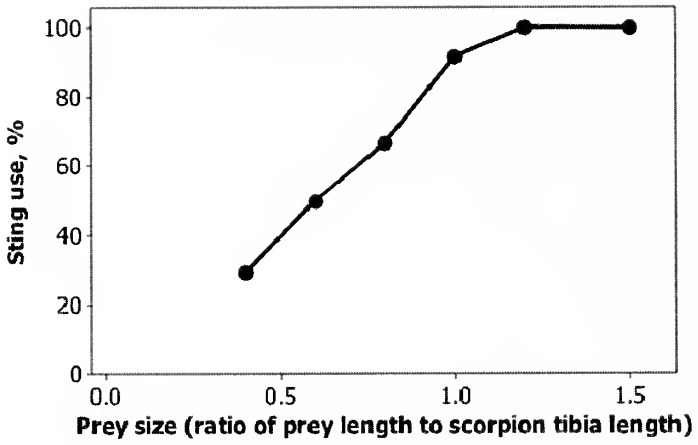

Figure 1.—Sting use by *Hadrurus spadix* increased with prey size. Sting use was measured as the percentage of cases in which the sting was deployed.

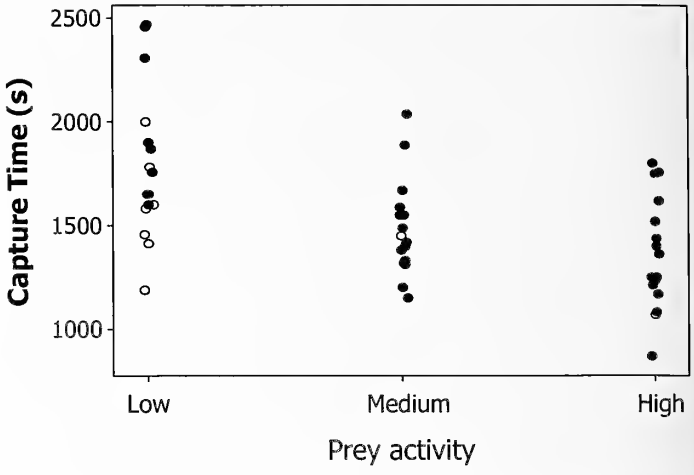

Figure 2.—Capture time and sting use in relation to prey activity. Prey activity was manipulated to be low, medium or high by keeping prey in a refrigerator for 15, 10 or 5 mins, respectively. ● = sting used, o = sting not used. Jitter has been applied to x coordinates to make overlapping points visible.

−4.58, $df = 1$, $P < 0.0005$: the response variable was the proportion of the three trials in which the sting was used). When the smallest prey were offered, stings occurred in only 29% of 24 cases, but this rose to 100% when the prey items were larger than the scorpions' patella lengths. There was no variation in sting use between scorpions ($X_7^2 = 2.37$, $P > 0.05$).

In Experiment 2, prey were rendered less active by keeping them for short periods (5, 10 or 15 min) in a refrigerator. Sting use increased during encounters with more active (i.e., less cooled) prey, as shown in Fig. 2 (Binary Logistic Regression: $Z = -2.70$, $df = 1$, $P = 0.007$). Interestingly, it took longer to catch less active (more cooled) prey (Fig. 2, General Linear Model: $F_{1,15} = 31.3$, $P < 0.0001$). For prey kept in the refrigerator for 15 min, capture time was longer when the sting was deployed than when it was not (General Linear Model: $F_{1,7} = 10.2$, $P = 0.015$). The results are consistent with the hypothesis that sting use is reserved for prey that are difficult to subdue, due to their large size (Fig. 1) or high activity levels (Fig. 2). Less active prey were less likely to be stung and more likely to simply be grasped in the pedipalps. This corresponds to Rein's (2003) suggestion that scorpions sting reluctantly, and only if a prey item struggles, in order to minimize the use of venom and thus its metabolic costs. It is interesting that the sting was invariably used when the prey length exceeded that of the pedipalp patella, suggesting that prey cannot then be reliably held in the pedipalps. Quinlan et al. (1995) reached a similar conclusion from the observation that sting use is more frequent in *Urodacus armatus* Pocock 1888 than in its longer-clawed relative *U. novaehollandiae* Peters 1861.

Surprisingly, the scorpions took longer to capture less active prey items than more active ones (Fig. 2). In situations where prey had not been cooled or had only been cooled for 5 min, the scorpions adopted the alert stance as soon as the prey began to move. When a prey item had been cooled for a greater period of time, however, it took longer to begin moving and/or made reduced movements. In these situations, the scorpions appeared initially ignorant of the presence of the cricket and were slower to locate them once the prey had begun to move. This is consistent with the idea that scorpion species that frequent sandy environments, such as *H. spadix*, locate their prey on the basis of vibrations in the substrate picked up both by their pectines (Brownell, 1977; Mineo & Del Claro 2006) and by the basitarsal compound-slit sensillae of the distal leg segments (Brownell & Farley 1979). The nocturnal scorpion *Smeringurus mesaensis* Stahnke 1957 has been shown to be sensitive to vibrations from a distance of up to 50 cm (Brownell & Farley 1979). By reducing the activity of the prey, there were fewer vibrations in the sand for the scorpions to detect, and this increased capture time (Mineo and Del Claro 2006).

After prey capture, the scorpions were sometimes observed to enter an inactive phase in which they remained motionless with the prey item still grasped in their pedipalps, sometimes for several minutes. This inactive phase is puzzling. Equilibrium species such as *H. spadix* are thought to have evolved low metabolic rates and low levels of surface activity due to constraints placed on them by predation (Polis 1990). By minimizing the period of time required outside of the safety of the burrow, scorpions also minimize their exposure to predators, so it would be logical for scorpions to catch and consume prey as quickly as possible. No function has been suggested for the inactive phase sometimes displayed here (and also reported by Bub and Bowerman (1979) and Rein (2003)), but perhaps the scorpions need to wait for the venom to subdue the prey completely before eating (M. R. Graham, pers. com.).

Wigger et al. (2002) proposed a venom optimization theory to account for the amount of venom injected into prey by spiders. They found that the volume of venom injected into the prey increased with prey size and in prey that were difficult to overwhelm; e.g., in those displaying defensive behavior. These relationships are similar to those reported here. This raises the question, can scorpions control the amount of venom they inject into their prey and thus further conserve this costly resource?

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SHORT COMUNICATION

The chemical defense of the Texas cave harvestman *Chinquipellobunus madlae*: first report on the family Stygnopsidae and on a North American troglobiont harvestman (Opilones: Gonyleptoidea)

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Abstract. The stygnopsid harvestman *Chinquipellobunus madlae* (Goodnight and Goodnight 1967) is known from numerous caves in eleven counties in Texas and is a highly adapted troglobiont (Cokendolpher 2004). Adult and juvenile specimens were extracted in methanol, and the major volatile component of their chemical defense secretion was identified as 2-methyl-5-ethylphenol; a minor component was 2, 5-dimethylphenol. Methylethyl phenols and dimethyl phenols have also been identified in other grassatorid Opiliones, but this is the first report of defensive chemistry from a member of the family Stygnopsidae and from a North American troglobiont harvestman.

Keywords: Secretion, 2-methyl-5-ethylphenol, 2, 5-dimethylphenol

Harvestsemen are well known for their chemical defenses (Gnaspini & Hara 2007). Despite the great diversity within the order Opiliones, many common and phylogenetically significant families of harvestmen remain unstudied for this ecologically important character. As part of an ongoing survey of the order, we describe below the first observations on the chemistry of the repugnatorial secretion of a stygnopsid harvestman, *Chinquepellobunus madlae* (Goodnight & Goodnight 1967).

Six living specimens of *Chinquipellobunus madlae*, two adult females and four immatures, collected from six caves in Bexar Co., Texas, USA (Bunny Hole, B-52 Cave, Dos Viboras Cave, MARS Shaft, Pain in the Glass Cave and Platypus Pit), from 21 to 28 October 2008, were dropped into less than 1 ml USP methanol in glass screw-cap vials with Teflon cap liners for extraction. Each individual extract was analyzed separately. GC/MS analysis of the methanol extract was carried out using a Shimadzu model 2010 GC/MS equipped with an RTX-5, 30 m × 0.25-mm i.d. GC FTIR spectra were obtained using a Hewlett-Packard model 5965B detector interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a 30 m × 0.25 mm ZB-5 30 m × 32 mm i.d. column. The specimens are now preserved in 70% ethanol and will be deposited as vouchers in the collection of the Virginia Museum of Natural History, Martinsville, Virginia.

Two volatile components were observed in the methanol extract of the specimens, in an average ratio of 17:1. The major component (94.2%) was identified as 2-methyl-5-ethylphenol by direct comparison with an authentic sample (Morgan & Pettit 1934). The mass spectrum and chromatographic retention time of the extract component and the authentic sample were identical, as was the vapor phase infrared spectrum. The minor component (5.8%) had a mass spectrum consistent with a dimtheylphenol. Comparison with commercial samples of the possible dimethylphenols narrowed the possibilities to 2,4-dimethylphenol and 2,5-dimethylphenol. Since these isomers proved inseparable by gas chromatography under a variety of conditions, the mixture was acetylated (acetic anhydride/ pyridine) and compared to acetates of 2,4-dimethylphenol and 2,5-dimethylphenol, which have different retention times. The gas chromatographic retention time of the natural dimethylphenol acetate matched that of the 2, 5-dimethyphenol acetate. In addition, in the FTIR spectra the frequency of absorption for the carbonyl groups is 1784 cm^{-1} for the unknown, 1785 cm^{-1} for the 2,5-dimethyl isomer and 1781 cm^{-1} for the 2,4-dimethyl isomer.

2-Methyl-5-ethylphenol has been detected before in seven species of harvestmen, namely *Cynorta astora* Goodnight & Goodnight 1942 from Panama (Eisner et al. 1977); *Eucynortula albipunctata* (Piekard-Cambridge 1904) from Costa Rica (Roach et al. 1980); *Pachyloidellus goliath* Acosta 1993 from Argentina (Acosta et al. 1993); *Camarana flavipalpi* B. Soares 1945 from Brazil (Machado & Pomini 2008); *Stygnomma spinifera* (Packard 1888) from Florida, USA (Duffield et al. 1981); *Bishopella laciniosa* (Crosby & Bishop 1924) from North Carolina, USA; and *Texella bifurcata* (Briggs 1968) from Oregon, USA (Shear et al. in press). The first two named are in the family Cosmetidae, *P. goliath* is in the subfamily Pachylinae and *C. flavipalpi* in the subfamily Tricommatinae of the family Gonyleptidae, and *S. spinifera* is a member of the family Stygnommatidae. The last two species named are both members of the family Phalangodidae. Gonyleptidae and Cosmetidae are grouped in the superfamily Gonyleptoidea, Phalangodidae in Phalangodoidea, and Stygnommatidae is a family of Samooidea (Giribet & Kury 2007). The phylogenetic relationships of these families are not well resolved. Stygnopsidae, the family to which *Chinquepellobunus madlae* belongs, is currently placed in Gonyleptoidea, and so the presence of 2-methyl-5-ethylphenol is not surprising and is consistent with this taxonomic and phylogenetic placement. In the two cosmetids, 2-methyl-5-ethylphenol was accompanied by 2,3-dimethylphenol, and in *P. goliath* by 2,3-dimethylphenol, 2,3-dimethyl-5-ethylphenol and three benzoquinones. In *S. spinifera*, the compounds 2,3-dimethylphenol and 2,3-dimethyl-5-ethylphenol were also detected, while 2-methyl-5-ethylphenol was the sole extractable component in *C. flavipalpi*, *B. laciniosa* and *T. bifurcata* (Gnaspini & Hara 2007; Machado & Pomini 2008; Shear et al. 2009). 2,5-dimethylphenol, the minor component in *C. madlae* secretion, was reported earlier by Hara et al. (2005) from *Daguerria inermis* Soares & Soares 1947, like *P. goliath*, a pachyline gonyleptid. In *D. inermis*, 2,5-dimethylphenol was the major component, making up 61.8% of the secretion; other compounds were not identified (Hara et al. 2005).

Machado and Pomini (2008) suggested that the use of 2-methyl-5-ethylphenol might have evolved in parallel in the two superfamilies Gonyleptoidea and Samooidea. The finding that this compound occurs in at least one species of Stygnopsidae, considered basal in Gonyleptoidea, and also in the superfamily Phalangioidea raises the possibility that it evolved in an ancestor of the two superfamilies. More data on secretions in a wider phylogenetic sampling is obviously needed.

Stygnopsids are endemic to Central America, Mexico and southern Texas, USA (Mendes & Kury 2007). The family consists of 35 species grouped in eight genera. Phylogenetically, stygnopsids may be basal in the Gonyleptoidea, sister to the other families in the superfamily (Kury & Cokendolpher 2000); however, a more complete data set for grassatorean harvestmen is presently being compiled by Adriano B. Kury and will likely result in changes in the tree (A. Kury pers. comm. to WAS 2009). *Chinquepellobunus* was originally described in Phalangodidae (Goodnight & Goodnight 1944), then transferred to Stygnopsidae (Goodnight & Goodnight 1945), synonymized with *Hoplobunus* Banks (Goodnight & Goodnight 1953), and revalidated by Cokendolpher (2004). *Chinquepellobunus madlae* is a troglobiont known from many caves in Bandera, Bexar, Comal, Edwards, Kendall, Kerr, Kinney, Medina, Terrell, Uvalde and Val Verde counties in Texas. Cokendolpher (2004) remarks that this exceptionally broad distribution of a highly troglomorphic harvestman, which could never survive surface conditions long enough to move between caves not connected underground, is difficult to accept. He notes that the same caves occupied by *C. madlae* are also home to a variety of species of other cave-adapted arthropods, none of which is found over such a vast area as *C. madlae*. He speculates that we are actually dealing with a superspecies, made up of a number of reproductively isolated populations convergent on the same troglomorphic habitus and thus inseparable by their anatomy (Cokendolpher 2004). Genetic data could resolve this problem. Since our specimens came from six different caves, we mention this possibility. However, all specimens showed essentially identical chemical profiles.

Twenty-one of the 35 described species in Stygnopsidae have been collected in caves, most of them exclusively, though not all of these are real troglobionts. Stygnopsids are presumably predatory, the cave-dwelling species feeding on other arthropods found in their habitat. The chemical defense of only one other troglobiotic species, *Goniosoma spelaeum* (Mello-Leitao 1932), a gonyleptid from Brazil, has been studied. In that case the secretions consisted of two benzoquinones (Gnaspini & Cavalheiro 1998). The retention of chemical defenses in troglobionts is interesting, since predator pressure on these harvestmen might be expected to be relaxed in a cave ecosystem, where predators aside from the harvestmen themselves may be absent. It may suggest that their presence in that habitat and their physieal adaptations to it are relatively recent.

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SHORT COMMUNICATION

Stealing for love? Apparent nuptial gift behavior in a kleptoparasitic spider

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Abstract. The presentation of nutritional resources as nuptial gifts before or during the mating process is well known among insects, but has only rarely been documented in spiders. Here, we report on observations and a series of photographs made during field studies in Fortin de las Flores, Veracruz, Mexico, which, although a single anecdotal report, represent a potentially significant finding. A male of the kleptoparasitic spider *Argyrodes elevatus* Taczanowski 1873 (Araneae, Theridiidae) was observed stealing a prey item from within a communal web of its host, the colonial orb-weaver *Metepeira incrassata* F.O. Pickard-Cambridge 1903 (Araneae, Araneidae). The male *A. elevatus* then carried and presented the prey item to a female, waited nearby until she began feeding, and copulated with her as she fed upon it. As far as is known, this is the first report of kleptoparasitic *Argyrodes* apparently utilizing a prey item stolen from a host spider as a nuptial gift.

Keywords: *Argyrodes*, nuptial feeding, colonial spiders, *Metepeira*

In many arthropod species, the courtship and mating process includes some form of provision of nutritional resources, either as nuptial feeding or nuptial gifts offered before, during or immediately following copulation (Boggs 1990; Vahed 1998; Gwynne 2008). Presentation of nuptial gifts has only rarely been documented in spiders, and is limited to two families, the Pisauridae and Trechaleidae. Nuptial gifts are best known for *Pisaura mirabilis* (Clerck 1757) (Stålhandske 2001, 2002), but similar nuptial offerings during courtship have been reported for two additional pisaurids, *Pisaurina lama* Bosenberg and Strand 1906 (Itakura 1993) and *Perenethis fascigera* (Bosenberg and Strand 1906) (Itakura 1998). Most recently, a study of nuptial gifts in two species of the closely-related family Trechaleidae, *Paratrechalea azul* Carico 2005 and *Paratrechalea ornata* (Mello-Leitao 1943) has revealed a similar provisioning behavior during courtship (Costa-Schmidt et al. 2008).

During our field studies in Mexico, we observed the unusual behavior described below, which represents the first report of kleptoparasitic *Argyrodes* apparently utilizing a prey item stolen from a host spider as a nuptial gift. Spiders of the genus *Argyrodes* are well known as kleptoparasites on other spiders and are frequently found in association with many web-building spider species (see reviews in Higgins & Buskirk 1998; Whitehouse et al. 2002; Agnarsson 2002). A number of species of *Argyrodes* are associated with colonial and social spiders and may represent a cost of group-living from prey loss and predation (Elgar 1989; Cangialosi 1991; Whitehouse & Lubin 2005). This is certainly true for the colonial orb-weaver *Metepeira incrassata* F.O. Pickard-Cambridge 1903, which serves as host to six species of Argyrodinae (McCrate & Uetz 2009).

