(ISSN 0161-8202)

The Journal of ARACHNOLOGY

OFFICIAL ORGAN OF THE AMERICAN ARACHNOLOGICAL SOCIETY

VOLUME 35 2007 NUMBER 2

The Journal of Arachnology (ISSN 0161-8202), a publication devoted to the study of Arachnida, is published three times each year by *The American Arachnological Society*. **Memberships (yearly):** Membership is open to all those interested in Arachnida. Subscriptions to *The Journal of Arachnology* and *American Arachnology* (the newsletter), and annual meeting notices, are included with membership in the Society. Regular, $40; Students, $25; Institutional, $125 . Inquiries should be directed to the Membership Secretary (see below). **Back Issues:** Patricia Miller, P.O. Box 5354, Northwest Mississippi Community College, Senatobia, Mississippi 38668 USA. Telephone: (601) 562-3382. **Undelivered Issues:** Allen Press, Inc., 810 E. 10th Street, P.O. Box 368, Lawrence, Kansas 66044 USA.

Cover photo: Female *Satrapanus grayi* (Pseudoscorpiones, Chernetidae) endemic to Lord Howe Island, Australia. Photo by Mark Harvey.

Publication date: 11 September 2007

♾ **This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).**

2007. The Journal of Arachnology 35:215–226

GEOGRAPHICAL DISTRIBUTION OF TWO SPECIES OF *MESOBUTHUS* (SCORPIONES, BUTHIDAE) IN CHINA: INSIGHTS FROM SYSTEMATIC FIELD SURVEYS AND PREDICTIVE MODELS

Cheng-Min Shi,[1,2] **Zu-Shi Huang,**[1,2] **Lei Wang,**[1,2] **Li-Jun He,**[1,2] **Yue-Ping Hua,**[1,2] **Liang Leng**[1,2] and **De-Xing Zhang**[1,3]: [1]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China; [2]Graduate University of the Chinese Academy of Sciences, Beijing 100049, China

ABSTRACT. Although *Mesobuthus* scorpions in China have become endangered in recent years, they are largely underinvestigated. Even the baseline data on their distributions are lacking. Here the geographical distributions of two *Mesobuthus* scorpions in China are provided through a combined study of systematic field surveys and GIS-based ecological niche modeling using 227 surveyed point occurrence data across an area of ca. 2800 × 1700 km^2 and validated historical records. *Mesobuthus martensii* (Karsch 1879) appears to be restricted to latitude south of 43°N and the north side of the Yangtze River, bordered by the Helan Mountains and the Tengger and Mo Us sand desert in the west and limited by the sea in the east. *Mesobuthus eupeus* (C.L. Koch 1839) reaches the east side of the Helan Mountains and the west edge of the Loess Plateau, extending westward along the northern slope of the Qilian Mountains and ultimately penetrating to the northern part of the Junggar Basin. The former is mainly found in semi-humid and humid regions while the latter is an arid and semi-arid dweller. The two species show a parapatric distribution on the whole with a contact zone formed at the boundary of their ranges across the big turning of the Yellow River in the central-western part of Inner Mongolia, Ningxia and the middle part of the Gansu Province. This pattern of distribution is shaped both by the fundamental ecological niche constraint of the species and possibly by the biological interactions between the two species. Some diagnostic features for the two species are also provided for quick identification.

Keywords: *Mesobuthus martensii*, *Mesobuthus eupeus*, ecological niche modeling, contact zone

Formal description of the scorpion fauna of China began in 1840's (Gervais 1844). This was followed by occasional reports of new species, records, or amendments throughout the last two centuries mostly by non-Chinese scholars (Simon 1880; Karsch 1881; Pocock 1889; Kraepelin 1899, 1901; Birula 1898, 1904, 1911, 1917, 1925; Kishida 1939; Vachon 1952; see Shi & Zhang 2005 for review). Wu (1936) was the first Chinese scholar who studied the scorpion fauna of China; since then there have been few additional reports in this field. In fact, scorpion taxonomy and biogeography still remained a virgin field in China until very recently. This is rather surprising given that scorpions have long been used in traditional Chinese medicine (e.g., it was already well described in the medical code *Peaceful Holy Benevolent Prescriptions* compiled between 978–992 AD in the Song Dynasty).

Recently, Zhu et al. (2004) compiled a checklist of the scorpions in China, and Shi & Zhang (2005) reviewed scorpion systematics with special emphasis on the buthids in the region. The species diversity of Chinese scorpions seems to be rather low in comparison with scorpion faunas of other regions of the world. The nineteen species and subspecies reported in China as listed in Zhu et al. (2004) belong to 9 genera and 5 families. For comparison, 16 species belonging to 9 genera and 3 families were recorded from Israel and Sinai (Levy & Amitai 1980), and more than 60 species (11 genera and 4 families) in Baja California, Mexico and adjacent islands (Williams 1980). Considering the large size of

[3] Corresponding author. E-mail: dxzhang@ioz.ac.cn

China and the ecological diversity of its different regions, it is quite possible that the scorpion fauna in China has been largely underestimated (Lourenço et al. 2005a). This suspicion was justified by the recent discovery of more than 10 new species from Tibet, Yunnan, and Hainan Province (Qi et al. 2005; Lourenço et al. 2005a, 2005b). Therefore, additional new species can be expected, especially in the arid areas.

Among the 19 scorpion species and subspecies listed in Zhu et al. (2004), the *Mesobuthus* scorpions are the most well known species in China. The genus *Mesobuthus* Vachon 1950, with some 15 species reported so far (Fet & Lowe 2000; Gantenbein et al. 2000; Lourenço et al. 2005a), is widespread in the Palaearctic Region from Turkey to Korea. In fact, *Mesobuthus* species are the most common and abundant scorpions in a variety of arid habitats, from sand deserts to high mountains, with the centers of diversity in Central Asia and Iran (Fet et al. 2000; Gantenbein et al. 2003). Historically, three species had been reported in China, viz.: *M. martensii* (Karsch 1879) (Simon 1880; Karsch 1881; Pocock 1889; Birula 1911; Kishida 1939; Song et al. 1982; Qi et al. 2004), *M. eupeus* (C.L. Koch 1839) (Birula 1911, 1925; Schenkel 1936; Fet 1989, 1994), and *M. caucasicus* (Nordmann 1840) sensu Fet 1998 and Gantenbein et al. 2003 (Fet 1989, 1994). A fourth species, *M. songi* Lourenço et al. 2005 was just described from Tibet (Lourenço et al. 2005a). Of the aforementioned scorpions, *M. martensii*, commonly called the "Chinese scorpion", was the most studied and the most popularly used species in traditional Chinese medicine. Over the past decade, more than 70 different peptides, toxins, or homologues have been isolated from venom of this species (Goudet et al. 2002). Even so, its taxonomic status is still blurred due to dubious synonymization (Karsch 1881) and misleading discrimination where different type specimens were examined (Pocock 1889). Another species, the mottled scorpion, *M. eupeus* is widely distributed with a dozen or so subspecies being recognized mainly based on coloration and morphometric characteristics (Fet 1994). Two subspecies, *M. eupeus mongolicus* (Birula 1911) and *M. eupeus thersites* (C.L. Koch 1839), occur in northwest China (Birula 1911, 1925; Fet 1989, 1994; Zhu et al. 2004; Shi & Zhang 2005). This species has also been used in Chinese medicine in recent years (CMS & DXZ pers. obs.). Both species are now threatened by human overexploitation and habitat loss. Fortunately, *M. martensii* has now been listed in the China Species Red List and is considered vulnerable (Wang & Xie 2005), a timely action for protecting this species in China. However, the lack of some basic knowledge about these species greatly impairs conservation efforts. For example, distribution records of *M. martensii* often appear to be a broad-brush description such as "from Inner Mongolia to Korean Peninsula." The same treatment was given to *M. eupeus* in China but with an even vaguer outline. Thus, up-to-date baseline data on the geographical distribution of these species, which are fundamental for conservation planning (Ferrier et al. 2002; Funk & Richardson 2002; Rushton et al. 2004; Elith et al. 2006), are critically needed.

During the last 6 years, we have conducted a systematic sample collection and an extensive field survey of the geographical distribution of two mesobuthid scorpions in China. This allows us to draw a detailed picture of the biogeographical distribution of these species and predict their potential distribution ranges using some ecological modeling methods, such as the Genetic Algorithm for Ruleset Prediction (GARP, Stockwell & Nobel 1992), in a geographic information system (GIS) environment. Here we report the results of our combined study with emphasis on the field survey data, which are unique in scale and density in the study of the two *Mesobuthus* species.

METHODS

Occurrence data and field survey.—The literature dealing with *M. martensii* and *M. eupeus* was consulted to extract distributional records, which served as the start point for our field survey. Extensive geographical surveys were carried out from May to September during 2001–2006, using the following strategies: first, the places with historical records were visited; then we extrapolated our survey outward from around the areas where scorpions were successively collected, according to the similarity of the topography and climatic parameters in 50–100 km intervals; finally, we explored the areas of different landscape adjacent to the outermost sample localities. The

above survey was complemented by irregular collections from the putative distributional areas.

Scorpion collection was carried out either by a stone-rolling method in daytime or UV-light collection at night (Williams 1968). The sample localities were positioned using a GPS receiver (Garmin International), with their longitude, latitude, and elevation recorded. The animals obtained were either deposited in 99.7% ethanol or brought back to the laboratory alive. A subset of specimens was preserved in 75% alcohol for subsequent morphological analysis under a Nikon SMZ1500 stereomicroscope. All the materials are deposited at the Laboratory of Molecular Ecology and Evolution, Institute of Zoology (MEE-IOZ), Chinese Academy of Sciences, Beijing.

Modeling the species' potential distribution.—A species' presence in a place can often be assured when it was successfully sampled there, but its absence cannot be deduced simply from its not being collected in an area. Therefore, a field survey alone is not enough for delimiting a species' distribution range. Modern computational and GIS technologies provide opportunities to use species' presence-only locality data to predict their potential distributions. All the point occurrence data collected by field surveys and literature reviews were geo-referenced to the nearest 0.1° of latitude and longitude and used for ecological niche modeling. Ecological niche models use known occurrence point locations for a species and values for environmental variables (i.e., temperature and precipitation) at those point locations to generate an approximation of the fundamental ecological niche for that species. This ecological niche can then be projected onto a map of the study area in order to predict where that species might occur. Based on ecological niche models, species' distributions were modeled using the Genetic Algorithm for Rule-set Prediction (GARP) (Stockwell & Noble 1992). The occurrence points are divided evenly into training and test data sets. GARP models based on presence-only data, and absences are included in the modeling exercise via sampling of pseudo-absence points from the set of pixels where the species has not been detected. A method is chosen from a set of possibilities (e.g., logistic regression, bioclimatic rules) and then applied to the training data to develop or evolve a rule through an iterative process of rule selection, evaluation, testing, and incorporation or rejection (Peterson et al. 2006). Predictive accuracy is evaluated based on the testing data. The change in predictive accuracy between iterations was used as the criterion to evaluate whether particular rules should be incorporated into the model. The algorithm runs 1000 iterations or until convergence.