The observations reported here were made at a coffee plantation near Fortin de las Flores, Veracruz, Mexico (18°53′54.30″N, 96°59′30.92″W), used in several previous studies of colonial *M. incrassata* (see Uetz & Hodge 1990; Rayor & Uetz 1990, 1993, 2000; Uetz et al. 1994; Uetz & Hieber 1994; Jakob et al. 2001; Uetz et al. 2002; Hieber et al. 2002). Observations were made during mid-day on 13 July 1991, in a colony of approximately 300 *M. incrassata*, as part of a study of *Argyrodes* kleptoparasitism (A. McCrate, M.S. Thesis 1996). Photographs of the behaviors described below were taken using a Nikon FM 35mm SLR film camera and represent a sequence of 23 slides, taken over a period of several minutes. The specimens were captured and preserved, and later identified by Scott Larcher and Dawn Southard at the Smithsonian National Museum of Natural History as *Argyrodes elevatus* Taczanowski 1873.

A male *A. elevatus* was observed stealing a recently captured and wrapped prey item from a host spider within a communal web of the colonial orb-weaver *M. incrassata*. The male *A. elevatus* carried the prey item (Fig. 1a) in the manner typical for members of this genus, trailing behind the spider on silk attached to the spinnerets (Vollrath 1979a, 1984; Whitehouse 1997). The male moved toward a female *A. elevatus* within the colony that was initially moving in a direction away from the male at a distance of 30–40 cm, following her path along strands of the communal web. The male approached the female to a distance of approximately three body lengths (1–2 cm) and stopped. At this time, the male removed the prey item from its trailing position behind the abdomen with the forelegs and used its mouthparts to position the prey item on the silk line ca 1.0 cm directly in front of the female (Fig. 1b). The female turned around, and the male left the prey item behind (Fig. 1c), then waited nearby (< 1.5 cm). The female approached the prey item, commenced palpating the prey with her mouthparts, and began feeding, after which the male approached (Fig. 1d). The male then assumed a face-to-face mating position (Fig. 1e) and copulated with the female while she fed. Feeding by the female during copulation was intermittent, but intromission continued even when the female released the prey item (Fig. 1f). This behavior is different from pre-contact courtship behavior reported for other *Argyrodes* species (Cangialosi 1990; Whitehouse 1994; Whitehouse & Jackson 1994), and to our knowledge, this apparent nuptial gift behavior is the first report for *A. elevatus* or any other *Argyrodes* or theridiid species.

An alternative explanation (to nuptial gift behavior) might be that this observation represents a means of avoiding aggression, i.e., the smaller male abandoned its prey item in the presence of a larger female. Or, it might also be possible that *A. elevatus* males sometimes engage in mating behavior by approaching and copulating with females while they are feeding. However, both mating and agonistic interactions among *Argyrodes*, described in detail for other species, usually involve complex behaviors not seen here (Cangialosi 1990; Whitehouse 1994; Whitehouse & Jackson 1994). We believe that this unique observation represents nuptial gift exchange in *A. elevatus* for several reasons: 1) the male followed and approached a moving female, as is typical in the first phases of *Argyrodes* courtship (Cangialosi 1990; Whitehouse 1994; Whitehouse & Jackson 1994); 2) the male behaviors observed are clearly different from those seen in other phases of courtship and mating described for *Argyrodes* species, which typically include leg-waving, rotary probing and other

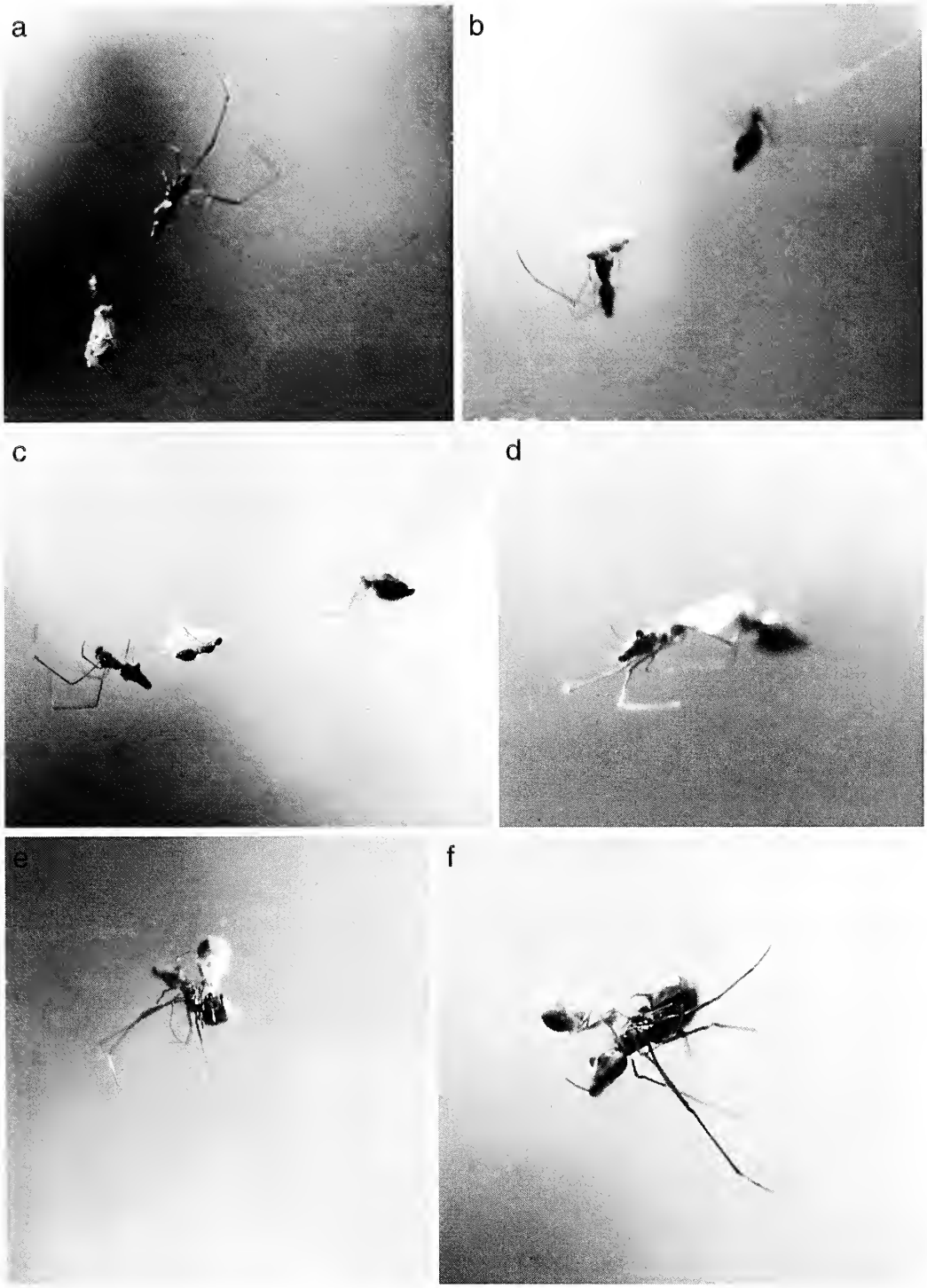

Figure 1.—Selected photographs from a sequence taken in the field illustrating male nuptial gift behavior in *A. elevatus*: a. male dragging stolen prey item (an unidentified hymenopteran); b. male presenting prey item in a position near the female; c. male leaving prey item to wait nearby; d. male approaching female as she accepts, palpates and and begins to feed upon the prey item; e. male positioning to insert palp; f. copulation.

behaviors (Cangialosi 1990; Whitehouse 1994; Whitehouse & Jackson 1994), which were not seen in this event; 3) the presentation was obvious and appeared intentional (i.e., the male removed the prey item from the spinnerets, held it with mouthparts and directed it toward the female); and 4) although different from other reports of nuptial gift behavior in spiders (Anderson et al. 2008), acceptance of the gift in this manner by the female was atypical of *Argyrodes* foraging behavior, as she swiftly turned to face the male, grasped the prey item and began feeding without carrying it away (Vollrath 1979a,b, 1984; Cangialosi 1990, 1991; Whitehouse 1994, 1997). Given that this observation is so distinctly different from the well-documented mating behavior patterns of other *Argyrodes* species, we suggest that the apparent nuptial gift of *A. elevatus* could represent an alternative mating tactic for *A. elevatus*.

The contribution of a nuptial gift may enhance male fitness, and several hypotheses have been offered to explain their adaptive value

for arthropods, including spiders: 1) paternal investment via nutritional contribution to offspring fitness (Thornhill 1976); 2) sexual selection for male mating effort, resulting in increased fertilization success via prolonged copulation (Austad & Thornhill 1986; Stålhandske 2001; Huber 2005); 3) male exploitation of female sensory biases (Stålhandske 2002; Sakaluk et al. 2006; Bilde et al. 2007; Vahed 2007); and 4) protection from sexual cannibalism (Bristowe 1958; Vahed 1998; Stålhandske 2001). Recent experimental evidence for *P. miriabilis* supports the mating effort hypothesis, while refuting the defense against cannibalism and paternal investment in offspring fitness hypotheses (Anderson et al. 2008). The question of whether a nuptial gift of a wrapped prey item represents sensory exploitation by its resemblance to a female's egg sac is doubtful, as female *P. miriabilis* more readily accepted unwrapped prey (Anderson et al. 2008). However, these hypotheses may not be mutually exclusive, as presentation of a prey item as a nuptial gift may exploit sensory aspects of the female's foraging behavior, thereby occupying her attention while the male attempts mating (Stålhandske 2002; Vahed 2007; Bilde et al. 2007; Anderson et al. 2008). While most of these hypotheses apply to our observation, it is not possible to refute any with a single observation such as this. We might speculate that a combination of several of these hypotheses applies here. For example, it is possible that our observation of *A. elevatus* represents some form of sensory exploitation, as this species is kleptoparasitic, and exploits prey wrapped in silk by host spiders and hanging in the web. While a wrapped prey item suspended in the *M. incrassata* colonial web near a female might be a likely target for kleptoparasitism, a male presenting a prey item in this manner might also produce vibratory stimuli that attract female attention, and thereby exploit a pre-existing sensory bias of the female. By presenting a nuptial gift in this manner, male *Argyrodes* might avoid female aggressive attacks or potentially cannibalism (Whitehouse 1994) and subsequently allow copulation while the female feeds. Ultimately, since the female fed upon the prey item while copulating, the apparent nuptial gift would likely contribute material resources to offspring (Bilde et al. 2007).

Given that this is a single observation, any adaptive explanation is speculative and should be interpreted with caution. We observed this behavior only once in thousands of hours of observation of *Metepeira incrassata* (and hundreds of hours by ACM observing *Argyrodes*) in the field, although our primary interest was the other behaviors, and thus it is hard to gauge its true frequency. However, a recent (and independent) observation of similar behavior by *A. elevatus* in a colonial orb-weaver colony in South America (Cobbold & Su, unpubl. manuscript) would appear to corroborate that our observation was not an artifact. Although this anecdotal report of a single observation raises many questions that cannot be answered here, we believe it represents a significant finding worthy of future investigation.

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SHORT COMMUNICATION

The host becomes dinner: possible use of *Cyclosa* as a nuptial gift by *Argyrodes* in a colonial web

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Abstract. Nuptial gifts in spiders are poorly documented. We report on an observation and on photographs of a colonial web in San Vicente de Chucurí, Santander, Colombia, which suggest a likely case of nuptial gift behavior in a male of the kleptoparasitic spider *Argyrodes elevatus* Taczanowski 1873 (Araneae: Theridiidae). Pictures of a male *A. elevatus* holding a dead *Cyclosa huila* Levi 1999 (Araneae: Araneidae) wrapped in silk, in close proximity to a female *A. elevatus* facing him, document the couple at various moments at dusk and early the following morning. While no copulation is seen, these pictures suggest an attempt by the male to deliver a nuptial gift. Our observations support a recent report of apparent nuptial gift behavior in *A. elevatus* and raise questions on the foraging behavior of kleptoparasitic spiders in communal webs.

Keywords: *Argyrodes elevatus*, kleptoparasite, nuptial feeding, *Cyclosa huila*, communal web

While nuptial gift offering during courtship and mating is well documented in insects (Vahed 1998), it has rarely been seen in spiders (Costa-Schmidt et al. 2008). Male offerings of nuptial gifts in the form of a prey item wrapped in silk are currently known in only two spider families: Pisauridae (Stahlhandske 2001, 2002; Itakura 1993, 1998) and Trechaleidae (Costa-Schmidt et al. 2008). Recently, Uetz et al. (2010) described in the theridiid *Argyrodes elevatus* Taczanowski 1873 (Araneae: Theridiidae) what may be the first nuptial gift behavior seen in a spider family outside the Lycosoidea clade. Since we still lack examples of nuptial gift behavior in spiders, our ability to understand the evolutionary origin of this behavior and its potential role in sexual selection in spiders remains limited (Costa-Schmidt et al. 2008).

During a visit to the Reserva Reinita Cielo Azul (6°50′47″N, 73°22′30″W), located in the Cordillera Oriental of Colombia, a male *Argyrodes* was observed approaching a female, positioning himself to face her while holding a prey item in his chelicerae, and remaining in close proximity, an unusual activity reminiscent of nuptial gift behavior. Spiders of the genus *Argyrodes* are cosmopolitan kleptoparasites on other spiders, especially web-building species (Agnarsson 2002). Most *Argyrodes* steal silk and food from their host by scavenging prey items from the web, but they sometimes kill and eat the host itself (Wise 1982; Whitehouse et al. 2002; Kerr 2005).

The observations and pictures that we describe here were taken while documenting the spider species composition of a relatively large communal spider web (length 323 cm, height 107 cm and width 132 cm) found on a fence by a dirt road. Photographs were taken with a Panasonic digital camera on 30 Dec 2008 at dusk and on 31 Dec 2008 at dawn, after which all spiders were captured and preserved in ethanol. We later identified the *Argyrodes* pair and the host species as *Argyrodes elevatus* Taczanowski 1873 (2 males, 9 females) and *Cyclosa huila* Levi 1999 (Araneae: Araneidae) (38 females). The date and time at which each picture was taken were recorded by the camera and used to build a sequence of pictures. Based on the relative numbers of species and the communal web structure, a tight concentration of smaller interconnected webs, it seems that *C. huila* created the primary aggregation of orb webs, which was subsequently invaded by other species, including *A. elevatus*.

The prey item displayed by the male *A. elevatus*, lightly wrapped in silk, was larger than he was. When the male was approximately 3 cm from the female, he came to a stop facing the female, which by then was also oriented toward him. At this time (17:22 h), a picture of the pair was taken (Fig. 1a). The pair remained facing each other at this distance for at least 2 min, as indicated by a picture taken after. A picture taken 42 min later (Fig. 1b) shows the male still holding the prey item in his chelicerae, but the female is located a few cm further from the male than in the previous picture, and both individuals face in opposite directions.

Close-ups of the prey item (Figs. 1a, b) displayed by the male depict an eight-legged arthropod whose pointed abdomen and dorsal pattern are strongly evocative of *C. huila* (Fig. 2). Since the male *A. elevatus* was not seen attacking and killing the host, we cannot determine whether the item was preyed upon by *A. elevatus* or scavenged after its death.

A picture of the web taken the following morning at 06:12 h shows the *A. elevatus* couple no longer interacting, as indicated by the resting position (Fig. 1c). The male remains relatively close to the female (about 4 cm) but faces away from her and no longer carries the prey item seen the night before.

Although these pictures do not document copulation, three features are indicative of an attempt of the male *A. elevatus* to deliver a nuptial gift to the female: 1) the initial approach of the male holding a wrapped prey item in his chelicerae, an unusual behavior since *Argyrodes* typically carry their prey attached to the spinnerets (Vollrath 1979; Cangialosi 1990); 2) the orientation of the couple where the two individuals face eaeh other; and 3) the persistent close proximity of the male to the female.

The mating behavior of *Argyrodes* follows a consistent sequence, where the male orients toward the female and approaches her while producing courting vibrations, before eventually copulating (Whitehouse & Jackson 1994). Except for mating behavior, intraspecific interactions in *Argyrodes* in the host web are normally of short duration (e.g., Whitehouse & Jackson 1994). For instance, in *Argyrodes antipodiana* O. Pickard-Cambridge 1880, male-male grappling interactions range from 3 s to 6 min (Whitehouse 1997), whereas courtship-copulation interactions last between 2 and 8 h (Whitehouse & Jackson 1994). Given that the interaction that we report lasted at least 44 min and that the male held the prey item in his chelicerae during the entire interaction, we believe that this behavior was deliberate and that it represents an unusual courting interaction in which the male used its host *C. huila* as a gift to entice a female.