GARP modeling was carried out on DesktopGARP (http//www.lifemapper.org/desktopgarp), which offers much improved flexibility in choice of predictive environmental data layers. The 14 environmental data layers, in the form of raster grids with 0.1° resolutions, were obtained from the website http//www.lifemapper.org/desktopgarp. An environmental layer jackknifing procedure is performed to determine what environmental factors are more significant or important than others for our species (Peterson & Cohoon 1999). Based on the evaluation of commission and omission errors as well as accuracy values, the environmental layers are incorporated or discarded in subsequent modeling.

To optimize model performance, we developed 100 replicate models of ecological niche for each species. The 10 best models were selected from the replicate models according to the procedure developed by Anderson et al. (2003) using "Best Subset Selection Parameters" option with the "extrinsic" omission limit set to 10%. Model quality was tested via the testing data. Chi-squared tests were employed to determine whether test points fall into regions of predicted occurrence more often than expected by chance (Peterson 2001; Anderson et al. 2002a, b). Finally, these 10 models were imported into ARCVIEW GIS 3.2 (Environmental Systems Research Institute, Inc. 1999) and overlaid to create one consensus predictive range map. Grid cells that were predicted as present by at least 9 of these models were extracted to give an optimal model for *M. martensii* and *M. eupeus*, respectively. Sympatric zone was obtained by superimposing the optimal models of the two species in ARCVIEW GIS 3.2 with spatial analyst extension.

RESULTS

The scorpions.—The adults of the two species, *M. martensii* and *M. eupeus*, can be easily recognized based on size and coloration,

and the contrasts are especially obvious when they are observed together (for example, when they occurred at the same locale or were in the same collecting jars). Generally, *M. martensii* is larger, with carapace and mesosomal tergites yellowish-brown to blackish-brown. Metasomal segments I–IV are yellowish, while metasomal segment V has conspicuous darkish-brown to blackish spots, especially on the ventral and lateral surfaces. *Mesobuthus eupeus* is smaller, with the whole body yellow to yellowish-brown and with the coloration relatively uniform on the carapace, mesosomal tergites, and metasomal segments. Metasomal V has only slightly brownish pigmentation. Mesosomal tergites often have irregular longitudinal blackish-brown stripes.

In the laboratory, the two species can be easily distinguished by the following characters: in *M. martensii*, the ventromedian carinae of the metasomal segment II and III are crenulate and the granules are evenly developed; the ventrolateral carinae of the metasomal segment V are serratocrenulate and the granules are evenly developed or slightly increasing in size posteriorly; the pedipalp chela is slender with Cl/Cw = 4.45 ± 0.23 (Cl = chela length, Cw = chela width, mean ± SD, n = 65) for the female and 3.71 ± 0.24 (n = 43) for the male, and both the fixed finger and the movable finger having 12–13 rows of oblique granules. In *M. eupeus*, the ventromedian carinae of the metasomal segment II and III are serratocrenulate with the granules increasing in size posteriorly; the ventrolateral carinae of the metasomal segment V are crenulate, and the granules are irregularly increasing posteriorly with 1–3 of them significantly enlarged and lobate; the pedipalp chela is strong with Cl/Cw = 3.47 ± 0.25 (n = 52) for the female and 3.23 ± 0.27 (n = 26) for the male, the fixed finger and the movable finger having 10 and 11 rows of oblique granules, respectively.

Occurrence and distribution.—Our survey covers 16 provincial administrative regions and we succeeded in collecting scorpions from 211 sites belonging to 174 counties across an area of ca. 2800 × 1700 km². Information about the voucher specimens' exact localities is available from authors on request for scientific purposes only. We reserve the right not to release these data for public review for conservation consideration. These two species have been overexploited for commercial purposes and these activities have become rampant in recent years (CMS & DXZ pers. obs.). Releasing our data will make the situation even worse. However, below we present broad information about the two species' ranges.

Mesobuthus martensii (Karsch 1879)*:* historical records of the distribution of *M. martensii* are rather cursory. Many literature sources gave some very broad descriptions, such as Northwest and/or North of China or just provincial names. We were only able to specify 12 localities to recognizable administrative regions (Table 1), which are shown by triangles in Fig. 1.

We have collected *M. martensii* from 175 sites belonging to 15 provincial regions: Anhui (2 sites), Beijing (3), Gansu (16), Hebei (17), Henan (16), Hubei (2), Inner Mongolia (4), Jiangsu (1), Liaoning (10), Ningxia (9), Qinghai (1), Shandong (46), Shaanxi (27), Shanxi (20), and Tianjin (1) (squares in Fig. 1). The northernmost locality where we have collected this scorpion is Beipiao (41.83°N, 120.61°E), Liaoning Province, and the westernmost sample site is Guide (36.01°N, 101.40°E), Qinghai Province. We successively collected specimens on eight islands of Miaodao Archipelago and from Wafangdian (39.37°N, 121.50°E) of Liaodong Peninsula. The elevations of the sample sites range from < 10 m (Shandong and Liaodong Peninsula) to more than 2300 m (Qinghai Province) above sea level. Almost all the sites lie in the mountain areas, great or small. Thus this species is a typical "mountaineer," and shows strong lithophilism in the plain regions. On the Loess Plateau the species may be found in the crevices and some burrows of other invertebrates or small mammals and reptiles. On three occasions we found this species in human dwellings.

Mesobuthus eupeus (C.L. Koch 1839)*:* documented localities of this species in China are very few (Table 1). Two subspecies had been recorded. The subspecies *M. eupeus mongolicus* was described from Gansu, Gobi-Altai and Alashan regions by Birula in 1911, and later reported from Tianshan (Tien-shan) Mountains near Urumchi (Schenkel 1936), Xinjiang Uygur Autonomous Region. Birula (1911, 1925) had recorded that this subspecies was found in Lanzhou, Gansu Province, but

Table 1.—Historical occurrences of *Mesobuthus martensii* and *M. eupeus* in China.

Species	Locality	Lat. °N	Long. °E	References
M. martensii	Beijing (Pekin)	39.9	116.4	Simon 1880, Karsch 1881, Pocock 1889
	Yantai (Tchefou) Shandong	37.5	121.4	Simon 1880, Pocock 1889, Wu 1936
	Tianjin (Tientsin)	39.1	117.2	Karsch 1881
	Alashan, Inner Mongolia	38.8	105.7	Birula 1911
	Wulingshan, Hebei	40.6	117.4	Kishida 1939
	Chaoyang, Liaoning	41.5	120.4	Kishida 1939
	Lingyuan, Liaoning	41.2	119.3	Kishida 1939
	Chengde, Hebei	40.7	118.1	Kishida 1939
	Kaifeng, Henan	34.7	114.4	Wu 1936
	Xuanhua, Hebei	40.6	115.0	Wu 1936
	Donghai, Jiangsu	34.5	118.7	Wu 1936
	Changshandao, Shandong	37.9	120.7	Wu 1936
M. eupeus	Gobi-altai, Mongolia	42.5	104.0	Birula 1911
	Alashan, Inner Mongolia	40.5	103.0	Birula 1911
	Lanzhou, Gansu (Lantsho-fu, Kan-su)	36.0	103.7	Birula 1911, 1925
	Tianshan, Xinjiang (Tien-shan Urimchi)	43.8	87.6	Schenkel 1934

we failed to find this species there or in adjacent areas whereas *M. martensii* were found instead (Lanzhou: 36.12°N, 103.72°E; Yuzhong: 36.35°N, 104.28°E; and Gaolan: 36.33°N, 103.90°E). Another subspecies, *M. eupeus thersites*, was also observed in northwest China (Fet 1989, 1994). Here we just identified specimens to species, with no effort to further identify them to subspecies.

We collected *M. eupeus* from 36 sites (circles in Fig. 2), spanning Gansu (12 sites), Ningxia (12), Xinjiang (1) and Inner Mongolia (11). The southernmost site is Jingyuan (36.50°N, 104.60°E; Gansu), and the easternmost one is Urad Qianqi (40.67°N, 108.73°E; Inner Mongolia). The majority of sites lie in the Gobi desert and bald mountains. There are three exceptions: one site is in a deserted human dwelling, one site lies in the interdunal zone of sand desert, and the third is in a degraded pasture.

Contact Zone: In 8 localities (bisected circles in Figs. 1–3) we collected *M. martensii* and *M. eupeus* at the same site: Jingyuan (36.5°N, 104.6°E) and Jingtai (37.2°N, 104.3°E) of Gansu province; Qingtongxia (37.7°N, 106.0°E), Siyanjing (37.7°N, 105.7°E) and Shikong (37.7°N, 105.5°E) of Ningxia Autonomous Region; and Alashan Zuoqi (37.8°N, 105.5°E), Urad Qianqi (40.7°N, 108.7°E) and Urad Zhongqi (41.3°N, 108.6°E) of Inner Mongolia. All these sites are near or on the litho-mountains.

Prediction of potential distribution and sympatric zone.—In total, 227 occurrence points, 187 for *M. martensii* and 40 for *M. eupeus*, were used for modeling the potential distributions. Of the 14 environmental layers six were excluded from modeling because of their high omission and commission errors and low predictive accuracy. The eight environmental layers used in the predictive modeling are annual means of frost days, solar radiation, precipitation, minimum temperature, mean temperature, maximum temperature, water vapor pressure and wet days. The chi-square test yielded significant results for all the models produced ($P < 10^{-34}$ for *M. martensii* and $P < 10^{-5}$ for *M. eupeus*).

Projections of models onto maps permit visualization of the ecologically potential distribution ranges of the two species. Thus, *M. martensii* appears restricted to the latitude south of 43°N and to the north side of the Yangtze River, bordered by the Helan Mountains in the west and limited by the Yellow Sea and the East China Sea in the east (Fig. 1); an area covering the North China Plain in the east, the Loess Plateau in the west, and the Liaohe Plain in the north.

The occurrence points for *M. eupeus* are concentrated in a relatively small sub-area of

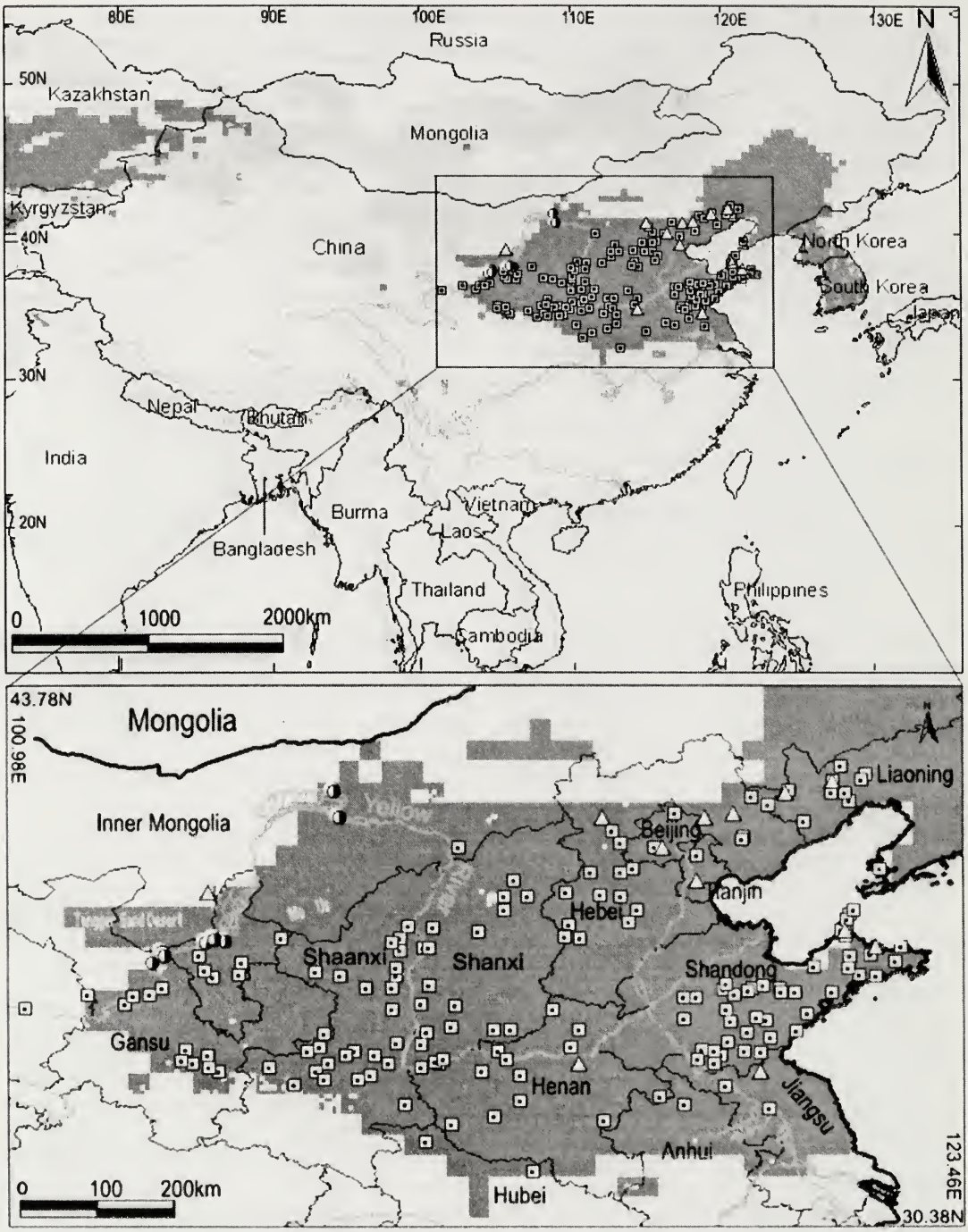


Figure 1.—Ecological niche-based prediction of potential distribution of *M. martensii*. Squares, present data; triangles, historical records. Bisected circles show the sites where *M. martensii* and *M. eupeus* co-occurred.

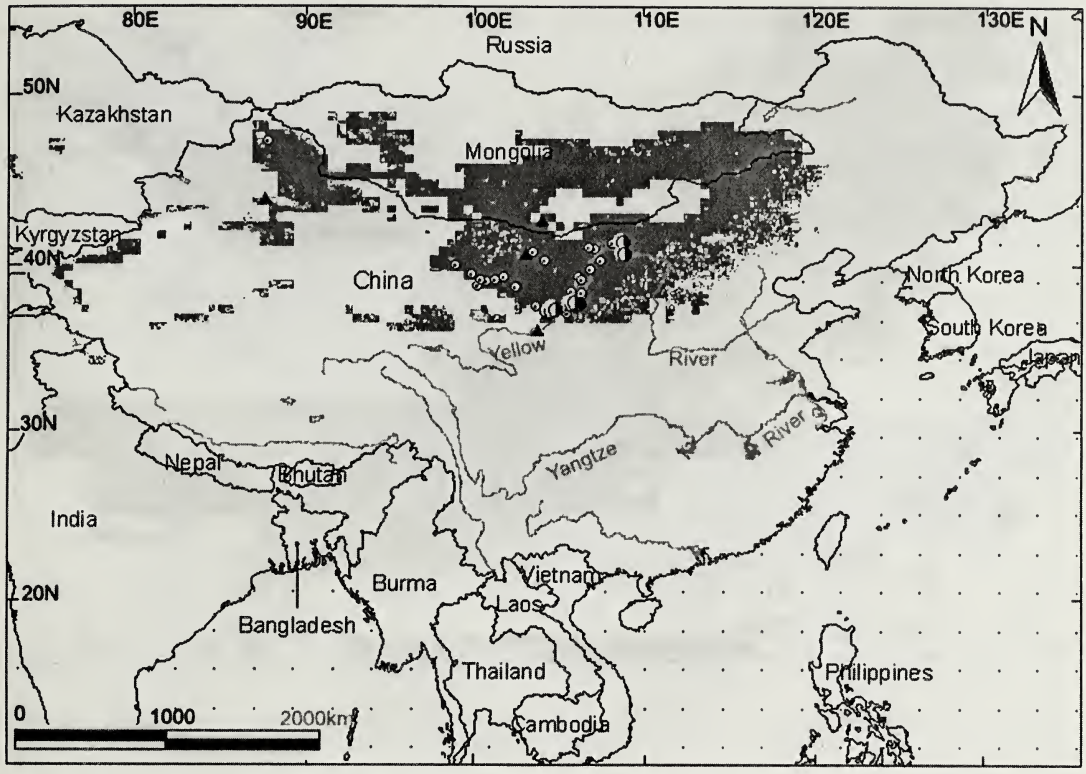


Figure 2.—Ecological niche-based prediction of potential distribution of *M. eupeus*. Circles, present data; triangles, historical records. Bisected circles show the sites where *M. eupeus* and *M. martensii* co-occurred.

its range, being less optimal for ecological niche modeling. A circular potential distribution in the region 36–48°N and 94–120°E (Fig. 2) was predicted, plus some patchy appearance in Xinjiang, west Mongolia, and Kazakhstan and Kyrgyzstan. This is in accordance with historical records of this species there. Outside China, this species ranges from central Anatolia through Caucasus and Turkey to Mongolia and has been found in Afghanistan, Armenia, Azerbaijhan, Georgia, Iran, Iraq, Kazakhstan, Kyrgyzstan, Pakistan, Russia (Astrakhan region), Tajikistan, Turkmenistan, and Uzbekistan (Fet 1989, 1994; Karatas & Karatas 2001, 2003).

Superimposition of the two species models reveal limited areas of potential sympatry (Fig. 3). The potential sympatric zone lies across the big turning of the Yellow River in central-western Inner Mongolia, Ningxia and the middle part of the Gansu Province. Altogether, 24 sites lie in the potential sympatric zone. Ten of those represent collections of *M. eupeus* and six of those *M. martensii*. The remaining eight sites where two species co-occurred fell exactly into the predicted sympatric zone.

DISCUSSION

Our data indicate that *M. martensii* and *M. eupeus* show a parapatric distribution with a contact zone formed at the boundary of their ranges. *Mesobuthus martensii* mainly occurs in the humid and semi-humid regions where the mean annual rainfall exceeds 300 mm. Its range spans the so-called second and third geographical cascades of mainland China, with the average altitude descending eastward from more than 2500 m to less than 10 m above sea level, and includes the littoral zone (Remy & Leroy 1933; Millot & Vachon 1949) and some islands of Bohai Sea. The northern limit of the Chinese scorpion might not exceed 43°N. This is in accordance with Kishida (1939), who recorded that there was no scorpion in Ongniud Qi (42.9°N, 119.0°E). The

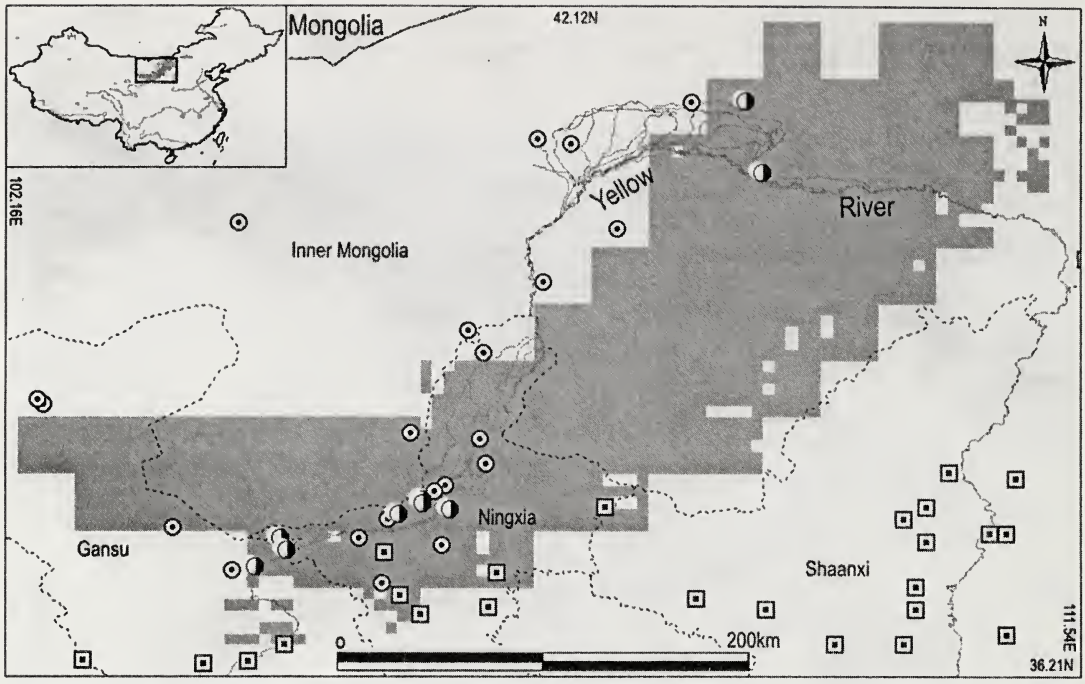


Figure 3.—Ecological niche-based prediction of potential range of the contact zone between *M. martensii* and *M. eupeus*. Squares, *M. martensii*; circles, *M. eupeus*. Bisected circles show the sympatric sites.

Tengger and Mo Us sand desert constitute the northwestern distributional boundary for *M. martensii*.

In contrast, *M. eupeus* is an arid dweller living in the arid and semi-arid environment, as recorded in the literature. All our sampling sites are in or near the desert regions, ranging from the central western area of Inner Mongolia to western Gansu Province, and proceeding further west- and northward to the Altai region, Xinjiang. Although our data about *M. eupeus* are relatively restricted, our models indicated the potential distribution of *M. eupeus*, especially in China, ranges from the east side of the Helan Mountains, expanding westward along the northern slope of the Qilian Mountains, ultimately penetrating to the northern part of the Junggar Basin. We think the sample sites presented here largely cover the whole range of its distribution in China. This species is abundant in the central-western part of Inner Mongolia and Gansu and the west part of Ningxia where the average rainfall is less than 300 mm or even less than 100 mm in some areas.