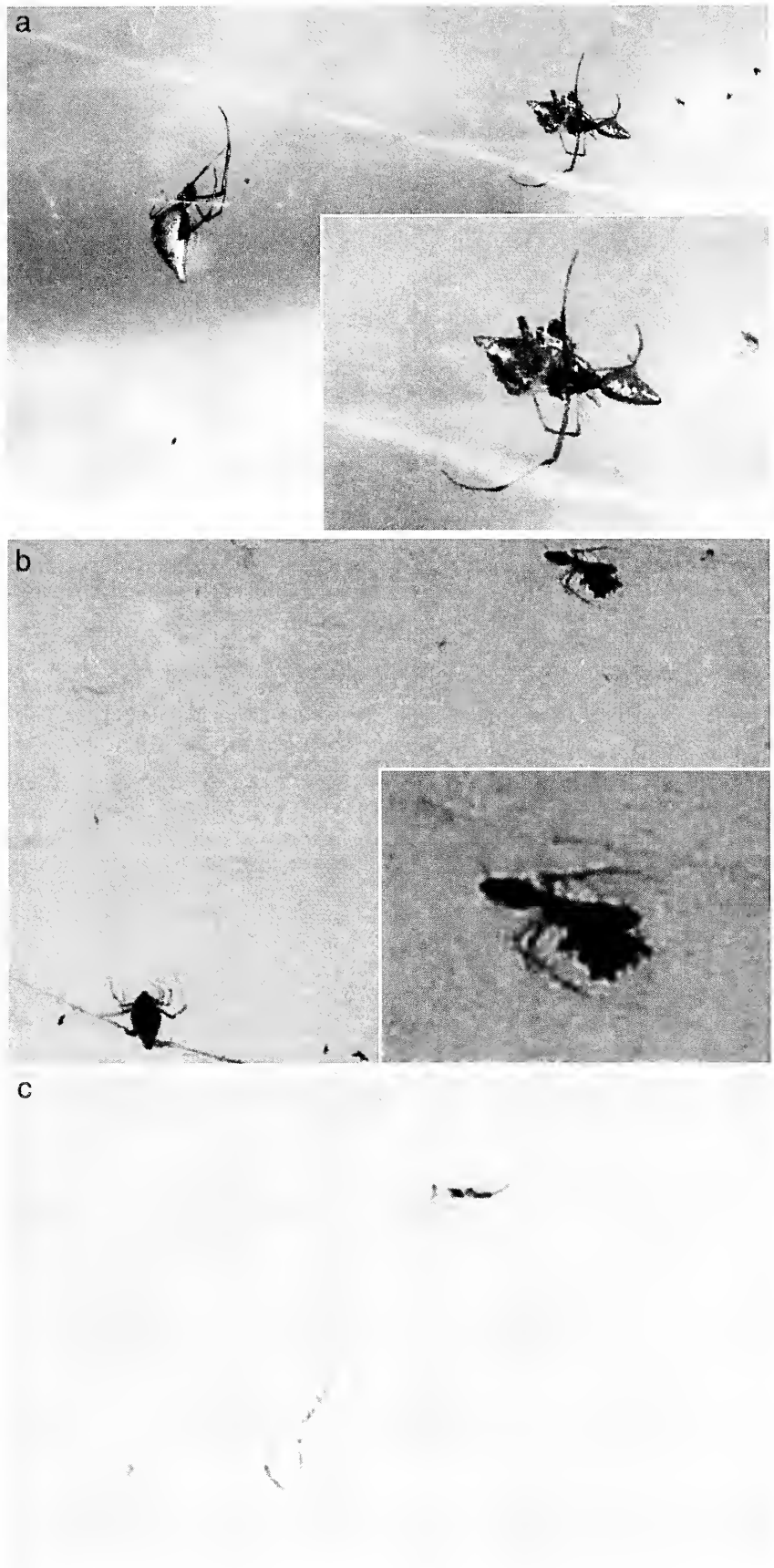

Figure 1.—Sequence of photographs taken in the field illustrating possible male nuptial gift behavior in *A. elevatus*. a. Male with prey item, facing female (picture taken at 17:22 h); b. male and female facing away from each other, male still holding prey item (picture taken at 18:04 h); c. male and female at rest the following morning, male with no prey item (picture taken at 06:12 h).

Figure 2.—Live *Cyclosa huila* from the same communal web as the *A. elevatus* pair.

The evolutionary relationships within *Argyrodes* remain poorly understood, but the genus is currently composed of six monophyletic clades, each with a typical feeding behavior (Exline & Levi 1962; Whitehouse et al. 2002). *A. elevatus* belongs to the Argyrodes clade, which specializes on kleptoparasitism rather than araneophagism (Whitehouse et al. 2002). The apparent use of *C. huila* as prey by *A. elevatus* exposes the variable nature of the relationship between these parasites and their hosts.

Obligatory kleptoparasites depend on resources provided by their host, and they rarely migrate to other webs once a suitable web has been found because suitable webs are rare resource patches (Agnarsson 2003). Since communal hosts provide more resources to *Argyrodes* than the webs of solitary hosts (Agnarsson 2003), episodic araneophagy in the Argyrodes clade may be more likely to occur on communal hosts than on solitary hosts, because eliminating a solitary host would deplete the resource income of *Argyrodes* and result in an energetic cost to search for a new host. In contrast, eliminating an individual of a communal host species would not deplete the resource income, because other individuals of the host species would still remain. In our case, the host was *C. huila*, a social species (Levi 1999) of the territorial periodic-social category (Avilés 1997), in which each *C. huila* spun its own orb web, and all individuals were adults. We have no evidence that the *C. huila* was killed by *A. elevatus*, but our observations generate questions for which answers would improve our understanding of the links between the phylogeny of *Argyrodes* and the diversity of foraging behaviors in the genus. For instance, we could gain insight into these links by determining if the unusual behavior reported here is rooted in episodic araneophagy and if so, if the use of the host by *A. elevatus* is related to the social nature of *C. huila*.

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SHORT COMMUNICATION

Insect attraction by webs of *Nephila clavipes* (Araneae: Nephilidae)

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Abstract. Although well studied, the role of spider webs in attracting prey and the role of web ornaments remain open questions. We carried out a field study to determine whether webs of *Nephila clavipes* (Linnaeus 1767) attract insects. *Nephila* builds large orb-webs with debris-decoration that host kleptobiotic *Argyrodes* spiders. We studied the potential prey of *Nephila* with sticky traps placed in two similar linear plots. One plot contained 20 *Nephila* webs, and the other was cleared of *Nephila* webs. We measured the number and size of the insects caught in the traps. We compared the size of the trapped insects with prey caught by *Nephila* and gleaned by *Argyrodes*. In the plot with *Nephila* webs we collected 314 individuals versus 105 individuals in the plot without *Nephila*. Species of Diptera and Coleoptera were captured most frequently. Four saprophagous families, Phoridae and Sciaridae (both Diptera), Staphylinidae and Elateridae (both Coleoptera), were more abundant in the plot with *Nephila* webs. We show for the first time under natural conditions that prey attraction is most efficient for saprophagous insects, suggesting that the debris-decoration in *Nephila* webs attracts this guild. We also found that the size of some insects captured does not correspond to the range of prey consumed by *Nephila*, but to that of kleptobiotic *Argyrodes* spiders. We hypothesize that the debris-decoration may be used by *Nephila* as a strategy to limit food competition with *Argyrodes*.

Keywords: Prey attraction, debris-decoration, kleptobiosis, food competition

Among theories proposed to explain the existence of ornamentation on spider webs, the prey attraction hypothesis has been most extensively tested and discussed (Blackledge & Wenzell 1999; Herberstein et al. 2000). However, Gonzaga & Vasconcellos-Neto (2005) and Chou et al. (2005) showed that the linear detritus stabilimenta built by *Cyclosa* species (Araneidae) do not increase prey capture, but rather have an anti-predator function. Another function of stabilimenta, described for *Gasteracantha cancriformis* (Linnaeus 1758) (Araneidae), is a warning to large animals that could destroy webs (Jaffé et al. 2006). Champion de Crespigny et al. (2001) showed that *Nephila edulis* (Labillardière 1799) (Nephilidae), a species with a relatively permanent web, incorporates a prey cache on which it feeds during periods of food shortage. Most studies, however, describe stabilimenta as a strategy to attract prey. For example, the experimental study of Bjorkman-Chiswell et al. (2004) showed that a band of decaying carcasses and plant matter built by *N. edulis* attracts sheep blowflies.

In *Nephila clavipes* (Linnaeus 1767) (Nephilidae) adults build stabilimenta made of decaying matter (Fig. 1), generally insect carcasses (Hénaut et al. 2005). These authors observed that numerous insects captured by the web are too small to be consumed by *Nephila* but are gleaned by kleptobiotic *Argyrodes* spiders (Theridiidae). Our field study tested the role of *N. clavipes* webs in attracting insects and looked at the possibility that *Nephila* has a strategy to provide a supply of food to the kleptobiotic spiders. To approach these questions we identified the trophic characteristics (at family level) and size of the potential prey in the environment to determine which guilds of prey are attracted, and also, if they fall within the range of prey sizes consumed by *Nephila* or *Argyrodes* spiders.

The work was conducted at the edge of a coffee plantation in Southern Mexico. The study area was established along big trees and barbed wire fences. For further details on the study area, see Hénaut et al. (2005). The *Nephila* webs were distributed regularly in a row along the fence, built on the fences or between the fences and trees. The area was a 200-m long, homogeneous linear transect with 40 *Nephila* webs. We divided the area into two consecutive plots of 100 m each (20 *Nephila* webs in each plot). The first plot was called "with *Nephila*" (*Nephila* spiders and their webs were left in this plot), the other was called "without *Nephila*" (20 *Nephila* webs with their spiders were removed). Identification of experimental spiders was based on voucher specimens deposited in the collection of the Laboratorio de Ecoetología de Artrópodos in Ecosur, Tapachula, Mexico.

The study was carried out at the end of the rainy season (November 2003), when *N. clavipes* and their prey were numerous. At this time *Nephila* spiders are adult, and their webs are not destroyed by heavy rain.

To determine the capture rate of potential prey, eight sticky traps per plot were set up. The traps, similar to those used by Hénaut et al. (2006), were hung one meter above the ground, less than one meter from one side of each *Nephila* web on the plot with *Nephila*, and every 10 meters in the plot without *Nephila*. The sticky traps were made of a transparent plastic board (30 × 20 cm) coated with Tangle Foot © (The Tanglefoot Company, Grand Rapids MI 49504 USA). Captures were repeated over two 24-h periods using different traps and renewed webs.

Trapped insects were preserved in 70% ethanol before being counted, identified, and measured in the laboratory under a binocular microscope. We determined the number of individuals per order for each plot (with or without *Nephila*). Individuals were identified to the family level only for the orders in which the number of individuals was significantly different between the two plots. Some prey individuals (49 insects in the plots with *Nephila* and 30 insects in the plots without *Nephila*) could not be identified. We measured the length of each prey item from the extreme anterior point of the head to the hindmost part of the abdomen. The mean body length of insect families (mean ± SE) was also calculated for the most frequent families.

Figure 1.—A web of *Nephila clavipes*. A general view and a focus on debris-decoration: a = plant remains, b = prey remains.

The total number of insects per trap, the number of insects of the most abundant orders, the number of saprophagous insect families, and the number of insects of the two most abundant saprophagous families were compared between the two experimental plots using one-way ANOVA after square root transformation of the response variables.

We collected three times more insects in the plot with *Nephila* (363 individuals: 10 orders and 42 families) than in the plot without *Nephila* (135 individuals: 9 orders and 28 families). The mean number of insects per trap was significantly greater in the plot with *Nephila* than in the plot without *Nephila* (22.7 ± 1.9 *vs.* 8.1 ± 1.0 respectively; $F_{1,30} = 38.89$, $P < 0.001$). The number of individuals per order was also always higher in the plot with *Nephila* (Table 1). For five orders with more than 10 individuals captured, the difference was statistically significant (Table 1).

For orders that presented a significant difference between plots, we analyzed the number of individuals per family. Few families presented significantly more individuals in the plot with *Nephila* (Diptera: Phoridae, Sciaridae, Dolichopodidae; Hymenoptera: Formicidae; Homoptera: Cicadellidae) and only one family of Diptera (Chironomidae) presented significantly more individuals in the plot without *Nephila* (Table 2).

From the five families that differed in abundance between plots, three were saprophagous (Phoridae, Seiaridae, and Dolichopodidae) according to Borror & DeLong (1981). Four other saprophagous families (Otitidae, Drosophilidae, Sphaeroceridae, Mycetophelidae)

Table 1.—Comparison of the total number of invertebrates of 11 orders captured in traps on two plots. Comparisons by means of ANOVA were made only for orders represented by more than 10 individuals.

Order	*Nephila* present	*Nephila* absent	ANOVA
Diptera	213	62	$F_{1,30} = 41.63$, $P < 0.001$
Coleoptera	68	44	$F_{1,30} = 1.87$, $P = 0.181$
Hymenoptera	32	8	$F_{1,30} = 12.04$, $P = 0.002$
Homoptera	22	12	$F_{1,30} = 2.28$, $P = 0.141$
Hemiptera	13	2	$F_{1,30} = 10.58$, $P = 0.003$
Orthoptera	2	0	-
Lepidoptera	2	1	-
Psocoptera	7	3	-
Zoraptera	1	0	-
Strepsiptera	0	1	-
Araneae	3	2	-

were trapped, but at very low abundance. When pooled together, the mean number of individuals from saprophagous families per trap was significantly higher in the plot with *Nephila* ($n = 175$) than in the plot without *Nephila* ($F_{1,30} = 67.76$, $P < 0.001$). This difference was due mostly to two families: Phoridae and Sciaridae (Table 2).

The mean body length of insects trapped in the plot with *Nephila* (2.02 ± 0.05 mm; range: 0.8–11 mm) was significantly smaller ($F_{1,416} = 10.3$, $P = 0.001$) than in the plot without *Nephila* (2.36 ± 0.09 mm; range: 0.8–5 mm). The sizes of trapped individuals belonging to the three saprophagous families that presented a significant difference between both plots were Phoridae (1.4 ± 0.04 mm, $n = 106$); Sciaridae (1.7 ± 0.06 mm, $n = 74$) and Dolichopodidae (2.5 ± 0.2 mm, $n = 7$). None of these insects fit in the range of prey sizes caught by *Nephila*, but they do fit in the range of prey sizes exploited by *Argyrodes* spiders (Hénaut et al. 2005).

Our study provides the first evidence under natural conditions that webs of *Nephila clavipes* attract a larger number and higher diversity of insects than control sites. Both plots were in a similar environment (architecture, floral composition, orientation, climate), so the greater number of insects in the plot with *Nephila* webs could not reflect environmental variation. Furthermore, traps were placed at the height of *Nephila* webs sufficiently far from webs so that prey were unlikely to steer away from the webs onto the traps, all the more so since the prey of *Nephila* webs tumble to escape from the web (Zschokke et al. 2006). Therefore, we conclude that the presence of *Nephila* webs increased the number of insects that stuck to the traps.

Several studies have shown that the presence of debris-decoration made of silk on the webs of orb-web spiders attracts prey. For instance, *Argiope* spider web ornaments increased prey capture rate (Herberstein 2000; Bruce et al. 2001). In a field study, Tso (1998) showed that the stabilimentum-ornamented webs of *Cyclosa conica* (Pallas 1772) (Araneidae) trapped significantly more insects (150%) than undecorated webs. However, few field studies have been carried out to study the effect of debris-decoration containing detritus (animal and/or plant). Among these studies, Gonzaga & Vasconcellos-Neto (2005) and Chou et al. (2005) argued against the prey attraction hypothesis in research carried out both in the field and laboratory with *Cyclosa morretes* Levi 1999 and *C. fililineata* Hingston 1932 (Araneidae). These two spiders build debris-decorations that include linear and spiral silk structures and detritus. On the other hand, Bjorkman-Chiswell et al. (2004) observed that decaying matter in *N. edulis* webs do indeed attract saprophagous insects. Among all the insects we observed, particularly small saprophagous insects belonging to two families of dipterans were more abundant in both the traps and webs. Therefore, we suggest that the presence of decaying organic material in the *N. clavipes* webs is the possible

Table 2.—Total number of individuals (sum for all traps excluding non-identified individuals) for each family of Diptera, Hemiptera, Hymenoptera, and Homoptera in both plots. Comparison was done using ANOVA only for families with more than 10 individuals.

Order / Family	*Nephila* present	*Nephila* absent	ANOVA
Diptera			
Ceratopogonidae	0	1	-
Chamaemyiidae	11	9	$F_{1,30} = 0.002$, $P = 0.96$
Chironomidae	2	14	$F_{1,30} = 5.54$, $P = 0.025$
Clusidae	2	1	-
Dolichopodidae	6	1	$F_{1,30} = 5$, $P = 0.033$
Drosophilidae	3	1	-
Empididae	0	3	-
Lauxaniidae	1	1	-
Muscidae	1	0	-
Mycetophilidae	1	0	-
Otitidae	1	1	-
Phoridae	96	10	$F_{1,30} = 34.02$, $P < 0.001$
Sciaridae	62	12	$F_{1,30} = 13.53$, $P = 0.001$
Simulidae	3	2	-
Sphaeroceridae	3	0	-
Tephritidae	0	1	-
Tipulidae	1	1	-
Trixoscelididae	1	0	-
Hemiptera			
Anthocoridae	4	0	-
Miridae	4	2	-
Pentatomidae	1	0	-
Hymenoptera			
Bethylidae	4	0	-
Braconidae	2	1	-
Ceraphronidae	2	0	-
Chalcididae	1	0	-
Encyrtidae	4	0	-
Eucharitidae	2	0	-
Eulophidae	1	0	-
Eupelmidae	0	1	-
Formicidae	15	6	$F_{1,30} = 5.25$, $P = 0.029$

explanation for the high abundance of saprophagous catches. Less numerous hymenopterans and homopterans were also attracted to the web, probably by the bright yellow color of the silk and the spider, as described by Craig (1994) and Tso et al. (2004).