Distribution patterns of species are shaped by a number of factors, including barriers to dispersal, physical and biological factors that make particular regions of habitat unsuitable for viability and/or reproduction, etc. (Burton 1998). Potential distribution predicted by GARP ecological niche modeling relied on the fundamental niche (Soberón & Peterson 2005). The actual geographic distribution is a modification of the fundamental niche because it is defined by the complex interaction of the realized environment (as well as some biological and historical realities) and the fundamental niche (Brown et al. 1996; Patterson 1999; Peterson et al. 1999; Anderson et al. 2002a, b; Anderson & Martínez-Meyer 2004). This may be why some regions in Central Asia are predicted to be suitable for *M. martensii*, but it is unlikely that this species could have occurred there in reality. There is no report of *M. martensii* from this region and it cannot escape the attention of the scorpiologists' investigation given that it is a "hotspot" region of *Mesobuthus*. Other explanations also exist for its absence: 1) the populations there have gone locally extinct and 2) *M. martensii* has failed to disperse to this region because the existence of *M. eupeus* acts as an effective

biological barrier preventing *M. martensii* from dispersing further west.

Biological interaction as a constraint for species distribution is possible in the case of *M. eupeus*. Ecological models predict that *M. eupeus* can survive further south and east in the middle part of Inner Mongolia, but this species is restricted to the northwest possibly due to the existence of *M. martensii*. We suggest that in the central western region of Inner Mongolia, Ningxia and the middle part of Gansu the two species mutually limit the range expansion of their counterparts, probably due to the competition for food and other resources (such as shelters).

The absence of *M. martensii* in the area north of mid-Liaoning may be the result of population extinction due to some undefined reasons in relatively recent time. This is in accordance with our field survey at Tieling (42.3°N, 123.8°E) where we failed to spot a scorpion. However, several elderly residents ascertained the occurrence of the scorpion there when they knocked down old houses before the 1970's, but they have not seen any since the 1980's. The abuse of insecticides and other poisonous chemicals may be an explanation for its disappearance to some extent, but it cannot account for all. Scorpions are nocturnal obligatory predators but with poor optical sensitivity, detecting their prey through substrate vibration using the tarsal sensilla (Brownell 1977; Brownell & Farley 1979a, b, c; Brownell & Hemmen 2001; Foelix & Schabronath 1983) and/or via air borne vibrations by the trichobothria on the pedipalps (Hjelle 1990). These biological features effectively reduced their exposure to poison because the scorpions will not prey on the poisoned dead creatures that produce no vibration. Actually, *M. martensii* is abundant under the fence stones of farmland in Haiyang (36.83°N, 121.03°E), Shandong Province. Some other anthropogenic activities, such as house rebuilding and large-scale soil cultivating, which cause habitat loss and recent exceptional climate change may serve as alternative explanations for the absence of this species in Tieling. This deserves further investigation.

The two *Mesobuthus* scorpions were found on either side of the Yellow River. The majority of sympatric sites (7 of 8) lie on the outer (western and northern) bank of the river (Fig. 3). This observation suggests that the Yellow River does not constitute an effective barrier for the two species and the present patterns of distribution are the results of historical processes, an interesting topic meriting further investigation by phylogeographical approaches.

Our results cast further doubt about the identity of *M. martensii* (Kishida 1939; Qi et al. 2004). The type specimens upon which *M. confucius* was described by Simon (1880) were collected from Yantai (Tchefou) and Beijing (Pékin). With no convincing evidence, *M. confucius* was then synonymized with *M. martensii* by Karsch (1881) when he examined specimens collected from Beijing and Tianjin. The holotype locality of *M. martensii* is presumably Singapore (Karsch 1879). However, none of our predicting models showed that Singapore is a suitable area for the survival of *M. martensii*. There are two possible interpretations: 1) the type specimen of Karsch (1879) represents a different taxon from that found in Singapore but the name (then *Buthus martensii*) was wrongly given to the Chinese scorpion, i.e., *M. martensii* and *M. confucius* are two different species; 2) the type specimen of Karsch (1879) is of Chinese origin. Karsch (1879) recorded "Exemplum singulum typicum in Mus Berol. asservatum a Prof. de Martens in Singapore collectum"; there was the possibility that the specimen was initially transported by Chinese immigrants from China to Singapore possibly for medical use. It is a pity that neither Qi et al. (2004) nor we could have examined that specimen; thus this issue remains to be clarified in the future.

ACKNOWLEDGMENTS

We would like to thank the following friends, schoolmates and colleagues for their assistance with various aspects of field survey: Jia Chen, Hongqiang Dong, Yujun Dong, Tinggui Feng, Ruidong Han, Ruicai Huo, Yajie Ji, Junlin Kang, Jun Lei, Jiyuan Li, Huatao Liu, Fei Lu, Yudi Liu, Chengjun Lü, Jiancang Ma, Conghai Tang, Lijuan Tang, Duohong Wang, Junping Wang, Ronghua Wang, En Wu, Yuchun Wu, Yongfeng Xu, Ruisheng Yang, Bowei Zhang, Deyi Zhang, Rui Zhang, Xincheng Zhao, Guangjian Zhu. We thank Professors Hongzhang Zhou and Aiping Liang for providing specimens from Inner Mongolia and Tibet. We are grateful to Professors Dax-

iang Song and Suigong Yin for providing valuable information about scorpion researches in China, Professors Naxin Bei and Yuanhua Wu for accommodation during our field survey and Professor Mingsheng Zhu for help with some publications. We thank Dr. Søren Toft, Dr. Victor Fet and an anonymous referee for their constructive comments on an earlier version of the manuscript and Dr. Fet, in particular, for linguistic improvement. We are in debt to Paula Cushing, the journal's managing editor, for the thorough linguistic and format-and-style corrections on the manuscript. This research was supported by the Natural Science Foundation of China (NSFC grant no. 30570246), the CAS Knowledge Innovation Program (grant no. KZCX2-YW-428) and the CAS "Bai Ren Ji Hua" Professorship.

LITERATURE CITED

Anderson, R.P., M.P. Gómez-Laverde & A.T. Peterson. 2002a. Geographical distributions of spiny pocket mice in South America: insights from predictive models. Global Ecology and Biogeography 11:131–141.

Anderson, R.P., A.T. Peterson & M. Gómez-Laverde. 2002b. Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. Oikos 98:3–16.

Anderson, R.P., D. Lew & A.T. Peterson. 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. Ecological Modeling 162:211–232.

Anderson, R.P. & E. Martínez-Meyer. 2004. Modeling species' geographic distributions for preliminary conservation assessments: an implementation with the spiny pocket mice (*Herteromys*) of Ecuador. Biological Conservation 116:167–179.

Birula, A.A. 1898. Miscellancea scorpiologica. III. Zur Synonymie der russischen Skorpione (Fortsetzung). Annuaire du Musée Zoologique de l'Académie Impériale des science de St. Petersbourg 3:295–299.

Birula, A.A. 1904. Miscellanea scorpiologica. VI. Ueber einige *Buthus*-Arten Centrasiens nebst ihrer geographischen Verbreitung. Annuaire du Musée Zoologique de l'Académie Impériale des Sciences de St.-Pétersbourg 9:20–27.

Birula, A.A. 1911. Arachnologische Beiträge. I. Zur Scorpionen- und Solifugen-Fauna des Chinesischen Reiches. Revue Russe d'Entomologie 11(2):195–199.

Birula, A.A. 1917. (Byalynitskii-Birulya A.A.) Arthrogastric Arachnids of Caucasia. Part 1. Scorpions. Annals of the Caucasian Museum. Series A, No. 5. Translated from Russian. Israel Program for Scientific Translation, Jerusalem 1964. 170 pp.

Birula, A.A. 1925. Skorpiologische Beiträge. 10. Zur geographischen Verbreitung zweier weitverbreiteter Skorpionen-Arten Palaearcticums. Zoologischer Anzeiger 63(1–2):93–96.

Brown, J.H., G.C. Stevens & D.M. Kaufman. 1996. The geographic range: size, shape, boundaries, and internal structure. Annual Review of Ecology and Systematics 27:597–623.

Brownell, P.H. 1977. Compressional and surface waves in sand: used by desert scorpions to locate prey. Science 197:479–482.

Brownell, P.H. & R.D. Farley. 1979a. Prey-localizing behaviour of the nocturnal desert scorpion, *Paruroctonus mesaensis*: orientation to substrate vibrations. Animal Behaviour 27:185–193.

Brownell, P.H. & R.D. Farley. 1979b. Detection of vibrations in sand by tarsal sense organs of the nocturnal scorpion, *Paruroctonus mesaensis*. Journal of Comparative Physiology A 131:23–30.

Brownell, P.H. & R.D. Farley. 1979c. Orientation to vibrations in sand by the nocturnal scorpion *Paruroctonus mesaensis*: mechanism of target localization. Journal of Comparative Physiology A 131:31–38.

Brownell, P.H. & J.L. van Hemmen. 2001. Vibration sensitivity and a computational theory for prey-localizing behavior in sand scorpions. American Zoologist 41:1229–1240.

Burton, R. 1998. Intraspecies phylogeography across the Point Conception biogeographic boundary. Evolution 52:734–745.

Elith, J., C.H. Graham, R.P. Anderson, M. Dudík, S. Ferrier, A. Guisan, R.J. Hijmans, F. Huettmann, J.R. Leathwick, A. Lehmann, J. Li, L.G. Lohmann, B.A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J.M. Overton, A.T. Peterson, S.J. Phillips & N.E. Zimmermann. 2006. Novel methods improve prediction of species' distribution from occurrence data. Ecography 29:129–151.

Ferrier, S., G. Watson, J. Pearce & M. Drielsma. 2002. Extended statistical approaches to modeling spatial pattern in biodiversity in northeast New South Wales. I. Species-level modeling. Biodiversity and Conservation 11:2275–2307.

Fet, V. 1989. A catalogue of scorpions (Chelicerata: Scorpiones) of the USSR. Rivista del Museo Civico di Scienze Naturali "Enrico Caffi" (Bergamo) 13(1988):73–171.

Fet, V. 1994. Fauna and zoogeography of scorpions (Arachnida: Scorpiones) in Turkmenistan. Pp. 525–534. *In* Biogeography and Ecology of Turkmenistan (V. Fet & K.I. Atamuradov, eds.). Kluwer Academic Publishers, Dordrecht, Netherlands.

Fet, V. 1998. Case 3026. *Androctonus caucasicus* Nordmann, 1840 (currently *Mesobuthus caucasicus*; Arachnida, Scorpiones): proposed conservation of the specific name. Bulletin of Zoological Nomenclature 55:14–16.

Fet, V., B.E. Hendrixson, W.D. Sissom & G. Levy. 2000. First record for the genus *Mesobuthus* Vachon, 1950 in Israel: *Mesobuthus nigrocinctus* (Ehrenberg, 1828), comb. n. (Scorpiones: Buthidae) from Mt. Hermon. Israel Journal of Zoology 46:287–295.

Fet, V. & G. Lowe. 2000. Family Buthidae C.L. Koch, 1837. Pp. 54–286. *In* Catalog of the Scorpions of the World (1758–1998) (V. Fet, W.D. Sissom, G. Lowe & M.E. Braunwalder, eds.). The New York Entomological Society, New York.

Foelix, R.F. & J. Schabronath. 1983. The fine structure of scorpion sensory organs. I. Tarsal sensilla. Bulletin of the British Arachnological Society 6: 53–67.

Funk, V. & K. Richardson. 2002. Systematic data in biodiversity studies: use it or lose it. Systematic Biology 51:303–316.

Ganbentein, B., C. Kropf, C.R. Largiader & A. Scholl. 2000. Molecular and morphological evidence for the presence of a new Buthid taxon (Scorpiones: Buthidae) on the Island of Cyprus. Revue Suisse de Zoologie 107:213–232.

Ganbentein, B., V. Fet & A.V. Gromov. 2003. The first DNA phylogeny of four species of *Mesobuthus* (Scorpiones: Buthidae) from Eurasia. Journal of Arachnology 31:412–420.

Gervais, P. 1844. Remarques sur la famille des scorpions. Archives du Muséum d'Histoire Naturelle, Paris 4:201–240.

Goudet, C., C-W. Chi & J. Tytgat. 2002. An overview of toxins and genes from the venom of the Asian scorpion *Buthus martensii* Karsch. Toxicon 40:1239–1258.

Hjelle, H.T. 1990. Anatomy and morphology. Pp. 9–63. *In* The Biology of Scorpions (G.A. Polis, ed.). Stanford University Press, Stanford, California.

Karatas, A. & A. Karatas. 2001. First record of *Mesobuthus eupeus* (C.L. Koch, 1893) from central Anatolia (Scorpiones: Buthidae). Pp. 297–299. *In* Scorpions 2001. In Memoriam Gary A. Polis (V. Fet & P.A. Selden, eds.). British Arachnological Society, Burnham Beeches, Buckinghamshire, UK.

Karatas, A. & A. Karatas. 2003. *Mesobuthus eupeus* (C.L. Koch, 1893) (Scorpiones: Buthidae) in Turkey. Euscorpius 7:1–5.

Karsch, F. 1879. Scorpionologische Beiträge II. Mitteilunger der Münchener Entomologische, Ver. 3(2):97–136.

Karsch, F. 1881. Chinesische Myriopoden und Arachnoiden. Berliner Entomologische Zeitschrift 25:219–220.

Kishida, K. 1939. Arachnida of Jehol, Scorpiones. Pp. 1–66. *In* Report of the First Scientific Expedition to Manchoukuo. Sect.5, div.1.

Kraepelin, K. 1899. Scorpiones und Pedipalpi. Das Tierreich Deutslands 8:1–265.

Kraepelin, K. 1901. Catalogue des Scorpions des collections du Muséum d'Histoire Naturelle de Paris. Bulletin du Muséum d'Histoire Naturelle, Paris 7(6):265–274.

Levy, G. & P. Amitai. 1980. Fauna Palaestina Arachnida I: Scorpiones. The Israel Academy of Sciences and Humanities, Jerusalem. 130 pp.

Lourenço, W., J.-X. Qi & M.-S. Zhu. 2005a. Description of two new species of scorpions from China (Tibet) belonging to the genera *Mesobuthus* Vachon (Buthidae) and *Heterometrus* Ehrenberg (Scorpionidae). Zootaxa 98:1–16.

Lourenço, W., J.-X. Qi & M.-S. Zhu. 2005b. Description of a new species of *Isometrus* Ehrenberg 1828 (Scorpiones, Buthidae) from the island of Hainan, China. Boletin Sociedad Entomologica Aragonesa 36:57–63.

Millot, J. & M. Vachon. 1949. Ordre des Scorpions. Traité de Zoologie 6:386–436.

Patterson, B.D. 1999. Contingency and determinism in mammalian biogeography: the role of history. Journal of Mammalogy 80:345–360.

Peterson, A.T. 2001. Predicting species' geographic distributions based on ecological niche modeling. The Condor 103:599–605.

Peterson, A.T. & K.P. Cohoon. 1999. Sensitivity of distributional prediction algorithms to geographic data completeness. Ecological Modeling 117: 159–164.

Peterson, A.T., J. Soberón & V. Sánchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Peterson, A.T., V. Sánchez-Cordero, E. Martínez-Meyer & A.G. Navarro-Sigüenza. 2006. Tracking population extirpations via melding ecological niche modeling with land-cover information. Ecological Modeling 195:229–236.

Pocock, R.I. 1889. Notes on some Buthidae, new and old. The Annals and Magazine of Natural History 3(6):334–351.

Qi, J.-X., M.-S. Zhu & W. Lourenço. 2004. Redescription of *Mesobuthus martensii martensii* (Karsch, 1879) (Scorpiones: Buthidae) from China. Revista Ibérica de Aracnologia 10:137–144.

Qi, J.-X., M.-S. Zhu & W. Lourenço. 2005. Eight new species of the genera *Scorpiops* Peters, *Euscorpiops* Vachon, and *Chaerilus* Simon (Scorpiones: Euscorpiidae, Chaerilidae) from Tibet and Yunnan, China. Euscorpius 32:1–40.

Remy, P. & P. Leroy. 1933. Présence de Scorpions dans la zone subterrestre du littoral marin. Bul-

letin mensuel Société Linnéenne de Lyon 2(3): 3–42.

Rushton, S.P., S.J. Ormerod & G. Kerby. 2004. New paradigms for modeling species distributions? Journal of Applied Ecology 41:193–200.

Schenkel, E. 1936. Schwedisch-chinesische wissenschaftliche Expedition nach den nordwestlichen Provinzen Chinas. B. Scorpiones. Arkiv för Zoologi 27A(20):5.

Shi, C.-M. & D.-X. Zhang. 2005. A review of the systematic research on buthid scorpions (Scorpiones: Buthidae). Acta Zootaxonomica Sinica 30:470–477.

Simon, E. 1880. Arachnides recueills aux environs de Pékin. Annales Societe Entomologique de France, 5 série, Tome Dixié, 97–128.

Soberón, J. & A.T. Peterson. 2005. Interpretation of models of fundamental ecological niche and species' distributional areas. Biodiversity Informatics 2:1–10.

Song, D.-X., X.-X. Lü ;& J.-W. Shang. 1982. Morphology and biology of *Buthus martensii* Karsch, 1879. Bulletin of Biology 26:22–25.

Stockwell, D.R.B. & I.R. Nobel. 1992. Induction of sets of rules from animal distribution data: A robust and informative method of data analysis. Mathematics and Computers in Simulation 33: 385–390.

Vachon, M. 1952. Études sur les Scorpions. Algiers. Institut Pasteur d'Algérie. 482 pp.

Wang, S. & Y. Xie., eds. 2005. China Species Red List. Vol. III. Invertebrates. Higher Education Press, Beijing. Pp. 543–544.

Williams, S.C. 1968. Methods of sampling scorpion populations. Proceedings of the California Academy of Sciences, Series 4 36:221–230.

Williams, S.C. 1980. Scorpions of Baja California, Mexico and adjacent islands. Occasional Papers of the California Academy of Sciences 153:1–127.

Wu, H.W. 1936. A review of the scorpions and whip-scorpions of China. Sinensia 7:113–127.

Zhu, M.-S., J.-X. Qi & D.-X. Song. 2004. A checklist of scorpions from China (Arachnida: Scorpiones). Acta Arachnologica Sinica 13:111–118.

Manuscript received 27 April 2006, revised 31 January 2007.

2007. The Journal of Arachnology 35:227–237

CYTOGENETICS IN THREE SPECIES OF *POLYBETES* SIMON 1897 FROM ARGENTINA (ARANEAE, SPARASSIDAE) I. KARYOTYPE AND CHROMOSOME BANDING PATTERN

Sergio Gustavo Rodríguez-Gil: Laboratorio de Citogenética y Evolución, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Intendente Güiraldes y Costanera Norte, C1428EHA. Ciudad Autónoma de Buenos Aires, Argentina. E-mail: rodrigil@bg.fcen.uba.ar

María Susana Merani: Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, C1121ABG, Ciudad Autónoma de Buenos Aires, Argentina.

Cristina Luisa Scioscia: División Aracnología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Ángel Gallardo 470, C1405DJR, Ciudad Autónoma de Buenos Aires, Argentina.

Liliana María Mola: Laboratorio de Citogenética y Evolución, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Intendente Güiraldes y Costanera Norte, C1428EHA. Ciudad Autónoma de Buenos Aires, Argentina.

ABSTRACT. Species of *Polybetes* are known exclusively from South America. Currently there are 13 described species, 9 occurring in Argentina. Cytogenetic studies in spiders are scarce; the cytogenetics of only about 1% of nearly 39,500 described species are known. Within the Sparassidae, 38 species out of 1,009 (< 4%) have been cytogenetically analyzed; the most frequent chromosome number is $2n = 43/46$ (male/female), $n = 20 + X_1X_2X_3$, present in almost half of the species studied. Female diploid chromosome number is only known for four species: *Heteropoda venatoria* (Linnaeus 1767) ($2n = 44$); *Pediana regina* (L. Koch 1875), *Isopeda* sp. and *Olios* sp. ($2n = 46$). Within the genus *Polybetes*, only *P. pythagoricus* (Holmberg 1875) had been previuosly cytogenetically analyzed. In the present work, the karyotype, heterochromatin content and distribution, and silver stained nucleolus-organizer regions of *P. pythagoricus*, *P. rapidus* (Keyserling 1880) and *P. punctulatus* Mello-Leitão 1944 are described and compared. In *P. pythagoricus* the identification of the chromosome pairs by means of G-banding is also performed. Females of the three species show a chromosome complement of 44 telocentric chromosomes, with a similar karyotype. Males of *P. pythagoricus* show 42 telocentric chromosomes, the two sex chromosomes being the largest and of different size. In the three species, two pairs of telomeric NORs and small pericentromeric positive C-bands in all chromosomes were detected. This C-banding pattern seems to be characteristic of spiders. Comparative analysis of chromosome complements in Sparassidae indicates that $2n = 42/44$ ($X_1X_20/X_1X_1X_2X_2$) (male/female) may represent the ancestral karyotype for *Polybetes*.