In our field study, the *N. clavipes* webs mainly attracted small prey (smaller than 3 mm) that are not within the range of prey sizes captured by the spider (Hénaut et al. 2005). Moreover, this spider builds permanent webs, so it can hardly take advantage of eating small insects during web consumption as observed in other orb-weaving species (Hénaut et al. 2001). However, the small insects attracted by the web fit perfectly in the range of prey gleaned by kleptobiotic *Argyrodes* spiders that live on *Nephila* webs (Hénaut et al. 2005). Numerous small insects may prevent direct competition for food between *Nephila* and *Argyrodes*, which happens when kleptobiotic spiders steal prey from the host's reserves or eat at the same time (Hénaut et al. 2005). The attraction of numerous small saprophagous prey by *N. clavipes* webs may be a side-effect of the use of decaying matter in the debris-decoration to attract larger insects that are prey of *Nephila*. Alternatively, the construction of these decorations is a strategy of *Nephila* to provide abundant food to the kleptoparasitic spiders living on its web, hence avoiding direct competition with them.

This field study suggests that debris-decoration does attract saprophagous insects, but also offers a new perspective about the

function of these decorations in spiders. Further steps in this work would be to determine whether the presence of *Argyrodes* spiders actually induces the construction of the debris-decoration by *N. clavipes*.

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SHORT COMMUNICATION

Polymorphism in an ant mimicking jumping spider

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Abstract. *Myrmarachne bakeri* Banks 1930 is a polymorphic, generalized ant-mimicking jumping spider. In this study, variation in its polymorphic characters was observed and described. *Myrmarachne bakeri* varies in color, glossiness and patterns; and differs from other polymorphic ant-like spiders because it becomes polymorphic before adulthood. Morphological changes appear to have no set archetype, and few spiders revert to morphs previously observed. Polymorphism is widespread in *Myrmarachne*, but to date no species has been shown to exhibit the type of variation found in *M. bakeri*.

Keywords: Ant-like, visual discrimination, *Myrmarachne*, transformational mimicry

Spider coloration is typically seen as a result of selection by visually hunting predators (Oxford & Gillespie 1998). Apostatic selection occurs when a given phenotype is under-represented in a predator's diet when it is rare, but is over-represented when it is above a threshold abundance (Ruxton et al. 2004). This type of selection favors rarer morphs and may lead to polymorphism in characters recognized by the predator.

Many spiders are myrmecomorphic (ant-like) and, through their resemblance to ants, gain protection from predators that normally avoid ants because ants are dangerous and heavily defended (Nelson & Jackson 2006). This phenomenon, known as Batesian mimicry, in this case of ants, is especially common in the Salticidae (Cushing 1997). Several myrmecomorphic salticids are polymorphic (e.g., Oliveira 1988; Ceccarelli & Crozier 2007), but polymorphism can adopt various forms. Transformational mimics are those that change morphology at different instars, with each morph resembling a partieular model (see Cushing 1997). Other myrmecomorphs are polymorphic as adults and seem to have one particular model for each morph, while in other cases adults are sexually dimorphic and each sex mimics a different model (Oliveira 1988; Cushing 1997).

All species of *Myrmarachne* are Batesian mimics of ants (Edmunds 1993; Nelson & Jackson 2006; Nelson et al. 2006). *Myrmarachne*, like most salticids, are cursorial predators and rely primarily on vision to hunt their prey and to communicate with conspecifics (Richman & Jackson 1992; Nelson & Jackson 2007). Adults of the species studied here, *Myrmarachne bakeri* Banks 1930, do not appear to have any particular ant model; instead appearing to be generalized ant mimics. My first impression was that it was sexually dimorphic (like all species of *Myrmarachne*, adult males of *M. bakeri* have elongated chelicerae), with each sex having a "red" morph (Fig. 1A, B) and a "black" morph (Fig. 1C, D), leading me to believe that they mimicked two different ant models, one red and one black. However, it soon became clear that instead of simply having two distinct morphs, color variation in *M. bakeri* covers a whole spectrum of patterns, which I describe here.

I collected large juvenile and adult spiders in the vicinity of the International Rice Research Institute (IRRI) at Los Baños in the Philippines and housed them at IRRI and in the School of Biological Sciences at the University of Canterbury, New Zealand. Spiders were maintained in individual plastic cages, cleaned weekly, and provided humidity with a cotton roll through the bottom that dangled in a small cup of water. All spiders were fed twice a week. Spiders less than 3 mm were fed wild-caught whiteflies, eultured *Drosophila* and small cultured house flies (*Musca domestica*) thereafter.

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After collection, the adult sex was noted and juveniles were labeled as such. Spiders were then described in detail and sketched, paying particular attention to the spider's abdomen, as it was the abdomen that varied most. In descriptions of dermal morphology, "shiny" denotes that the spider glistened in the artificial light of the laboratory and did not have a 'hairy' or 'furry' appearance, while "dull" spiders did not glisten and had a 'hairy' or 'furry' appearance. Ventrally, *M. bakeri* exhibited changes of glossiness and darkening or lightening of parts, or all, of the abdomen. However, five individuals (4.8%) developed a ventral line along the sagittal plane. Morphs were considered different when there were visible changes in the colors on the dorsal side of the abdomen or changes in the number, or thickness, of dorsal abdominal lines. I did not consider a darkening of coloration after molting a different morph, as this may have been the product of the hardening of the exoskeleton.

Each time a spider was checked, described, and resketched, the cage was cleaned. I checked all spiders daily for molting, as evidenced by the exoskeleton in the cage. Each time a molt was found this was noted, along with any morphological changes in the spider. Regardless of whether molting occurred, each spider was described every three weeks until it died. In total, 105 spiders (70 females and 35 males) were described from collection until death, in many cases 6 months later.

Voucher specimens of all species have been deposited in the IRRI Taxonomy Laboratory in Los Baños, the Philippines, and in the Florida State Collection of Arthropods, Gainesville, Florida, USA.

Individual spiders changed in glossiness and markings. Surface glossiness, in which the individual sometimes appeared shiny and dull other times, changed in 17 (16.19%) individuals. Regardless of color, individuals might be shiny or dull (Fig. 1A, E), although more than 80% of black spiders were dull (Fig. 1C). Four spiders (3.81%) developed two white spots above the anterior medial eyes (Fig. 1F). These spots (termed 'eye spots') developed in both sexes and occurred in individuals of different colors. Once eye spots developed, they did not disappear, despite changes in overall coloring of the spider. Fourteen females (20%) developed black triangles on the sides and occasionally the middle of the abdomen toward the anterior end. In three individuals these triangles joined over time and became a new line on the abdomen. These triangles were always observed on the dorsal side of the abdomen (Fig. 1G, H).

Additionally, *M. bakeri* exhibited both color changes and polymorphism, although only four males (11.4%) changed morph once they became sexually mature adults (Fig. 1B, D, I, J). The chelicerae and cephalothoraces of males were black or dark red, although the cephalothorax and the chelicerae were not necessarily the same color (Fig. 1D, K). Overall, *M. bakeri* exhibited a large variety of colors that were expressed uniformly or in combination at

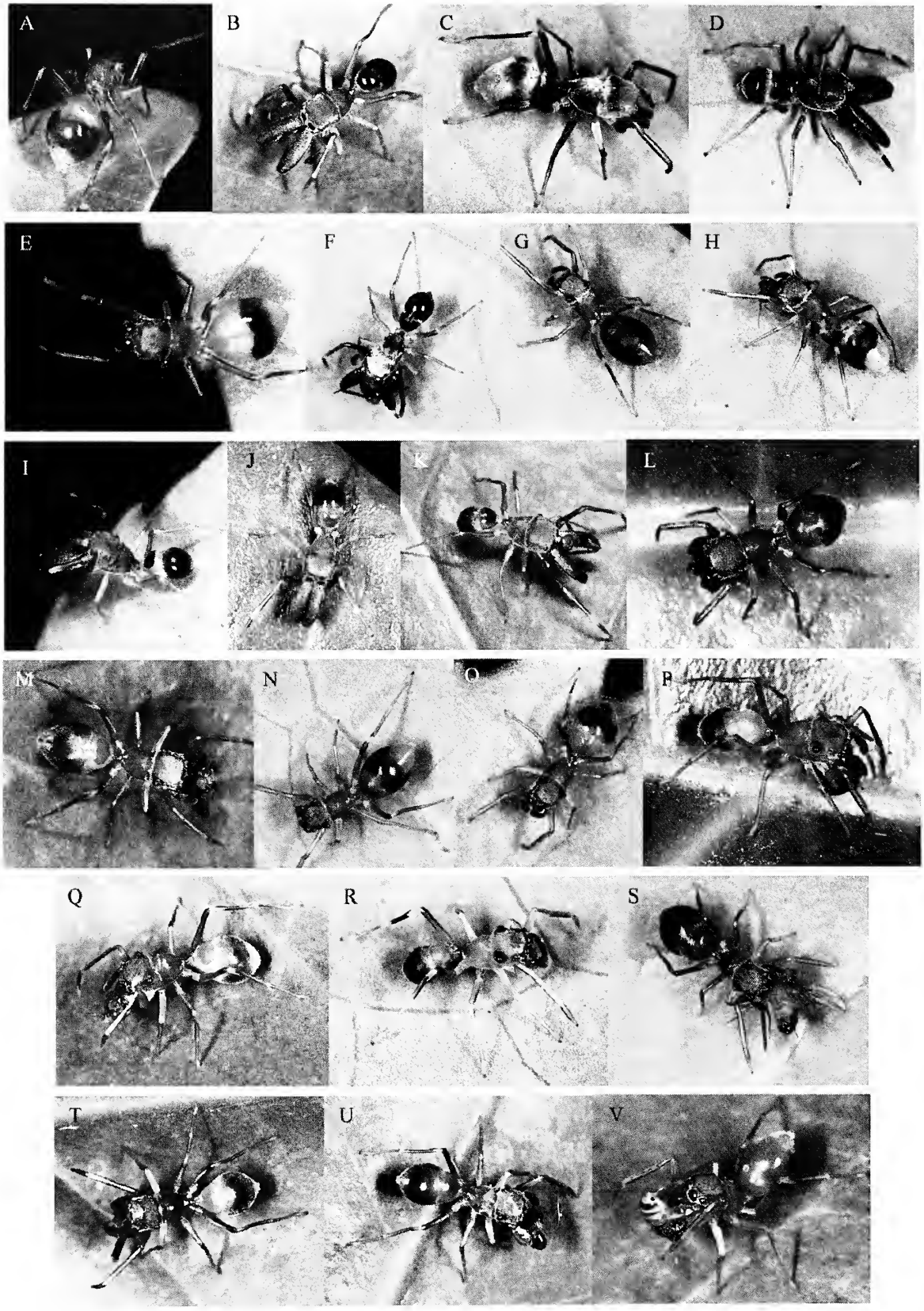

Figure 1.—Polymorphism in *Myrmaraclne bakeri*. Photos courtesy of Robert Jackson. A) 'Red' female (c.f. L, same individual); B) Shiny red male (c.f. J, same individual); C) Shiny black male; D) Shiny red female; E) Dull 'black' female; F) Black, shiny male with whitish eyespots; G) Brown female with triangles on the anterior part of the abdomen; H) Female with yellow abdominal tip and triangles at the anterior of her abdomen; I) Pale red male (adult of spider in P); J) Male with dark red coloration (c.f. B, same individual); K) Red male with black chelicerae (adult of spider in V); L) Dark red female (c.f. A, same individual); M) Red female (eating *Drosophila*). Note what may be pattern superimposition; N) Red female. Note what may be pattern superimposition; O) Red female; P) Orange subadult male (eating *Drosophila*) (adult is shown in I); Q) Orange female (eating *Drosophila*). Note what may be pattern superimposition; R) Yellow female; S) Brown female (eating *Drosophila*); T) Orange, red, and black female (eating *Drosophila*). Note what may be pattern superimposition; U) Female (eating *Drosophila*) with a golden abdominal tip; V) Shiny subadult male (eating *Drosophila*) (adult is shown in K).

some stage in their lives. These colors included light to dark red (74.2%; Fig. 1L, M, N, O), orange (36.19%; Fig. 1P, Q), yellow and gold (26.7%; Fig. 1H, R, U), ochre (1%), brown (40%; Fig. 1G, S), black (94.3%; Fig. 1C, T), and white (26.7%).

Most spiders (79.4%) changed morphs throughout the six-month observation period (Fig. 1K & V, B & J, I & P, A & L). From the total pool of spiders (n = 105), 28.9% exhibited two morphs, 25.8% exhibited three, 13.4% four, 8.3% five and 3.1% exhibited six different morphs. Spiders that went through three morphs or more were checked to see whether they had reverted to a previous morph. Just over a quarter of these (26.4%) were found to have reverted to a previous morph. Consequently, in *M. bakeri*, it appears that polymorphism occurs both between and within individuals. Furthermore, *M. bakeri* continued to change morphologically throughout the six-month period in the laboratory, where the feeding regime, as well as light, humidity, and temperature were controlled, suggesting that this polymorphism is not merely environmentally conditioned, and presumably has a genetic basis. Further research on the genetics underlying polymorphism in mimicry (Ceccarelli & Crozier 2007) may yield particularly interesting results.

Polymorphism in the Hawaiian happy-face spider (*Theridion grallator* Simon 1900) is controlled at one, or sometimes more, loci (depending on the island) (reviewed in Oxford & Gillespie 1998). In general, within patterned morphs, these spiders appear to have dominance of some alleles that are superimposed on the area of pigmentation controlled by another allele. Although in *M. bakeri* there are several morphs throughout life, a close look at the spiders does suggest what may be pattern superimposition. For example, some markings appear superimposed on others, such as the red 'spots' on the anterior of the abdomen (Fig. 1M, N, Q, T). Another analogy can be made between *T. grallator* and *M. bakeri*: both exist in volcanic archipelagos in which the possibility of population differentiation and speciation are particularly marked when compared to the mainland due to random events, such as a volcanic eruption.

Four virgin females were allowed to mate and were then observed at a later stage with egg sacs that hatched into spiderlings. This strongly suggests that they belonged to the same species as the males. However, *Myrmarachne* are notoriously difficult to rear in laboratory conditions, and the viability of the offspring could not be tested, as it was impossible to rear the young to adulthood. Nevertheless, all spiders that hatched were monitored until death. During the first two or three instars spiders exhibited only one morph (ant-like) consisting of a translucent body with a single black line on the abdomen. The subsequent two or more instars were also monomorphic, but consisted of a pale orange body with no lines or other markings (termed 'juvenile' morph). This suggests that *M. bakeri* has one particular ant model for each of these two morphs and that this may be a case of transformational mimicry, in which different morphs at different life stages resemble distinct ant models (Cushing 1997). However, due to my inability to successfully rear any individual through all instars into adulthood I was unable to determine how many instars are monomorphic (for either the early instar morph or the juvenile morph) and at what stage polymorphism within and between individuals occurs. However, a few individuals collected as large juveniles in the field became polymorphic three instars short of sexual maturity, with individuals exhibiting several different morphs. Overall, individuals changed morphology up to six times and few individuals had the same colors and patterns, suggesting that molting may not be a prerequisite to induce morphological changes in this species. While these results are merely suggestive, this aspect of *M. bakeri*'s polymorphism seems a particularly interesting avenue for further investigation.

Palatable Batesian mimics exploit the predator's aversion by evolving similar coloration to that of distasteful or dangerous animals, requiring them to be less numerous than the model. This frequency-dependent selection occurs because predators must encounter the unpalatable model more often than the palatable mimic in order to learn (or evolve, on a greater time scale) characteristic cues of the model and thereby avoid them (Ruxton et al. 2004; Nelson & Jackson 2006, Nelson et al. 2006).

Some animals, such as spiders and mantids, have an innate fear of ants (Nelson & Jackson 2006, Nelson et al. 2006). Just as in learned aversion, mimics that resemble models for which animals have evolved an innate fear may also benefit from being polymorphic. There must be considerable selection to maintain innate fear of dangerous prey and if there are many benign mimics, this pressure may be slackened. However, as with learned aversion, polymorphism (in the mimic) reduces the apparent number of the mimics per model. Therefore the selective pressure for aversion of the model is maintained because the characteristics of the prey continuously change and therefore predators may not develop innate mechanisms whereby they can distinguish the palatable mimic from its unpalatable model. Consequently, polymorphism is often expected among Batesian mimics (Ceccarelli & Crozier 2007) and may provide a selective advantage through an apparent reduction in the frequency of the mimic relative to the model (Oliveira 1988; Ritland 1995). This idea assumes that it is beneficial to parents to spread genes of different morphs so offspring do not appear common and are therefore not easily 'targeted' by predators. Indirect evidence for the possibility that polymorphism is selected for by (visual) predators lies in the marked difference between the observed polymorphism on the dorsal and ventral sides of the abdomen in *M. bakeri*. Predators would rarely view the spider from its ventral surface and therefore there would be little point in extending polymorphic characters ventrally.