Keywords: Chromosome number, telocentric chromosomes, heterochromatin, nucleolus-organizing regions

Species of *Polybetes* are known exclusively from South America. To date there are thirteen described species, nine of them occurring in Argentina (Platnick 2006): *P. germaini* Simon 1897, *P. martius* (Nicolet 1849), *P. obnuptus* Simon 1896, *P. pallidus* Mello-Leitão 1941, *P. punctulatus* Mello-Leitão 1944, *P. pythagoricus* (Holmberg 1875), *P. quadrifoveatus* (Järvi 1914), *P. rapidus* (Keyserling 1880), and *P. trifoveatus* (Järvi 1914). In nature, they are found under the bark of trees (e.g., *P. pythagoricus* is common under the bark of *Eucaliptus*), in the branches of trees (*P. rapidus*), and others are found in grasses such as *Cortaderia* spp. (*P. punctulatus*). *Polybetes pythagoricus* and *P. rapidus* are also common in

cities where they are found in parks, gardens or even in the roofs of buildings. They are nocturnal and sometimes enter houses on stormy days. Despite their usual aggressiveness, they possess venom of low toxicity that causes little local injury and is harmless to humans (Scioscia, personal observations).

Cytogenetic studies in spiders are scarce, with only approximately 1% of nearly 39,500 described species determined. Within the Sparassidae, 36 species out of 1,009 (< 4%) had been previously cytogenetically analyzed; male diploid chromosome numbers range from 21 to 44; female diploid chromosome number is only known for four species: *Heteropoda venatoria* (Linnaeus 1767) ($2n = 44$); *Pediana regina* (L. Koch 1875), *Isopeda* sp. and *Olios* sp. ($2n = 46$). The most frequent sex chromosome determination system is $X_1X_2X_30/X_1X_1X_2X_2X_3X_3$ (male/female) (Hackman 1948; Suzuki 1950; Suzuki & Okada 1950; Bole-Gowda 1952; Suzuki 1952; Mittal 1961; Díaz & Sáez 1966a, b; Mittal 1966; Benavente & Wettstein 1978; Olivera 1978; Datta & Chatterjee 1983; Rowell 1985; Srivastava & Shukla 1986; Parida & Sharma 1986, 1987; Rowell 1991a, b; Hancock & Rowell 1995; Platnick 2006). Within the genus *Polybetes*, only *P. pythagoricus* had been previously cytogenetically analyzed (Díaz & Sáez 1966a, b; Benavente & Wettstein 1978; Olivera 1978).

There are only a few studies in spiders that characterize banding patterns of chromosomes; the distribution of C heterochromatin is known in eleven Sparassidae, eight Araneidae, five Lycosidae, four Tetragnathidae, two Nephilidae, two Sicariidae, one Scytodidae, and one Salticidae species, and G-banding was performed only in *Lycosa thorelli* (Keyserling 1877) (Lycosidae) and *P. pythagoricus* (Brum-Zorrilla & Cazenave 1974; Olivera 1978; Brum-Zorrilla & Postiglioni 1980; Rowell 1985; Datta & Chatterjee 1988; Rowell 1991b; Gorlova et al. 1997; Silva et al. 2002; De Araujo et al. 2005a, b).

In the present work, the karyotype, heterochromatin content and distribution, and silver stained nucleolus-organizer regions (NORs) of *Polybetes pythagoricus*, *P. rapidus* and *P. punctulatus* are described and compared. Furthermore, in *P. pythagoricus* the identification of the chromosome pairs by means of G-banding was performed.

METHODS

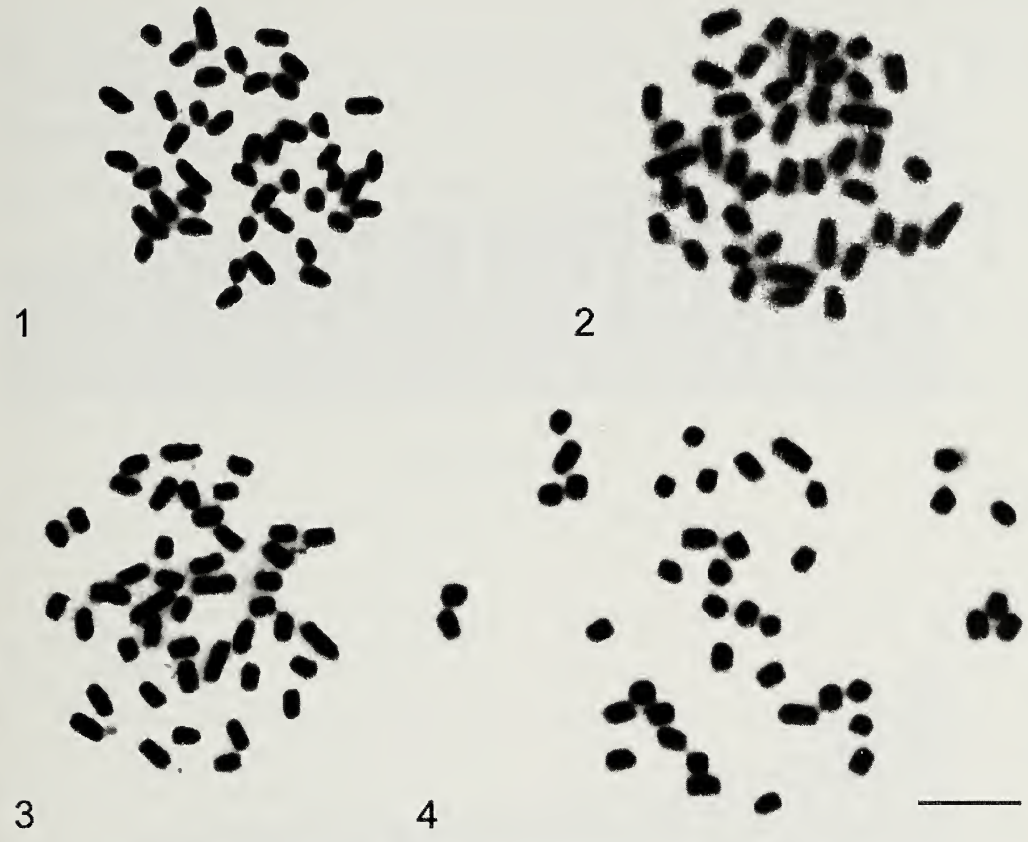
Adult females and males were collected in the field and were bred at the Arachnology Division of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN). Voucher specimens of all species in this study have been deposited in the National Collection of Arachnology (MACN-Ar, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Cristina Scioscia): *Polybetes pythagoricus*, five females and three males from Buenos Aires City, 34°35′15″S, 58°40′21″W, and Buenos Aires Province (Los Polvorines, 34°30′00S, 58°41′00″W; Villa Madero, 34°42′00″S, 58°30′00″W; San Isidro, 34°28′15″S, 58°31′43″W; and Martín García Island Natural Preserve, 34°11′15″S, 58°16′52″W); *P. rapidus*, six females from Buenos Aires Province (Bella Vista, 35°14′00″S, 59°53′00″W; Merlo, 34°40′12″S, 58°45′10″W; Villa Madero and Martín García Island Natural Preserve); *P. punctulatus*, one female from Martín García Island Natural Preserve and two immature females born in the lab.

For cytogenetic preparations, the specimens were cooled; and injected with 0.1 ml of 0.01% colchicine solution. After 1.25 h, several drops of hemolymph were removed from the coxal joints, and gonads together with some digestive tissues were dissected. Each sample was dispersed in 2 ml of hypotonic solution (KCl 0.56%) for 15 min, centrifuged at 800 rpm for 5 min, and fixed in 1 ml of 3 : 1 (methanol : acetic acid). The cell suspension was dropped onto clean slides, air-dried and stained with Giemsa 3% for chromosome counts and karyotyping.

C-band preparations were made following Sumner (1972) with some modifications: 0.2 N HCl for 1 h; saturated solution of $Ba(OH)_2$ for 30 s–1 min; 2 × SSC at 60° C for 1 h. Slides were air-dried and stained with 3% Giemsa.

G-bands preparations were made as follows: PBS solution for 15 min; 0.1% trypsin for 45 s–1 min. Slides were air-dried and stained with 3% Giemsa. NOR-banding was performed following Howell & Black (1980).

Eight to fifteen well-spread mitotic metaphases were measured to determine the karyotype of each species. Chromosome measurements were made using the computer



Figures 1–4.—Mitotic metaphases in spider chromosomes. 1. *Polybetes rapidus* female ($2n$ = 44); 2. *P. punctulatus* female ($2n$ = 44); 3. *P. pythagoricus* female ($2n$ = 44); 4. *P. pythagoricus* male ($2n$ = 42). Scale = 10 μm.

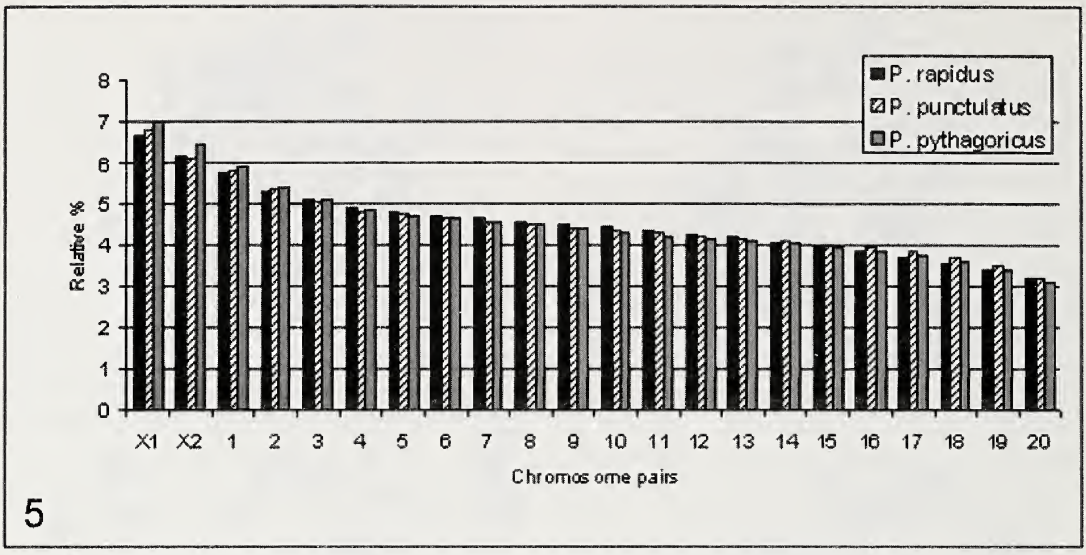
application Micromeasure version 3.3 (Reeves & Tear 2000). The total haploid complement length (TCL) in females was calculated by adding the mean value of each chromosome pair (in arbitrary units). In males of *P. pythagoricus*, the relative length of all chromosomes was analyzed to identify the two chromosomes that have no homologues (sex chromosomes), and TCL was afterwards calculated. The idiogram of each species was drawn on the basis of the relative percentage of each chromosome pair length to the TCL. Chromosome measurements were also made using a vernier calliper to estimate TCL in microns.