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SHORT COMMUNICATION

Reestablishment of the species *Poeciloneta bellona* (Araneae: Linyphiidae)

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Abstract. *Poeciloneta bellona* Chamberlin and Ivie 1943 is removed from synonymy established by Saaristo and Tanasevitch (2000) and re-described using new and existing specimens from the Rocky Mountains. *Incestophantes calcaratus* (Emerton 1909) is re-described and transfered to *Poeciloneta* Kulczyński 1894 and a lectotype is designated.

Keywords: Synonomy, *Poeciloneta*, *Incestophantes*, lectotype

The genus *Poeciloneta* Kulczyński 1894 is an uncommonly collected genus represented by 16 species worldwide, of which 12 species, including the two discussed in this paper, occur in North America (Platnick 2009). The spiders are found throughout Canada, in the northeastern United States, and in the mountains of the western United States. The spiders are usually collected from low branches and shrubs in coniferous forests.

Tanasevitch (1989) reviewed the palaearctic species in the genus and noted that *Poeciloneta bellona* Chamberlin and Ivie 1943 & *P. canionis* Chamberlin and Ivie 1943, based on the original descriptions, no longer belonged to *Poeciloneta*, but made no further note as to the placement of the two species. Later Saaristo and Tanasevitch (2000) redefined the *Bolyphantes-Poeciloneta* genus group and synonymized *P. bellona* with *I. calcaratus* (Emerton 1909) transferring the two the species to *Incestophantes* based on non-type specimens held at the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts.

Fresh specimens of *P. bellona* deposited at the Denver Museum of Nature and Science were collected from the Rocky Mountains of Colorado that closely matched Chamberlin and Ivie's (1943) original description and showed clear differences from existing descriptions of *P. calcaratus*. All applicable specimens were examined from the MCZ collection and it was found that no *P. bellona* were in the collection, which may account for the mistaken synonymy made by Saaristo and Tanasevitch. Additional specimens used for descriptions and comparisons came from the arachnid collection of the Denver Museum of Nature and Science, Denver, Colorado (DMNS); the Canadian National Collection, Ottowa, Canada (CNC); and the personal collection of Don Buckle, Saskatoon, Saskatchewan, Canada (DB). Inquiries were made as to the location of type material at the American Museum of Natural History, New York (AMNH); MCZ; University of Utah Natural History Museum, Salt Lake City, Utah; and the United States National Natural History Museum, Washington, DC. No declared type specimens for either species could be located, but specimens used by R. Emerton to describe *Bathyphantes (= Incestophantes) calcaratus* (Emerton 1909) were examined and lectotype and paralectotype specimens were designated. No speeimens identified as *P. bellona* by either R. Chamberlin or W. Ivie could be located.

Illustrations were made from digital photographs taken using an Olympus U-CMAD3 digital camera mounted on an Olympus SZX12 stereomicroscope. Label information is transcribed as written on the label; therefore, no additional information was added. Embolus illustrations were made by first soaking the palp in 10% KOH solution for 30 minutes, then immersing the palp in clove oil, which allowed for the removal of various parts of the embolus. Embolic illustrations are provided for the purpose of generic placement of the species. Because of the difficulty of the dissection and the high magnification requircd, the usefulness of thc dissection in species identification is limited and other characters are provided for identification purposes.

Abbreviations: Tm = metatarsus trichobothria, followed by the leg number; e = embolus; l = lamella; TA = terminal apophysis; sa = superategular apophysis; ds = dorsal scape; st = stretcher. Chaetotaxy as defined in Tanasevitch 1989, patterned as dorsal-prolateral-retrolateral-ventral.

TAXONOMY

Poeciloneta Chyzer & Kulczyński 1894
Poeciloneta bellona Chamberlin & Ivie 1943
Incestophantes calcaratus (Emerton 1909) Saaristo & Tanasevitch 2000: 260. **Synonymy rejected**

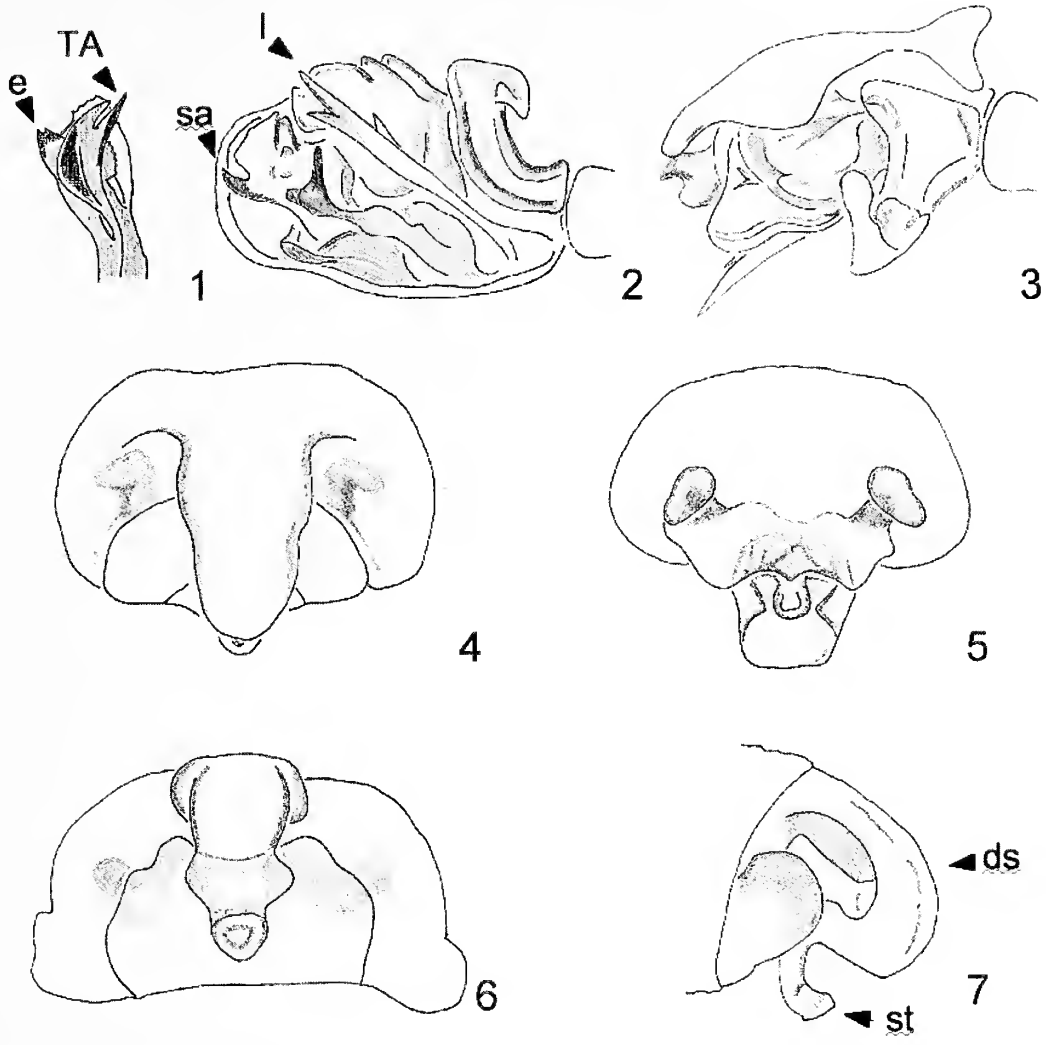
Type material.—Holotype female, USA: *Utah*: Dechesne County, Mirror Lake, 40°N, 110°W, 22 September 1932, Coll: Wilton Ivie. Unable to locate.

Other material.—3 males, 6 females. USA: *Colorado*: Clear Creek County, 1M, Hwy. 40 & Jones Pass Rd., 39°46′12″N, 105°49′31″W, 3266 m, 1 Jul 2002, beat sheet, B. Morrison (DMNS); 1F, Hwy. 40 Berthoud Pass, E/SE side, 39°47′48″N, 105°46′33″W, 3723 m, 2 July 2002, beat sheet, B. Morrison (DMNS); 1F, Squaw Mountain, 39°40′46″N, 105°30′15″W, 3305 m, 3 Sep 2005, beat sheet, P.E. Cushing (DMNS); 1F, Squaw Mountain, 39°40′55″N, 105°30′11W, 3356 m, 4 Oct 2005, beat sheet, J. Slowik (DMNS); 1F, Squaw Mountain, 39°41′05″N, 105°31′33″W, 3344 m, 3 Sep 2005, beat sheet, P.E. Cushing (DMNS); 1M, same locality, 8 Jul–5 Aug 2005, pitfall traps, J. Slowik (DMNS); Jefferson County: 1F, Jefferson County Open School, Lakewood, 39°43′59″N, 105°04′55″W, Sep 1999, C. Cummins (DMNS); Summit County: 1M, 1F, Eagles Nest near lower Boulder Lake, 8 Aug. 1999, S. Shiner (DMNS).

Diagnosis.—Male *Poeciloneta bellona* can be differentiated from all other *Incestophantes* and all *Poeciloneta* except *P. calcaratus* by the two-part, pointed, beak-shaped terminal apophysis (Fig. 1) and the narrow lamella (Fig. 2). They may be distinguished from *P. calcaratus* males by the longer more narrowed ventral terminal apophysis tip and the shape of the lamella, with the lamella of *P. bellona* forking later than *P. calcaratus* (0.85 of its total length). Females can be separated by the shape of the dorsal scape (Fig. 4) and the proximity of the stretcher when viewed laterally (Fig. 7).

Description.—*Male* (n = 3): total length, 2.40–2.90 mm; carapace length, 1.10–1.20 mm; carapace width, 0.90–1.00 mm. Tm I, 0.90–0.95; Tm IV, present. Chaetotaxy: F I, 0-1-0-0; F II–IV, 0-0-0-0; Pt I–IV, 1-0-0-0; Ti I, 2-1-1-0; Ti II, 2-0-1-0; Ti, III–IV,2-0-0-0; Mt I: 0-0-0-0.

Carapace smooth yellow, with dusky shield-shaped area located on the fovea with dark lines extending to the PLE. Carapace edge dusky, with darker areas extending mesially at each coxa. Sternum dusky, edges dark, chelicerae brown, labium and endites yellow with dark



Figures 1–7.—*Poeciloneta bellona* Chamberlin & Ivie 1943. 1. Terminal apophysis and embolus. 2, 3. Palp: 2. ventral view, 3. lateral view. 4–7. Epigynum: 4. ventral view, 5. dorsal view, 6. posterior view, 7. lateral view. e = embolus; TA = terminal apophysis; l = lamella; ds = dorsal scape; st = stretcher.

bases. Dorsal abdominal pattern consists of 9–10 dark chevrons with first 3 usually united on a white background. Laterally a dark line runs side to side from mid-abdominal length anteriorly above the pedicle. Lateral line followed posteriorly by two hash marks. Venter light yellow brown with a dark area around the epigastric plate, suffused with white spots. Legs light, with dark rings mid-length and at the end of the femur, patella, and tibia. Metatarsi with a dark ring at the distal end, tarsi without rings.

Lamella straight in ventral view, splitting into two spurs at 0.85 of total lamella length, dorsal fork equal to 0.08 of total lamella length, ventral fork equal to 0.20 of total lamella length (Fig. 2). Occasionally a third spur will exist, restricted to ventral side of lamella located below fork of other two major spurs; if present, 0.09 of total lamella length. Terminal apophysis two-part, pointed, long, beak shaped, tips as long as embolus thumb, directed at the lamella (Fig 1). Suprategular apophysis a broad hooked shape. Ventral edge or paracymbium hooked, bifurcate, both parts rounded (Fig. 3). Spur at base of cymbium broad, extending to or slightly beyond base of cymbium. Embolus proper terminal two-pointed, thumb large, embolus attached to terminal apophysis (Fig. 1).

Female ($n = 6$): total length, 2.40–2.90 mm; carapace length, 0.98–1.48 mm; carapace width, 0.77–0.88 mm. Tm I, 0.93–0.97; Tm IV, present. Chaetotaxy: same as male.

Body color and pattern similar to male.

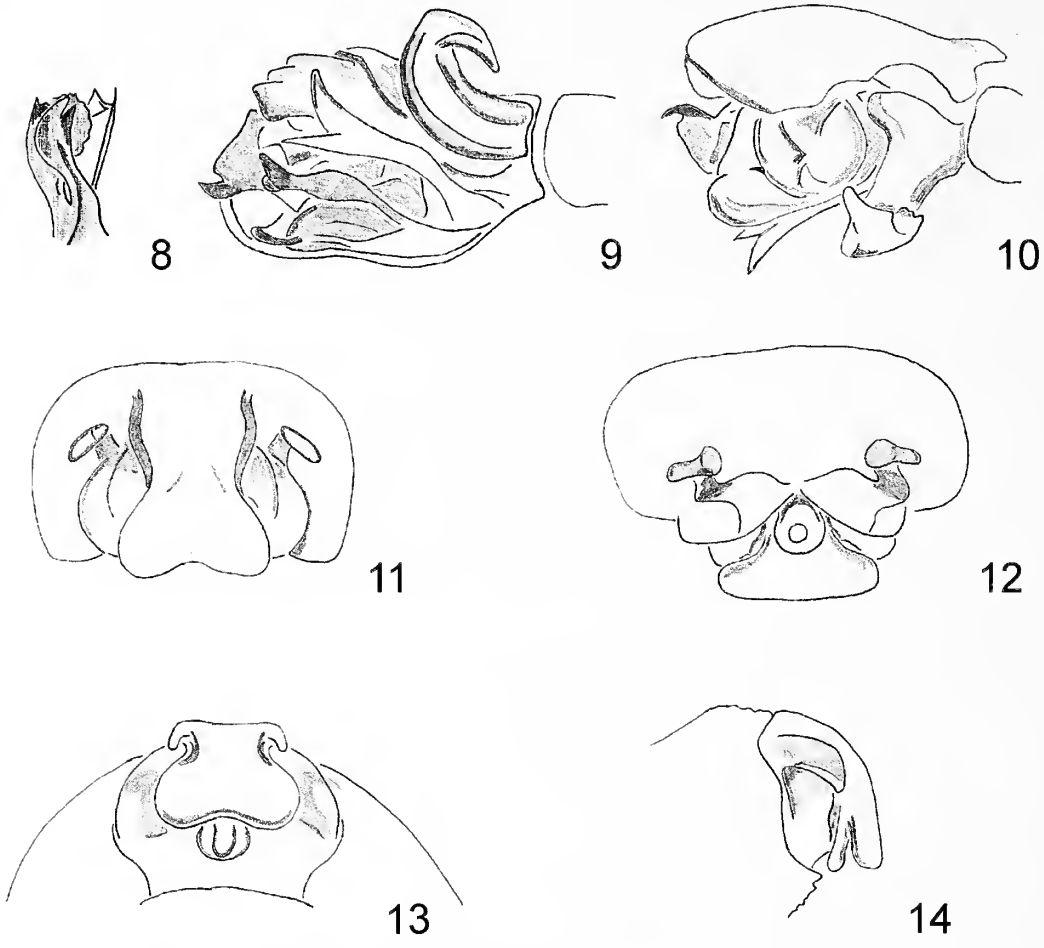
Epigynal plate oval, wider than long. Dorsal scape longer than wide, with one set of lateral bumps located proximal to mid-point (Fig. 4). Dorsal scape tip no wider than widest point of scape, smoothly curved. Stretcher separated from dorsal scape in lateral view (Fig. 7), visible in ventral view (Figs. 4, 6). Spermatheca kidney-bean-shaped, directed diagonally (Fig. 5).

Distribution.—USA, central Rocky Mountains, currently known only from Utah and Colorado (Fig. 15).

Habitat.—Spiders have been collected by beating conifers or in pitfall traps at about 3200 m in Colorado.

Discussion.—Upon examination of fresh specimens from the Rocky Mountains and comparisons of museum specimens, genitalic differences indicate that *P. bellona* is a distinct species. Furthermore, it is recommended that *P. bellona* be returned to *Poeciloneta* based on coloration, embolic shape and chaetotaxy as defined in Saaristo and Tanasevitch (2000). This species lacks a broad lamella and toothed paracymbium, and its chaetotaxy and coloration does not match that of *Incestophantes* as defined by Tanasevitch (1992).

Poeciloneta calcaratus (Emerton 1909) **New combination**
Bathyphantes calcaratus Emerton 1909:197
Lepthyphantes calcarata (Emerton 1909) Zorch 1937:874



Figures 8–14.—*Poeciloneta calcaratus* (Emerton 1909). 1. Terminal apophysis and embolus. 2, 3. Palp: 2. ventral view, 3. lateral view. 4–7. Epigynum: 4. ventral view, 5. dorsal view, 6. posterior view, 7. lateral view.

Incestophantes calcaratus (Emerton 1909) Saaristo & Tanasevitch 2000:260

Lepthyphantes calcaratus (Emerton 1909) Paquin & Duperre 2003:141

Type material.—*Lectotype*: male here designated—USA: *Maine*: Cumberland County, Portland, Long Island, 28 Aug 1906, J.H. Emerton (MCZ 20641). *Paralectotype*: male–*Piscataquis County*, Moosehead Lake, 7 Aug 1904, J.H. Emerton (MCZ 6 & MCZ 77053).