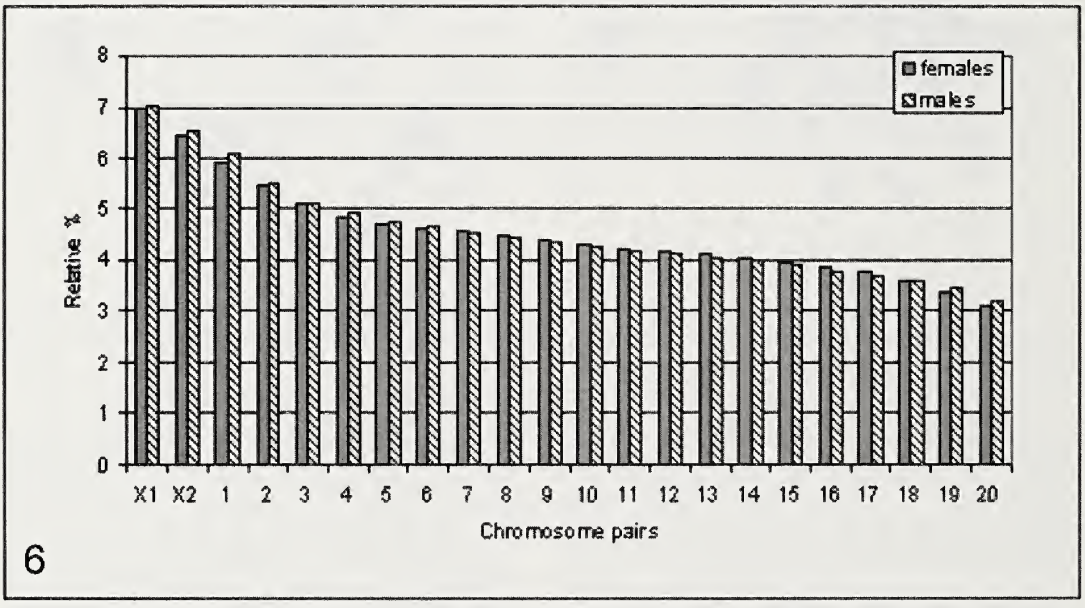
RESULTS

Chromosome complement.—Females of *P. rapidus*, *P. punctulatus* and *P. pythagoricus* show a chromosome complement of 44 telocentric chromosomes, and 42 telocentric chromosomes in males of *P. pythagoricus*. The sex chromosomes cannot be distinguished by their differential pycnosis in somatic metaphases of males and females (Figs. 1–4).

The total haploid complement length (TCL) is similar in the three species: 67.29 ± 4.91 μm in *P. rapidus*, 66.28 ± 6.91 μm in *P. pythagoricus* and 63.70 ± 1.52 μm in *P. punctulatus*.

Females of the three species show a similar karyotype: there are three large pairs of differently-sized chromosomes that can be distinguished; the rest of the chromosomal complement gradually decreases in size, except for the last pair that is slightly smaller. The largest chromosome pair shows slight size differences in the three species (*P. pythagoricus*, 7.00%; *P. punctulatus*, 6.80%; and *P. rapidus*, 6.63%), while the second pair is longer in *P. pythagoricus* (6.45%) (*P. punctulatus*, 6.12%; and *P. rapidus*, 6.16%) (Figs. 5, 7–9). In

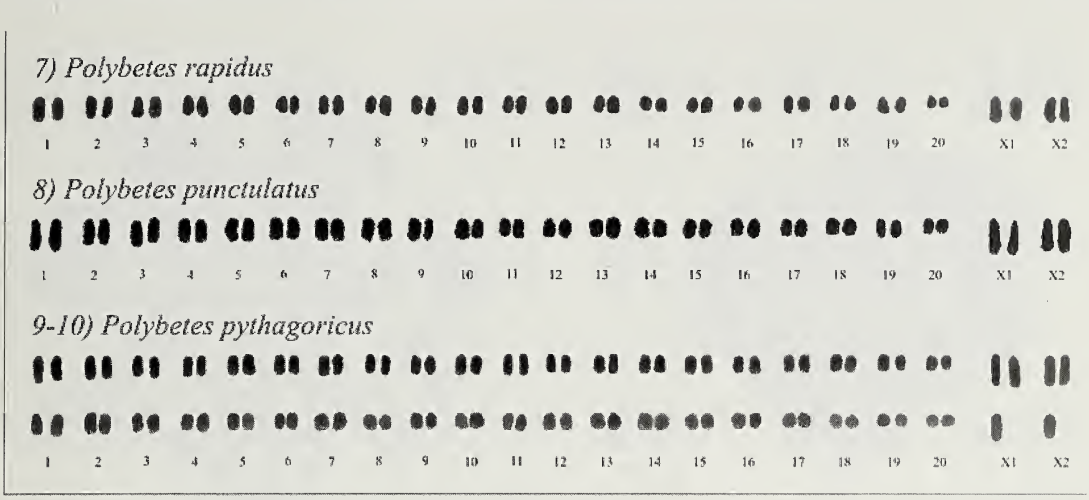




Figures 5, 6.—Comparative ideograms of spider chromosomes. 5. Females of *Polybetes rapidus*, *P. punctulatus* and *P. pythagoricus*; 6. Female and male of *P. pythagoricus*.

males of *P. pythagoricus*, the length analysis of all chromosomes of the complement makes it possible to determine that the two largest chromosomes of different sizes are the sex chromosomes X_1X_2 (Figs. 6, 10). Meiotic studies performed in males of *P. punctulatus* and *P. rapidus* (Rodríguez Gil 2006) demonstrated that in these species the sex chromosomes are also the largest of the complement.

C-banding and NORs silver staining.—In females of the three species, small positive C-bands in the pericentromeric region of all chromosomes were detected, except in the X_2 pair of *P. pythagoricus* where they are more prominent (Figs. 11, 12). The number of chromosomes with telomeric nucleolus-organizer regions (NORs) silver stained varied from 1 to 4 in different cells of the three species (Figs. 13, 14). It was not possible to determine precisely the NOR pairs; one pair was medium sized and the other was among the smaller ones.



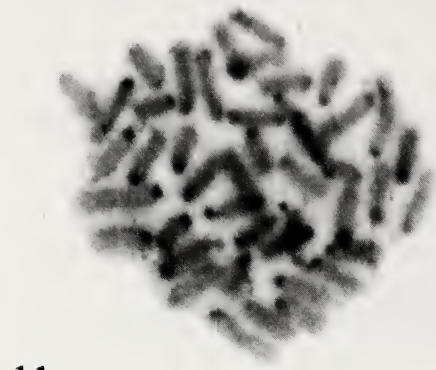
Figures 7–10.—Karyograms from spider cells depicted in Figures 1–4. 7. *Polybetes rapidus* female; 8. *P. punctulatus* female; 9. *P. pythagoricus* female; 10. *P. pythagoricus* male.

G-banding.—Despite applying the G-banding technique to the three species, only *P. pythagoricus* females yielded reproducible results, making it possible to determine the chromosome pair's identification (Figs. 16, 17). In *P. punctulatus*, only a few dark bands, with little contrast, were obtained in all the chromosomes (Fig. 15); therefore, the chromosome pair's identification was not possible. In *P. rapidus* no bands were obtained.

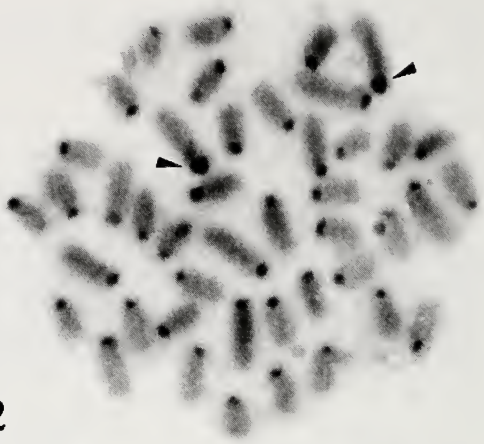
DISCUSSION

Chromosome complement and karyotype.—*Polybetes punctulatus* and *P. rapidus* were here cytogenetically characterized for the first time. *Polybetes pythagoricus* had been previously analyzed in Uruguayan populations; Benavente & Wettstein (1978) performed an ultrastructural study of sex chromosomes pairing in meiosis, and Díaz & Sáez (1966a, b) reported $2n = 42$ and $n = 20 + X_1X_2$ in males. However, Olivera (1978), in a preliminary report of *P. pythagoricus*, described contradictory data reporting a $2n = 40/42$ (male/female) with sex determination system $X_1X_2/X_1X_1X_2X_2$ at mitosis but in male meiosis described the presence of 10 chromosomes plus 2 Xs at one pole and 10 chromosomes at the other one in anaphase I. The three species of *Polybetes* analyzed in this work have $2n = 44 = 40 + X_1X_1X_2X_2$ in females and $2n = 42 = 40 + X_1X_2$ in *P. pythagoricus* males; they show very similar karyotype and total haploid complement length, with all the chromosomes telocentric. The sex chromosomes are the largest ones, the X_1 show slight size differences in the three species and X_2 is longer in *P. pythagoricus*. The conservative karyotype present in the three species could be considered characteristic for the genus.

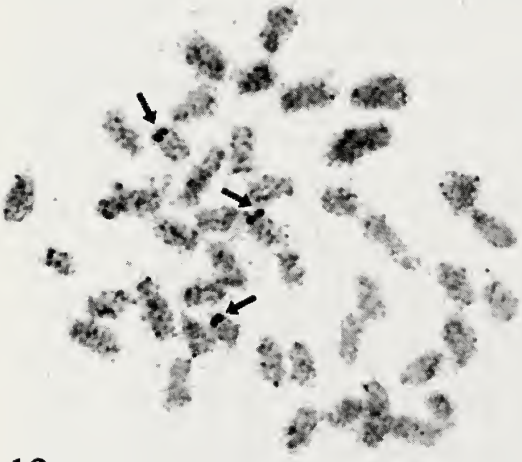
Currently, cytogenetic studies in Sparassidae have been performed on 38 species from 17 genera (Table 1). Usually the chromosomes are telocentric and one of the sex chromosomes is the largest of the complement. The predominant diploid numbers in this family are $2n = 43$, $n = 20 + X_1X_2X_3$, 15 species; and $2n = 41$, $n = 19 + X_1X_2X_3$, 10 species. In other genera, besides *Polybetes*, there seems to be karyotypic conservation as in *Heteropoda* ($n = 19 + X_1X_2X_3$, in 5 of 6 species studied) and *Isopeda* ($n = 20 + X_1X_2X_3$, in the 4 species analyzed). Bole-Gowda (1952) stated that *Heteropoda sexpunctata* Simon 1885 has a derived karyotype, $2n = 20 + X$ (male) with 19 metacentric (including the X chromosome) and two acrocentric autosomes, on the basis of Robertsons law. On the other hand, in the genus *Sparassus*, there is variation not only in chromosome numbers ($2n = 22$ to 44) but in the sex chromosome determination system as well (X_1X_2, $X_1X_2X_3$, $X_1X_2X_3X_4$); although none of the entities was identified at the species level, and it is possible that the genus may be misidentified in some of them (Parida & Sharma 1987). An-



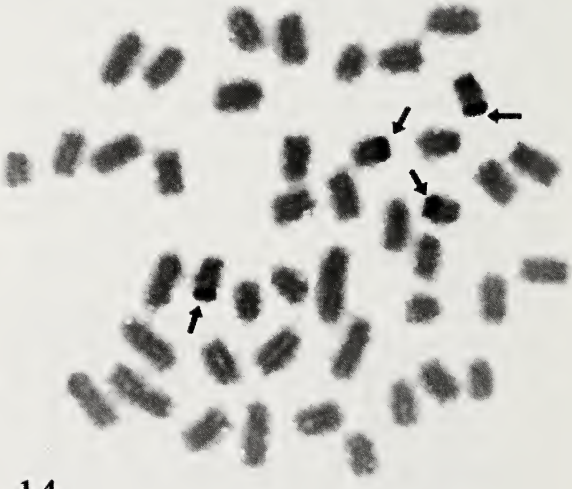
11



12



13



14

15

16

Figures 11–16.—C-banding in spider chromosomes: 11. *Polybetes punctulatus* female; 12. *P. pythagoricus* female (arrowheads point to prominent C-bands). NORs silver staining in spider chromosomes: 13. *P. rapidus* female; 14. *P. pythagoricus* female (arrows point to NOR regions). G-banding in spider chromosomes: 15. *P. punctulatus* female; 16. *P. pythagoricus* female. Scale = 10 μm.