Other material.—25 males and 12 females. CANADA: *Alberta*: 1F, 25 km SW Rock Mountain House, 52°14′N, 115°10′W, Jun 1996, H. Carcamo (DB); 1F, 25 km SW Rock Mountain House, 52°14′N, 115°10′W, 3–23 Aug 1995, pine forest, H. Carcamo (DB); 1F, 25 km SW Rock Mountain House, 52°16′N, 115°09′W, 23 Aug–23 Sep 1995, pine forest, H. Carcamo (DB); 1F, Lake Louise, 10 Sep 1982, B. Erickson & M. Dykes (DB); *British Columbia*: 4M, Alaska Hwy, 37 km West of Fort Nelson, 12 Jun–5 Sep 1984, aspen-spruce, S. & J. Peck (CNC); *Labrador*: 1M 1F, So. Labrador, Shefentika to Blanc Sablon, Jul 1915, C.W. Townsend (MCZ); *Nova Scotia*: 3M, Weymouth, Aug 1924, F. J. H. Emerton (MCZ); 1M, Barrington, Sep 1923, F. J.H. Emerton (MCZ); *Ontario*: 2M 1F, Canisbay Lake, Algonquin Provincial Park, 16–20 Aug 1972, Woodpile, C.D. Dondale (CNC); *Quebec*: 1F, 95 km N LaSarre, 49°36′23″N, 79°18′03″W, 21–28 Sep 1997, FIT (Flight interception trap), old growth black spruce, P. Paquin & N. Duperre (DB); *Saskatchewan*: 1F, Anglin Lake, 53°44′N, 105°56′W, 30 Jul 1996, wall of buildings, D.J. Buckle (DB); *Yukon*: 1M, Tatchun Lake, 62°17′N, 136°08′W, 7 Jul 2003, F. Levi (MCZ); USA: *Alaska*: 1M, Mile 64.3 Tok Cutoff, 62°43′N, 143°52′W, Jul 2003, F. Levi (MCZ); *Colorado*: Clear Creek County: 1F, Squaw Mountain, Canopy Site 3, 39°41′05″N, 105°31′32″W, 3289 m, 4–28 Oct 2005, pit traps, J. Slowik (DMNS); Eagle County: 1F, Gore Creek, Gore Mountains, 2591 m, 19 Aug 1962, Levi (MCZ); Las Animas County: 1F, Apilasa Tunnel dyke, 37.339°N, 104.998°W, 3109 m, 30 Aug 2006, 20:45–21:15 h, headlamp, J. Slowik (DMNS); Rio Grande County: 1M, Beaver Creek, San Juan Mountains, 2438 m, 13 Jul 1952, Levi (MCZ); *Michigan*: Keweenaw County: 1M, Keweenaw County, 8 May 1953, R.R. Dreisbach (MCZ); *Montana*: Glacier County: 1M, Glacier National Park, Cut Bank Creek, 1555 m, 15 Aug 1953, Levi (MCZ); *New Hampshire*: Grafton County: 1M, North Woodstock, Sep 1911, W. H. Fox (MCZ); Coos County: 4M, Mt. Washington, Glen Rd and Great Gulf, Aug 1910, J. H. Emerton (MCZ); Carroll County: 2M, Intervale, Jul–Aug 1910, Emerton (MCZ); *Utah*: Dechesne County: 1F, Mirror Lake, 40.708°N, 110.886°W, 2743 m, 17 Sep 2007, 09:30–10:30 h, beat conifers, J. Slowik (DMNS); *Vermont*: Chittenden County: 1M, Mt. Mansfield, 10 Jul 1911, Emerton (MCZ).

Diagnosis.—Male *Poeciloneta calcaratus* can be differentiated from other *Incestophantes* and *Poeciloneta* species except *P. bellona*, as described under diagnosis for *P. bellona* above. *P. calcaratus* males can be separated from *P. bellona* males by the fork of the lamella occurring earlier than in *P. bellona* (compare Figs. 2, 9) and tips of the terminal apophysis being shorter (Fig. 8). Females can be separated from other species of *Poeciloneta* by the shape of the scape (Fig. 11), in which posterior edge expands out into a bone shape. The stretcher, which lies up against the dorsal scape in lateral view (Fig. 14), can also be used to separate *P. calcaratus* from *P. bellona*.

Description.—Lectotype male: total length, 2.31 mm; carapace length, 1.10 mm; carapace width, 0.99 mm. All tibiae and metatarsi

Figure 15.—Map of specimen localities for *Poeciloneta bellona* Chamberlin & Ivie 1943 (squares) and *P. calcaratus* (Emerton 1909) (circles).

missing. Chaetotaxy: F I, 0-1-0-0; F II, 0-0-0-0; Pt I–IV, 1-0-0-0. Specimen light yellow, coloration and patterns light. Faint shield mark on carapace extending from fovea to PLE. Abdomen wrinkled due to dehydration. Eight chevrons on dorsum of abdomen. Venter light. Very faint rings on ends of femurs.

Variation.—Male (n = 10). Total length, 2.36–2.78 mm; carapace length, 1.13–1.33 mm; carapace width, 0.96–1.02 mm. Tm I, 0.84 (Tm I could be located on only one male specimen); Tm IV, present. Chaetotaxy: F I, 0-1-0-0; F II–IV, 0-0-0-0; Pt I–IV, 1-0-0-0; Ti I, 2-1-1-0; Ti II, 2-0-1-0; Ti III–IV, 2-0-0-0; Mt I, 0-0-0-0.

Coloration similar to *P. bellona* mentioned above.

Lamella with slight curve in ventral view, splitting into two spurs at 0.76 of total lamella length, dorsal fork 0.28 of total lamella length, ventral fork 0.20 of total lamella length (Fig. 9). Occasionally third spur will exist, restricted to ventral side below the fork for other two major spurs; if present, 0.10 of total lamella length. Terminal apophysis tip two-part, pointed; tip extends about half width of embolus thumb, directed at the lamella (Fig. 8). Suprategular apophysis hook-shaped. Ventral edge of paracymbium hooked, bifurcate, both spurs somewhat spatulate (Fig. 10). Embolus proper two-pointed, thumb large, embolus attached to terminal apophysis (Fig. 8).

Female (n = 10): total length, 2.42–3.14 mm; carapace length, 0.97–1.29 mm; carapace width, 0.81–0.97 mm. Tm I, 0.96 (Tm I could be located on only one female specimen); Tm IV, present. Chaetotaxy: Same as male except two females had Ti II, 2-1-1-0.

Color and pattern same as male.

Epigynal plate oval, wider than long. Dorsal scape almost as long as tip is wide, with one set of lateral bumps located toward end (Figs. 11, 13). Dorsal scape tip widest point of scape, smoothly curved, bone shaped. Stretcher slightly separated from dorsal scape in lateral view (Fig. 14), not visible in ventral view. Spermatheca oblong, directed laterally (Fig. 12).

Distribution.—North America north of 43°N, extending south along a finger into the Rocky Mountains to Colorado to 38°N (Fig. 15).

Habitat.—Spiders have been collected by beating conifers or in pitfall traps located in conifer forests. Rocky Mountain specimens were collected from conifer forests above 3200 m.

Discussion.—Emerton mentions the species being found from "Portland, Maine, Moosehead Lake, and the lower part of Mt. Washington" in the original species description. The specimen designated as the lectotype was found to precede the species description, was from one of the mentioned localities, and was identified by R. Emerton. The specimen had been held in the type holdings of the MCZ, but it had never previously held any type designation. Based on coloration, embolic shape and chaetotaxy as defined by Saaristo and Tanasevitch (2000) the species is moved to the genus *Poeciloneta*. As noted for *P. bellona*, this species shows incorrect lamella shape, paracymbium shape and chaetotaxy to be included in the genus *Incestophantes*.

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SHORT COMMUNICATION

A new species of blind subterranean *Tetrablemma* (Araneae: Tetrablemmidae) from Australia

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Abstract. The first blind Australian species of Tetrablemmidae and only the fourth in the world, *Tetrablemma alaus*, new species, is described from subterranean habitats in northwestern Australia. The total loss of eyes is correlated with its subterranean existence and is complemented by other troglomorphies including slightly elongated appendages and pale coloration.

Keywords: taxonomy, morphology, eye loss, troglomorphy

Spiders of the family Tetrablemmidae are found in tropical regions of the world where they inhabit leaf litter and soil, and are very occasionally found in caves. With 30 named genera and some 133 species (Platnick 2009), they are amongst the smallest of all spider families, but have attracted considerable interest and are moderately well known, at least at the generic level (Shear 1978; Lehtinen 1981; Tong & Li 2008). Two subfamilies are recognized, with the Pacullinae found in southeastern Asia, and the Tetrablemminae distributed worldwide. Amongst the most distinctive morphological features of tetrablemmids is the presence of strap-like sclerites situated between the dorsal and ventral scutes of the opisthosoma.

Eye reduction is a common feature amongst Tetrablemmidae, and although most species have six eyes, species with four eyes, two eyes or only one eye occur in many different genera (e.g. Shear 1978; Lehtinen 1981). Total eye loss is, however, very rare and limited to cave-dwelling troglobites from Thailand and Mexico (Shear 1978; Deeleman-Reinhold 1993). Amongst arthropods recently collected from subterranean ecosystems in northwestern Australia was a series of male tetrablemmids belonging to the genus *Tetrablemma* O. Pickard-Cambridge 1873 showing obvious troglobitic adaptations including total eye loss, pale coloration and slightly elongated legs. We present here a description of this unusual species, which is clearly a member of the widespread genus *Tetrablemma*. It is the first blind member of the genus and only the second fully blind tetrablemmid spider from the Old World.

METHODS

The *Tetrablemma* material was collected by staff from Subterranean Ecology using litter traps suspended down mining exploration drill holes. Litter traps are designed to capture terrestrial subterranean fauna and are made from PVC pipe (55 mm diameter × 140 mm in length) with aviary mesh over the top end to provide access to invertebrates and a PVC cap sealing the trap bottom. Each PVC cap had a hole drilled in it to allow any excess moisture to drain away to prevent the trap from becoming and remaining flooded in the event of surface water inflow. Each trap was packed with organic material, consisting mostly of spinifex, *Acacia* and some *Eucalyptus* litter sourced from the survey area. Prior to packing in the trap, organic material was sterilised in a microwave on high power for 10 min to ensure any invertebrates that may have been present were destroyed. Before deployment down drill holes, organic contents of each trap were moistened. Traps were left *in situ* for just over 7 wk (28 February–23 April 2008). On recovery, traps were individually sealed in ziplock bags for transport to the laboratory, where fauna was extracted from litter samples using Tullgren funnels and preserved in 100% ethanol.

Specimens were examined and measured using a Leica MZ16A microscope, and digital images were taken using a Leica DFC 500 digital camera and the software program AutoMontage Pro Version 5.02 (p). Legs and pedipalps were measured from a lateral aspect. Drawings were made under an Olympus BH-2 compound microscope with attached drawing mirror and then scanned and edited using the software program Adobe Photoshop Elements 2.0. All specimens are lodged in the Western Australian Museum, Perth (WAM) and the American Museum of Natural History, New York (AMNH).

TAXONOMY

Family Tetrablemmidae O. Pickard-Cambridge 1873
Subfamily Tetrablemminae O. Pickard-Cambridge 1873
Genus *Tetrablemma* O. Pickard-Cambridge 1873

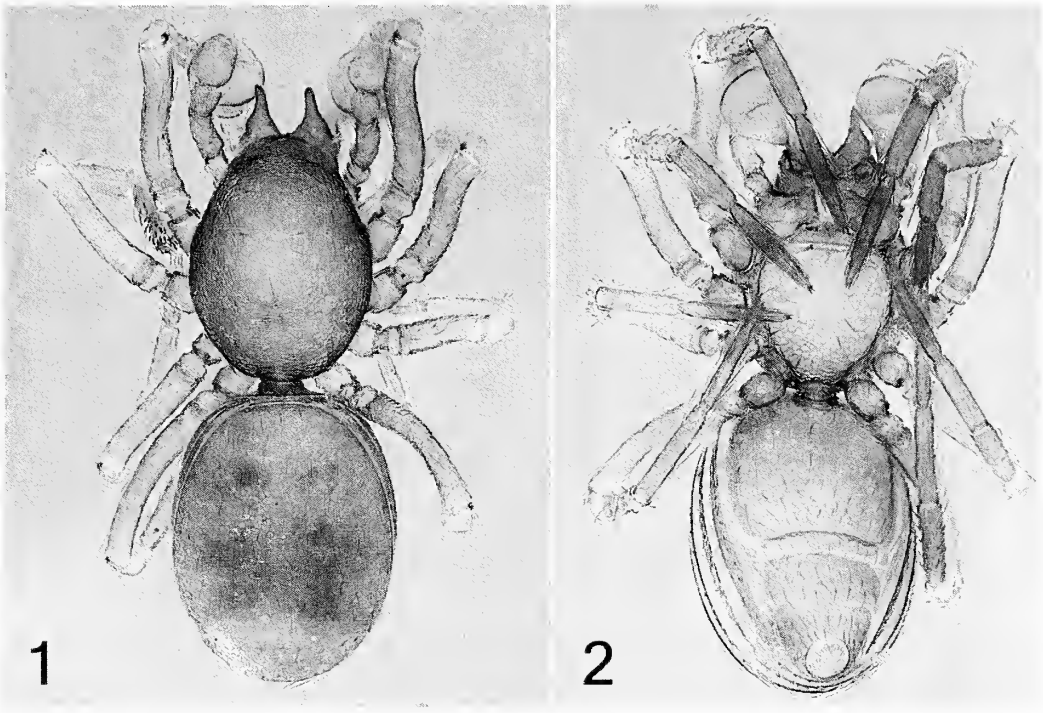
Tetrablemma O. Pickard-Cambridge 1873:114.

Type species.—*Tetrablemma mediooculatum* O. Pickard-Cambridge 1873, by monotypy.

Remarks.—*Tetrablemma* currently contains 20 species (Platnick 2009) and has been divided into three subgenera (Lehtinen 1981): *T. (Kumaonia)* Lehtinen 1981 with a single species from India; *T. (Indonops)* Tikader 1975 with 11 species from India, Samoa, Angola, Saint Helena, Seychelle Islands, Indonesia, Vietnam, Nepal, South Marianas; *T. (Tetrablemma)* with five species from Sri Lanka, India, Trinidad, Angola and Australia; as well as three recently described species that have not been included in either of these subgenera (Burger 2008; Tong & Li 2008; Labarque & Grismado 2009). Like these recent authors, we choose to disregard the subgeneric arrangement.

Tetrablemma alaus new species
Figs. 1–8

Material examined.—AUSTRALIA: *Western Australia*: *Holotype*: male, Callawa Ridge, Yarrie Station, hole #CA0022R, 20°38′36.4″S, 120°17′05.2″E, 28 February–24 April 2008, troglofauna trap (WAM T91751).



Figures 1–2.—*Tetrablemma alaus* new species, male holotype (WAM T91751). 1. Dorsal aspect. 2. Ventral aspect.

Paratypes: AUSTRALIA: *Western Australia*: 1 ♂, Cundaline Ridge, Yarrie Station, hole #CU0063R, 20°32′24.9″S, 120°09′18.5″E, 28 February–23 April 2008, litter trap (WAM T91745); 1 ♂, Callawa Ridge, Yarrie Station, hole #CA0023R, 20°38′25.9″S, 120°18′23.8″E, 28 February–23 April 2008, litter trap (WAM T91748); 1 ♂, same data (AMNH); 1 ♂, Cundaline Ridge, Yarrie Station, hole #CU0059R, 20°32′27.1″S, 120°09′17.3″E, 28 February–23 April 2008, litter trap (WAM T91744); 1 ♂, Cundaline Ridge, Yarrie Station, hole #CU0062R, 20°32′29.2″S, 120°09′23.7″E, 28 February–23 April 2008, litter trap (WAM T91746); 3 ♂, Callawa Ridge, Yarrie Station, hole #CA0024R, 20°38′24.2″S, 120°18′23.9″E, 28 February–23 April 2008, litter trap (WAM T91750). All specimens were collected by staff from Subterranean Ecology.

Etymology.—The specific epithet refers to the lack of eyes in this species; Greek: *alaos* = blind (Brown 1956).

Diagnosis.—*Tetrablemma alaus* is easily distinguished from all other species of Tetrablemmidae (except for *Bacillema leclerci* Deeleman-Reinhold 1993 from Thailand) by the complete lack of eyes or eye-spots. It differs from *B. leclerci* by the much shorter legs.

Description.—*Male:* color (in alcohol): prosoma, scuta of opisthosoma, chelicerae, palps and legs light orange; spinnerets pale yellow; membranous areas white.

Carapace: strongly elevated in lateral view, box-like (Fig. 3); entirely covered with fine mosaic-like pattern; without any long setae (Figs. 1, 3, 4). Eyes completely absent (Figs. 1, 3, 4). Clypeus steeply ascending.

Sternum and pleurae: sternum (Fig. 5) approximately as long as wide, covered with short setae and fine mosaic-like sculpture, separating coxae IV by slightly less than their diameter: pleurae sclerotized, fused with carapace and sternum.

Labium: somewhat triangular, anteriorly rounded, approximately twice as wide as long; separated from sternum (Figs. 2, 5).

Chelicera: basal segment anteriorly with hump extending into long, straight horn with slightly curved tip (Fig. 3); base of hump with slightly wrinkled cuticle; cheliceral horn approximately 0.6 times as long as basal segment; thin transparent lamina on mesal margin of basal segment protecting beyond tip of fang; fang with small teeth on inner side (Fig. 7).

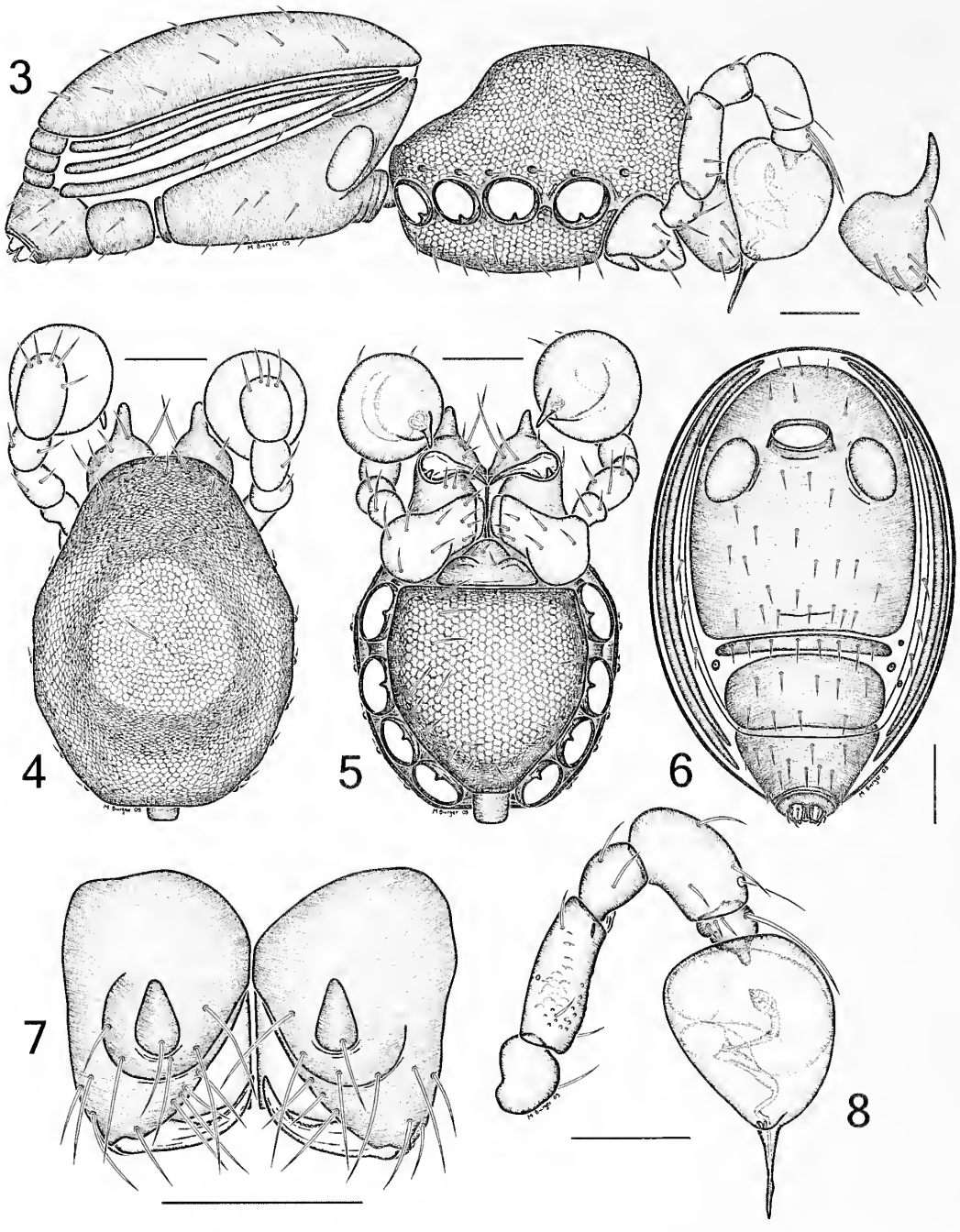
Palp: cuticle of femur partly squamous; tibia slightly enlarged, with one trichobothrium dorsally near distal end; cymbium short, with few long setae dorsally (Fig. 8). Palpal bulb: pyriform, longish, with convoluted sperm duct shining through; thread-like embolus straight, with simple tip (Fig. 8).

Legs: I-IV-III-II (from longest to shortest); femora with squamous cuticle and small teeth ventrally; patellae small; tibiae II–IV with three trichobothria dorsally, tibia I with four; metatarsi with one trichobothrium dorsally; tarsus with two dentate claws.

Opisthosoma: ovoid; large sclerotized plate covering dorsal surface (Figs. 1, 3); ventrally covered by four sclerotized plates (Figs. 2, 6): large pulmonary plate with rounded anterior margin, surrounding pedicel and bearing simple book-lung plates, followed posteriorly by short and broad postgenital plate, long and slightly less broad preanal plate, and conical anal plate surrounding spinnerets; few tiny perigenital plates near postgenital plate; laterally with four pairs of strap-like plates, most ventral pair very short and situated anteriorly, followed by three short strap-like posterior plates situated between dorsal plate and anal plate (Fig. 3).

Measurements (n = 1, male holotype): total length (without chelicerae) 1.210. Prosoma length 0.548, width 0.400, height 0.250. Opisthosoma length 0.818, width 0.517. Lengths of palp and leg segments: palp: femur 0.210, patella 0.061, tibia 0.130, tarsus 0.326, total 0.727; leg I: femur 0.416, patella 0.146, tibia 0.297, metatarsus 0.274, tarsus 0.250, total 1.383; leg II: femur 0.352, patella 0.129, tibia 0.270, metatarsus 0.146, tarsus 0.137, total 1.034; leg III: femur 0.278, patella 0.124, tibia 0.225, metatarsus 0.198, tarsus 0.218, total 1.043; leg IV: femur 0.378, patella 0.123, tibia 0.226, metatarsus 0. 238, tarsus 0.277, total 1.242.

Remarks.—The Australian tetrablemmid fauna consists of just two named species: *T. okei* Butler 1931 from Victoria and *T. magister* Burger 2008 from Queensland (Butler 1931; Burger 2008). Numerous new species of *Tetrablemma* and possibly other genera have been collected from tropical northern Australia, but are presently undescribed (Harvey unpubl. data). Due to the highly modified troglomorphic features and the localized distribution of the new species, we have chosen to describe this species separately from the epigean ones.



Figures 3–8.—*Tetrablemma alaus* new species, male paratype (WAM T91748). 3. body, lateral aspect. Lateral view of right chelicera on the right side. Only few hairs of opisthosoma shown. 4. Prosoma, dorsal aspect. 5. Prosoma, ventral aspect. 6. Opisthosoma, ventral aspect. 7. Chelicerae, anterior aspect. 8. Left palp, prolateral aspect. Scale lines = 100 μm.

As noted above, this is only the second completely blind Old World species of tetrablemmid, the first being *Bacillemma leclerci* from a cave in Thailand (Deeleman-Reinhold 1993). Eyeless and eyed populations have been reported for *Matta mckenziei* Shear 1978 and *Carainatta sbordonii* (Brignoli 1972), both from caves in Mexico (Brignoli 1972; Shear 1978; Lehtinen 1981).

The subterranean fauna of Western Australia is now known to be diverse and widespread, with numerous new species of many different animal groups described over the past 20 years. Whilst pseudoscorpions (e.g. Harvey 1991; Harvey & Mould 2006; Harvey & Edward 2007a; Harvey & Volschenk 2007; Edward & Harvey 2008; Harvey & Leng 2008a, 2008b), schizomids (Harvey 1988; Harvey & Humphreys 1995; Harvey 2001; Harvey et al. 2008) and scorpions (Volschenk & Prendini 2008) have received detailed taxonomic attention, there have been relatively few blind troglobitic spiders described to date. The fully blind trochanteriid *Desognanops humphreysi* Platnick 2008 was recently described from Millbillillie Station [ca 26°41′S, 120°20″E] (Platnick 2008), as were three blind oonopids from the Pilbara and Kimberley regions of Western Australia (Harvey & Edward 2007b). Gray (1973, 1981, 1992) recorded several blind stiphidiids of the genus *Tartarus* Gray, and the monotypic ctenid genus *Janusia* Gray 1973 from the Nullarbor Plain, in southern Australia. The highly troglobitic *Bengalla bertmaini* Gray & Thompson 2001 occurs in the deep limestone cave of the Cape Range Peninsula (Gray & Thompson 2001); although originally described within the Lycosoidea, it was later transferred to the family Tengellidae (Raven & Stumkat 2005).

The Callawa and Cundaline Ridge systems in the Yarrie Station area are located approximately 200 km east of Port Hedland on the northern margin of the Pilbara Craton. The ridges are mostly comprised of sandstones, shales and Archaean banded irons within which there are many fractures and fissures providing habitat for subterranean invertebrates. Callawa and Cundaline Ridges are known to contain a variety of troglobitic arachnid species including the blind pseudoscorpions *Lagynochthonius leemouldi* Edward and Harvey 2008 (Edward & Harvey 2008) and new species of *Tyrannochthonius* and *Indohya* (M. Harvey unpubl. data), and a blind oonopid belonging to a new genus similar to *Camptoscaphiella* (B. Baehr and M. Harvey unpubl. data).

The discovery of diverse assemblages of troglofauna in non-karstic formations in the Pilbara are largely due to the recent increase in mining exploration and associated subterranean fauna surveys required as part of environmental impact assessments. Previously terrestrial subterranean fauna in Western Australia was mostly known from karst-associated habitats only (Eberhard et al. 2008). The level of species richness recorded from the Yarrie area is comparable to other non-karstic formations surveyed in the Pilbara (Eberhard et al. 2008; Subterranean Ecology 2007).

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SHORT COMMUNICATION

Nephila clavipes females have accelerating dietary requirements

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Abstract. *Nephila* spiders are famous for extreme sexual size dimorphism, with females an order of magnitude larger than males. The proximal developmental mechanism for the sexual size dimorphism is extended development in females: they have many more juvenile instars than males. During an experimental rearing of *Nephila clavipes* (Linnaeus 1767) from two populations, we discovered that females cannot reach sexual maturity on diets that are qualitatively and quantitatively sufficient for male maturation. Here we describe the dietary regimes that produced sexually mature females and the life history implications of these requirements.

Keywords: Female gigantism, sexual size dimorphism, SSD, Araneae, Nephilidae

Spiders in the family Nephilidae are famous for extreme sexual size dimorphism, with males of many species an order of magnitude smaller than the females (Vollrath 1980; Christensen & Goist 1979; Hormiga et al. 2000; reviewed in Kuntner & Coddington 2009). This sexual size dimorphism originates developmentally through delayed maturation in females: they pass through several additional instars, while males mature between the fifth and eighth instar after emergence (pers. obs.; Hormiga et al. 2000). In order to better determine the developmental differences underlying extreme sexual size dimorphism, we developed a protocol for rearing female *Nephila clavipes* (Linnaeus 1767) (Araneae: Nephilidae) in the laboratory. Here, we describe the developmental trajectories, food requirements, and mortality patterns of females; male data have been presented elsewhere (Higgins & Goodnight unpubl. results). Most striking from these results is that in terms of the mass of food required for development (relative to body mass), females have accelerated dietary requirements after about the seventh instar, coinciding with a previously observed decline in orb-web investment (Higgins 2006) and likely reflecting accelerated growth rates associated with maturing early in strongly seasonal environments.

Seven egg sacs with unhatched eggs were collected in Los Tuxtlas Biological Research Station, Veracruz, Mexico, and shipped to Vermont, USA; all but one of these sacs failed to hatch, apparently due to desiccation of the eggs in transit. Five egg sacs were collected in Brazos Bend State Park, near Houston, Texas, USA (vouchers from these populations have been placed in the National Museum of Natural History, Smithsonian Institution, Washington, D.C.). All of these were hatched and had molted to the first true instar prior to shipping, and arrived alive. Dispersing spiderlings from the six egg sacs (one from Los Tuxtlas, five from Brazos Bend) formed the study population. Because of low survivorship of females during development, data are pooled across all families from the Brazos Bend population. To ease the burden of feeding large numbers of small spiders, we staggered the emergence of Brazos Bend spiderlings by holding egg sacs in cool, short-day conditions in a box lined with damp paper towels in a walk-in refrigerated chamber (4° C, 14:10 h D:L). When starting a new clutch, we hung an egg sac in a large box (31 cm wide x 23.5 cm high x 11 cm deep, Pioneer plastics) on 2.5 cm (= 1 in) chicken wire and placed a tube of high-protein *Drosophila melanogaster* (reared on instant fly food supplemented with high-protein dog chow: Mayntz et al. 2003) in the box, placing the box in warm long-day conditions (25° C, 10:14 h D:L, 75% RH) in a Percival incubator. In addition to releasing *D. melanogaster* into the boxes, we sprayed the spiderlings twice weekly with a dilute pollen solution (0.1 g organic "bee" pollen in 500 ml distilled water). Upon molting to the third instar, we moved spiders into individual boxes and randomly assigned each to a treatment group.

Spiders were fed biweekly most weeks; occasionally a weeks' worth of prey was provided at a single feeding. Initial treatments were: Low = 35% of post-molt body mass/week; Medium = 56%; High = 84%. These diets are in the middle of the range used by Higgins & Rankin (2001), which resulted in normal growth rates without the high mortality associated with overeating. Rather than removing spiders from their webs to weigh them, we estimated the mass of the spiders from the leg 1 tibia + patella length (TPL), abdomen length and abdomen width as in Higgins (1992: mass (mg) = 81 (TPL^3) + 784 (abdomen volume)). For the first four experimental instars, we based diets upon the mean size of the first 4–7 spiders reaching those instars, and spiders were fed *D. melanogaster*. After TPL $\geq$ 0.5 cm, we calculated diets individually for each animal immediately after each molt and fed spiders a mixture of *D. virilis* and *D. melanogaster*. All spiders in the same instar received the same quality of diet. Prey numbers used were calculated based upon the mean mass of each prey type: *D. melanogaster* (mean mass 0.748 mg, SD = 0.110, n = 11), *D. virilis* (mean mass 1.60 mg, SD = 0.239, n = 15). The *D. virilis* were not reared on protein-supplemented diets. At the eighth instar, we added commercially reared, high-protein house flies to the diets (*Musca domestica*, www.SpiderPharm.com; mean mass 11.65 mg, SD = 2.077, n = 10). The shifts in prey type were necessary for logistical reasons: if we had fed only *D. melanogaster* through the entire development, the number of flies provided in later instars would have numbered in the hundreds per week due to the large size of the juvenile females. In subsequent instars, the housefly proportion of the diet by mass increased (*D. virilis*: house flies – eighth instar: 3:7, ninth instar: 2:8, tenth or eleventh instar: 1:9).

As spiders molted to larger sizes, we moved them to accommodate their larger webs. When they molted to the 6th instar (TPL ca 0.3 cm), they were moved to a larger box (22 cm wide x 10 cm high x 10 cm deep), oriented horizontally for smaller spiders (0.3 cm $\leq$ TPL $<$ 0.5 cm) and vertically for larger ones (0.5 cm $\leq$ TPL$<$ 0.7 cm). All but three males reached sexual maturity while in this size range (prior to TPL = 0.6 cm). Juvenile females were moved to the largest box size when they molted to TPL $\geq$ 0.7 cm (31 cm wide x 23.5 cm high x 11 cm deep). To mimic environmental cues in natural populations, all spiders were moved to short-day conditions (11:13 h L:D) in a walk-in chamber 4 mo (= 138 days) after starting the experiment. Most

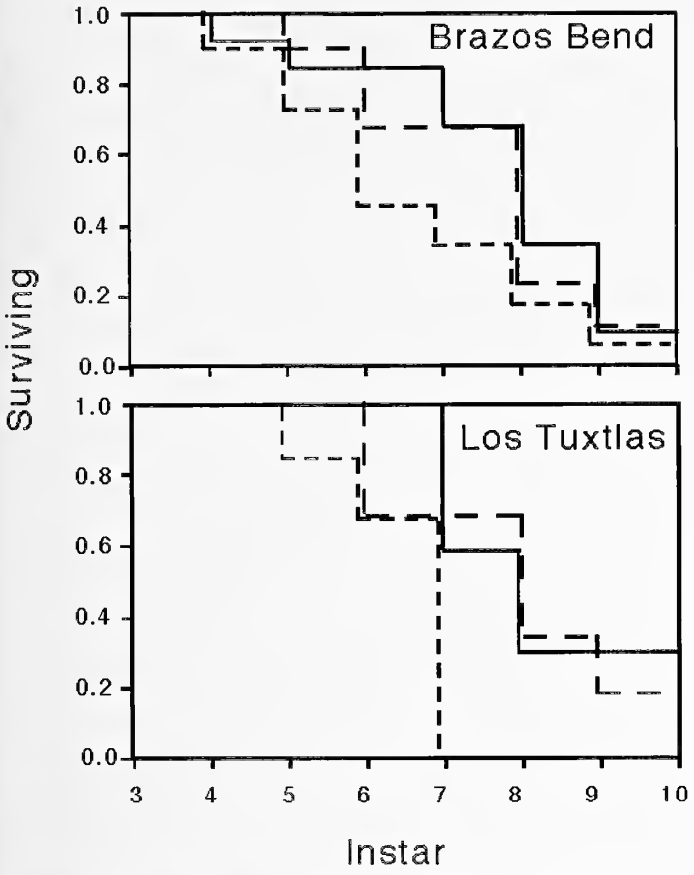

Figure 1.—Survivorship of unsexed juveniles (males and females to the 8th instar) and immature females on the three initial diets (L1: short dash; M1: long dash; H1: solid line), by instar. The survivorship calculations were censored by maturation (i.e., mature animals are removed from the calculation of survival).

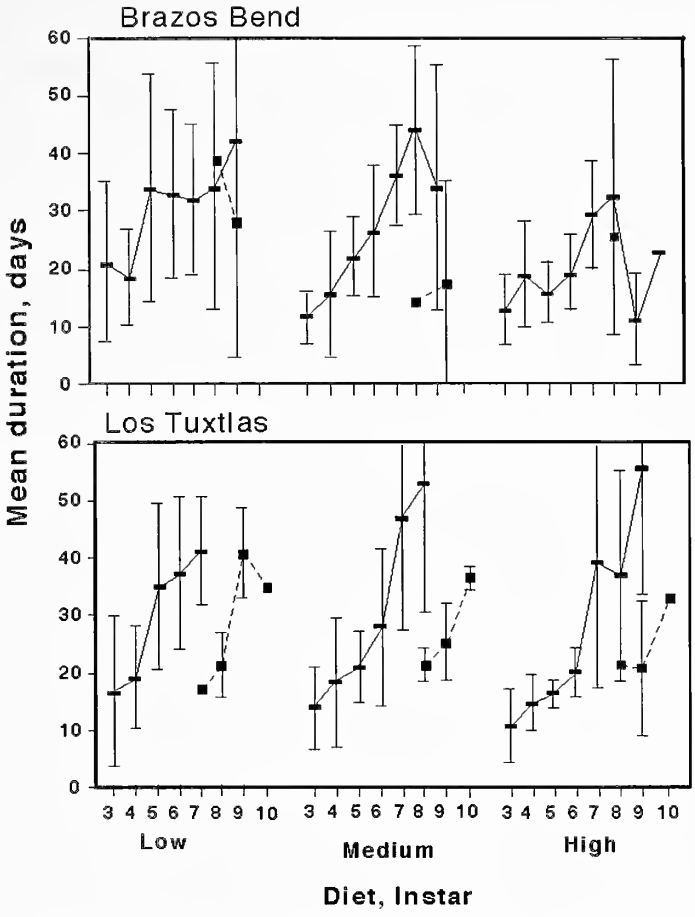

Figure 2.—Average instar duration of juvenile female spiders reared on low, medium, and high diets plotted against instar number. The trajectories on the initial diets (solid lines/bars) bifurcate at the seventh instar when half of the spiders were switched to higher diets (dashed lines/squares). Where no SD is indicated, only a single animal was observed. Note that for Brazos Bend in the H2 diet, only one individual in the eighth instar is represented because spiders molting to the ninth instar were all maturing, and sample sizes were very low.

males were sexually mature at the time of the move. Temperature and humidity in the walk-in chamber were less exactly controlled, but averaged 24° C and 72% RH.

We checked all spiders twice weekly, at which time we recorded and removed all uneaten dead flies, and recorded if the spider had molted. There were no differences in size at the first experimental molt (instar 4) across diets within populations. In addition to measuring the spiders, we retrieved the shed exoskeleton, which serves as a physical record of TPL of the prior instar.

All males from both populations reached maturity by the eighth instar (Brazos Bend: $n = 34$, range = 5–8; Los Tuxtlas $n = 6$, range = 5–7). Prior to the penultimate male instar, males and females cannot be distinguished, and thus the data for instars 3–7 include males and females. Penultimate males are not included in the survivorship analysis, as only 1 penultimate male (from Brazos Bend) died during the experiment.

About the time that males were reaching maturity, we noticed that juvenile female mortality was increasing (Fig. 1). Moreover, even spiders fed the highest diet spent much longer in the seventh instar (mean TPL = 0.58 cm) than the 14-day average for field-observed animals of this size (Higgins 1992; Fig. 2). Average eighth instar duration on the high diet was nearly 30 days for Brazos Bend animals and nearly 40 days for Los Tuxtlas animals. Despite being mid-run on the experiment, we decided to increase the diets of a random half of the individuals by 50% on 18 August 2006, when 22 Brazos Bend females and 21 Los Tuxtlas females were still alive. Because of the staggered start dates for different egg sacs and for spiderlings within an egg sac, the age of the spiders at the time of the shift varied (BB: mean age = 116.5 days, SD = 29.1; LT: mean age = 123.3, SD = 16.7). Most of the animals were in the seventh instar (fourth instar of experimental treatment; BB mean = 7.2, SD = 0.91; LT mean = 7.33, SD = 0.86). This resulted in six final diet treatments for females: low 1 (35%) and low 2 (switched to 56%), medium 1 and 2 (56%, 84%) and high 1 and 2 (84%, 126%). It is noteworthy that none of the animals in the eighth instar at the time of the diet shift reached maturity, even if they received the greater amount of food.

Instar duration shortened dramatically in the spiders experiencing the increase in food availability (Fig. 2). After log-transforming the data to normalize distributions, we tested the effect of diets on development by comparing the age and size of spiders entering the ninth instar for each population with separate MANOVA analyses, followed by individual ANOVA tests to determine how size and age were affected by diet (all statistical analyses performed on JMP 7.0.2). The separation of the two populations was necessary because none of the LT spiders on the L1 treatment survived to the ninth instar. For the Brazos Bend spiders, development to the ninth instar was significantly affected by diet ($n = 13$, partial correlation = 0.025; Roy's Maximum Root = 5.75, DFE = 7, $P = 0.0081$). In these spiders, age at the ninth instar was not altered by diet (ANOVA: $F_{(5, 12)} = 1.37$, $P = 0.34$), but spiders on higher diets were significantly larger (ANOVA: $F_{(5, 12)} = 7.55$, $P = 0.01$). The Los Tuxtlas spiders also showed a significant developmental response to diet and the two

developmental parameters were correlated with each other (n = 14, partial correlation = 0.53; Roy's Maximum Root = 5.64, DFE = 9, P = 0.015). The Los Tuxtlas spiders on higher diets showed significantly faster development to the ninth instar (ANOVA: $F_{(4, 13)} = 4.34$, P = 0.032; but no change in size with diet (ANOVA: $F_{(4, 13)} = 1.27$, P = 0.35).

A total of 14 females reached sexual maturity, four from Brazos Bend and ten from Los Tuxtlas. With the apparent difference in developmental response to diet, the data cannot be pooled across these two populations, and only the Los Tuxtlas sample is large enough to consider dietary effects on female size and age at maturation. We tested for an effect of diet by ranking the six diets from lowest to highest (L1, L2, M1, M2, H1, H2). Among these survivors, neither age nor size at maturity was affected by diet; however, no more than three animals survived from any diet group (standard least-squares regression - size: ln (TPL, mm): $F_{(1, 9)} = 0.72$, $P = 0.42$; age: ln (days since initiation of experiment): $F_{(1, 9)} = 2.49$, $P = 0.15$).

In light of the problems of synchronization of development in a species with female gigantism, where the gigantism is proximally caused by the addition of juvenile instars (Hormiga et al. 2000), it is perhaps not surprising that female dietary requirements accelerated at an intermediate developmental stage. These results also help to explain prior descriptions of declining relative investment into foraging by these spiders in Mexico (Higgins 2006): since prey capture rates are not tightly linked to orb-web size (Higgins & Buskirk 1992), spiders may be reducing foraging investment in order to shift resources to growth and development. Despite the high mortality of spiders even after the dietary shift, we do not believe that these results imply qualitative nutritional requirements being unmet for the following reasons. First, many kinds of spiders are regularly reared successfully on protein-enhanced fruit flies and house flies (Mayntz et al. 2003; C. Kristensen, Spiderpharm.com, pers. comm.), including *Nephila fenestrata* Thorell 1859 and *N. edulis* (Labillardière 1799) (N. Ruppel pers. comm.; L. Ceballos Meraz pers. com.). Second, few juveniles die before they can be sexed, and penultimate-instar males almost never die. It appears likely that large juvenile females are starving to death due to lack of food, rather than lack of nutrients in the food they are receiving. This sensitivity to food levels may be a price these spiders pay for the reproductive benefits of large female size.

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Rearing large numbers of spiders is time-consuming, and these experiments would not have been possible without the aid of volunteer undergraduates Sarah Wanamaker, Cynthia Quell, Rachel Taylor, Donald Kraft, Lauren Gauthier, Ashley Couture, and Ariel GallentBernstein. Collecting in Texas was hosted by Ruth Buskirk, and Juan Nuñez Fárfan and Jesus Vargas supplied the spider egg sacs from Los Tuxtlas. C. Kristensen, N. Ruppel, and L. Ceballos Meraz were generous with their time in discussing spider diets and these results. An anonymous reviewer, Matthias Foellmer, and Jeffrey Shultz provided comments that greatly improved the presentation of these results. This research was supported by a grant to LEH from the National Science Foundation (0233440).

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SHORT COMMUNICATION

An unusual setule on type IV urticating setae of *Homoeomma uruguayense* (Araneae: Theraphosidae)

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Abstract. We describe a new unusual setule on type IV urticating setae of the theraphosid spider *Homoeomma uruguayense* (Mello-Leitão 1946). These processes have a filiform stalk and a funnel-like apex that arise from the main axis of the urticating seta. The probable function of these structures in passive and active defense is discussed.

Keywords: Tarantulas, morphology

The presence of defensive urticating setae is exclusive to the Theraphosidae of Southern North America, Central and South America. These setae have attracted the attention of naturalists since the nineteenth century. Cooke et al. (1972) were the first to describe four types of theraphosid urticating setae and presented a detailed study of their morphology. After that, Marshall & Uetz (1990a) reported type V urticating setae located on the pedipalps in the genus *Ephebopus* Simon 1892, and Pérez-Miles (1998) described type VI abdominal urticating setae in the genus *Hemirrhagus* Simon 1903. Over the last decades, several papers on structure, function, and development of urticating setae have appeared. Also, it has become common practice for scientists to use urticating setae as systematic characters in phylogenetic analysis (Bertani 2001, 2002; Pérez-Miles 1992, 2000; Pérez-Miles et al. 1996).

Theraphosinae have small urticating setae of types I, III, IV (Fig. 1), and VI. These setae types are released by friction of the posterior legs against the abdomen. Urticating setae can be released when the tarantula is disturbed or can be incorporated into the shedding mat or cocoon (Marshall & Uetz 1990b; Pérez-Miles & Costa 1994) as passive defense against ants (Bertani & Guadanucci 2003). In Theraphosinae, types I and III can coexist and also type III and type IV, but types I and IV never occur together.

Homoeomma Ausserer 1871 has urticating setae types III and IV. Type III setae occupy a central dorsal area of the abdomen, while type IV are located in the periphery; at the border between both areas setae of intermediate morphology occur. In *Homoeomma uruguayense* (Mello-Leitão 1946), adult females lose type III urticating setae, whereas adult males maintain both types.

Having occasionally observed an unknown setular morphology on type IV urticating setae of *H. uruguayense,* we chose to study the morphology of type IV urticating setae on 23 males and 7 females of *H. uruguayense* from Montevideo, Canelones and Rio Negro, Uruguay (old and fresh material). We deposited the materials studied in the entomological collection, Facultad de Ciencias, Montevideo, Uruguay. Additionally, we studied a male of *Homoeomma brasilianum* (Chamberlin 1917) from Mairiporã, São Paulo, Brazil, deposited in the Instituto Butantan, São Paulo, Brazil (= IBSP); 3 males of *Homoeomma montanum* (Mello-Leitão 1923) from Itatiaia, Rio de Janeiro, Brazil, deposited at IBSP; a male (type) of *Homoeomma pictum* (Pocock 1903) from Caraz, Perú, deposited in The Natural History Museum, London, UK (= BMNH); and a male (type) of *Homoeomma villosum* (Keyserling 1891) from Taquara, Rio Grande do Sul, Brazil, deposited in BMNH.

We removed urticating setae with forceps from at least six areas of the abdomen (anterior, median and posterior; axial and lateral). At least 20 setae of each area were examined. We studied all specimens with optical microscopy and three with a scanning electron microscope; in these individuals, 43 setulae from 10 setae were measured.

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We found type IV urticating setae in all *Homoeomma* spp. examined. We discovered an undescribed structure (Fig. 2) on type IV urticating setae of all *H. uruguayense* individuals. These structures (setules) have a filiform stalk and a funnel-like apex. Setules measured 18.68 μm ± 3.39 SD in length. They are fixed to the main axis of the setae between the subconical barbs of type IV setae. A mean of 5.6 (± 2.5 SD, $n = 50$) setules per seta occurred only on barbed zones of urticating setae. Exceptionally we observed such setules on setae of intermediate morphology (III/IV). They were not present in the urticating setae of *H. brasilianum* , *H. montanum, H. pictum,* or *H. villosum.*

The function of the new setule remains obscure. Its morphology resembles structures associated with an adhesive function in insects and spiders (Nachtigall 1974; Rovner 1978; Stork 1980a; Roscoe & Walker 1991; Gorb 2001; Scherge & Gorb 2001; Gao & Yao 2004). For this reason, setules could represent contact elements that improve the action of adhesive forces (Kesel et al. 2003). Another possible explanation for setule function would be to increase the surface of the setae, facilitating flotation and transport through the air. A third alternative could be that setules help to maintain urticating setae on the substrate in a position with the penetration tip better exposed and ready for passive defense.

The finding of setules only on urticating setae of this species and not in others of the genus could be interpreted as an apomorphic acquisition of *H. uruguayense.*

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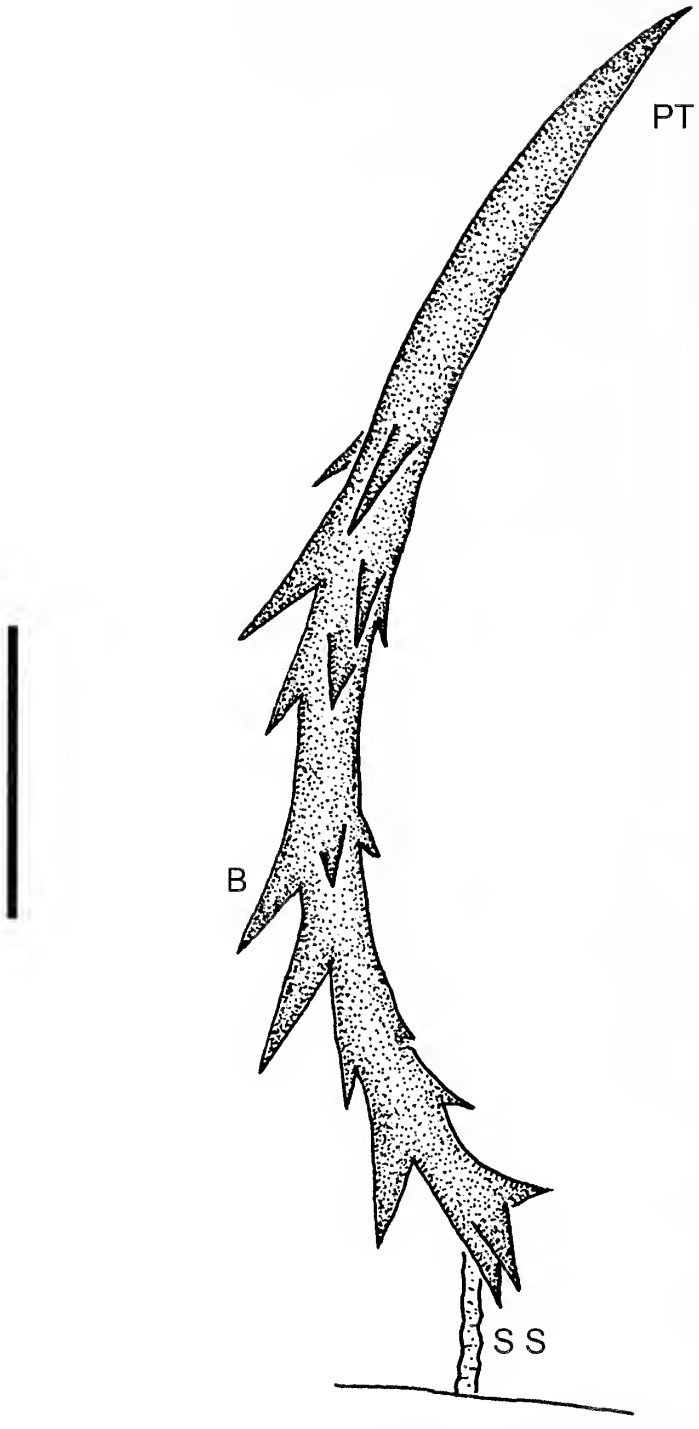

Figure 1.—Schematic representation of an usual abdominal type IV urticating seta of Theraphosinae showing the supporting stalk (SS), barbs (B), and penetration tip (PT). (Scale = 20 μm).

Figure 2.—Scanning electronic microscope photograph of the abdominal type IV urticating setae of *H. uruguayense* showing the setules (Scale = 10 μm). Insert: close-up of the setulae showing the flared apical tip (Scale = 2 μm).

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