Figure 17.—G-banding karyogram and idiogram of *Polybetes pythagoricus* chromosomes (from cell depicted in Fig. 16).

cestral populations of *Delena cancerides* Walckenaer 1837 also show $n = 20 + X_1X_2X_3$, but this species has a number of chromosomal races that differ by the presence of particular combinations of chromosomal fusions, either in homozygous or heterozygous condition (Rowell 1985, 1990, 1991a, b; Hancock & Rowell 1995). A reduced complement is also present in *Micrommata virescens* (Clerck 1757), but neither the chromosome number nor the sex chromosome complement is known with certainty (Hackman 1948).

Heterochromatin characterization.—The three species of *Polybetes* analyzed here show only small pericentromeric heterochromatic bands in all the chromosomes with no differential pycnosis of the sex chromosomes, although Olivera (1978) reported that "substantial" heterochromatic blocks were present in *P. pythagoricus* mitotic and meiotic chromosomes. *Polybetes pythagoricus* X_2 chromosomes showed a larger C-band than in the other two species, which may explain the differences in these chromosomes' size.

Since the pioneer characterization of *Schizocosa malitiosa* (Tullgren 1905) (Lycosidae) by Brum-Zorrilla & Cazenave (1974), few spider species have been analyzed with regard to the heterochromatin content and distribution. Our results fit with previous data of most of the other spider species analyzed that have a small amount of pericentromeric heterochromatin in the autosomes and sex chromosomes; this condition seems to be characteristic in spiders. In *Loxosceles intermedia* Mello-Leitão 1934 (Sicariidae) and *Isopeda* species, pericentromeric C-bands are more conspicuous (Brum-Zorrilla & Postiglioni 1980; Rowell 1985, 1991b; Datta & Chatterjee 1988; Gorlova et al. 1997; Silva et al. 2002). In a few species, telomeric localization of heterochromatin (telomeric C-bands) has also been described in some chromosomes of the complement; these bands have usually appeared in a polymorphic condition (Rowell 1985; Datta & Chatterjee 1988; Rowell 1991b; De Araujo 2005a). In *Nephilengys cruentata* (Fabricius 1775) (Nephilidae) interstitial C-bands are present in some autosomes; the same occurs in some autosomes and the sex chromosomes of one unidentified species of *Scytodes* (De Araujo et al. 2005a, b).

Table 1.—Karyotype characteristics and collecting locality of the Sparassidae species cytogenetically analyzed (f = female).

Species	2 n	n (male)	Locality	References
Bhutaniella sikkimensis (Gravely 1931)	42	19 + $X_1X_2X_3X_4$	India	Datta & Chatterjee 1983
Delena cancerides Walckenaer 1837 (ancestral karyotype)	43	20 + $X_1X_2X_3$	Australia	Rowell 1985, 1991a, b; Hancock & Rowell 1995
Delena sp.	43	20 + $X_1X_2X_3$		McIntosh *in* Suzuki 1952
Heteropoda leprosa Simon 1884	41	19 + $X_1X_2X_3$	India	Datta & Chatterjee 1983
Heteropoda phasma Simon 1897	41	19 + $X_1X_2X_3$	India	Srivastava & Shukla 1986
Heteropoda procera (L. Koch 1867)	41	19 + $X_1X_2X_3$	Australia	Rowell 1985
Heteropoda sexpunctata Simon 1885	21	10 + X	India	Bole-Gowda 1952
Heteropoda venatoria (Linnaeus 1767)	41–44 f	19 + $X_1X_2X_3$	India, Japan	Suzuki & Okada 1950; Bole-Gowda 1952; Srivastava & Shukla 1986
Heteropoda sp. nov.	41	19 + $X_1X_2X_3$	Australia	Rowell 1985
Holconia immanis (L. Koch 1867)	43	20 + $X_1X_2X_3$	Australia	Rowell 1991a, b (sub *Isopoda*)
Isopeda vasta (L. Koch 1867)	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Isopoda vaster* (sic))
Isopeda villosa L. Koch 1875	43	20 + $X_1X_2X_3$	Australia	Rowell 1991a, b (sub *Isopoda*)
Isopeda sp.	43–46 f	20 + $X_1X_2X_3$	Australia	Rowell 1985 (sub *Isopoda*)
Isopeda sp. nov.	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Isopoda*)
Isopedella leai Hogg 1903	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Isopoda tepperi* Hogg)
Micrommata virescens (Clerck 1757)	±35	16 + $X_1X_2X_3$ (?)	Finland	Hackman 1948 [sub *Micrommata viridissima* (De Geer)]
Neosparassus diana (L. Koch 1875)	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b (sub *Olios)*
Olios lamarcki (Latreille 1806)	42	20 + X_1X_2	India	Bole-Gowda 1952
Olios sp. 1	43	20 + $X_1X_2X_3$		McIntosh *in* Suzuki 1952
Olios sp. 2	43–46 f	20 + $X_1X_2X_3$	Australia	Rowell 1985
Parapalystes whiteae (Pocock 1902)	43	20 + $X_1X_2X_3$	India	Mittal 1961, 1966 (sub *Palystes*)
Pediana regina (L. Koch 1875)	43–46 f	20 + $X_1X_2X_3$	Australia	Rowell 1985, 1991b
Pediana sp. nov.	43	20 + $X_1X_2X_3$	Australia	Rowell 1991b
Polybetes punctulatus Mello-Leitão 1944	44 f	20 + X_1X_2	Argentina	this work; Rodríguez Gil 2006
Polybetes pythagoricus (Holmberg 1875)	42–44 f	20 + X_1X_2	Uruguay	Díaz & Sáez 1966a, b (sub *P. pitagorica* (sic))
			Argentina	this work; Rodríguez Gil 2006
	40–42 f		Uruguay	Olivera 1978 (sub *P. pithagorius* (sic))
Polybetes rapidus (Keyserling 1880)	44 f	20 + X_1X_2	Argentina	this work; Rodríguez Gil 2006

Table 1.—Continued.

Species	2 n	n (male)	Locality	References
Pseudopoda prompta (O. P.-Cambridge 1885)	41	19 + $X_1X_2X_3$	India	Srivastava & Shukla 1986 (sub *Heteropoda*)
Sinopoda forcipata (Karsch 1881)	41	19 + $X_1X_2X_3$	Japan	Suzuki 1952 (sub *Heteropoda*)
Sparassus sp. 1	44	21 + X_1X_2	India	Parida & Sharma 1987
Sparassus sp. 2	42	20 + X_1X_2	India	Parida & Sharma 1987
Sparassus sp. 3	41	19 + $X_1X_2X_3$	India	Parida & Sharma 1986, 1987
Sparassus sp. 4	41	19 + $X_1X_2X_3$	India	Parida & Sharma 1987
Sparassus sp. 5	22	10 + X_1X_2	India	Parida & Sharma 1987
Sparassus sp. 6	42	20 + X_1X_2	India	Datta & Chatterjee 1983 (sub *Parassus* sp. 1)
Sparassus sp. 7	44	20 + $X_1X_2X_3X_4$	India	Datta & Chatterjee 1983 (sub *Parassus* sp. 2)
Sparassus sp. 8	42	20 + X_1X_2	India	Datta & Chatterjee 1983
Spariolenus tigris Simon 1880	41	19 + $X_1X_2X_3$	India	Bole-Gowda 1952
Thelcticopis severa (L. Koch 1875)	43	Possibly $X_1X_2X_3$	Japan	Suzuki 1950, 1952 (sub *Thelticopis* (sic))

In spermatogonial mitosis, after C-banding, sex chromosomes have shown two different patterns. In three species of Lycosidae and in *Delena cancerides* the sex chromosomes were more darkly stained than the autosomes, while there was no difference in the sex chromosomes and autosomes in *Isopeda* and Araneidae species. In the three *Polybetes* species presented here and in four araneids, there is also no difference in the sex chromosomes and autosomes in female somatic and gonial cells. In one species, *Schizocosa malitiosa*, only one X chromosome was notable in that it exhibited complete heterochromatinization (Brum-Zorrilla & Cazenave 1974; Brum-Zorrilla & Postiglioni 1980; Rowell 1985; Datta & Chatterjee 1988; Rowell 1991b).

NORs silver staining and G-banding.—The variation in the number of chromosomes per cell bearing nucleolus-organizer regions observed in *Polybetes* species is common in the Ag-technique. It is characteristic of silver staining that not all the NORs are usually silver stained in species with multiple NORs, but only those transcriptionally active during the preceding interphase (Sumner 2003). It can be concluded that two chromosomal pairs with telomeric NORs are present in the three species here analyzed. Although the identification of the NOR pairs should be regarded as tentative, it seems possible that they correspond to the same pairs in the three species. Only a pair of NORs was detected at early somatic stages of *P. pythagoricus* Uruguayan specimens (Olivera 1978).

G-banding allows the precise identification of homologues and facilitates karyotypic comparisons between related species. Although good quality G-bands can be produced in reptiles, birds, mammals, in some fishes and amphibians, and in a few plants, this method does not yield consistent results in invertebrate chromosomes and only in a few species of insects have well-defined G-bands been obtained. The difficulty in obtaining good quality G-bands in invertebrates may reflect differences in mitotic chromosome substructure, e.g., tight compaction of the chromatin compared to vertebrates (Lorite et al. 1996; Appels et al. 1998; Baldanza et al. 1999; Sumner 2003). In spiders, G-banding had been performed in three species of Lycosidae (but only *Lycosa thorelli* showed consistent G-banding), and in *P. pythagoricus* from Uruguay (where only a few pairs could be identified) (Olivera 1978; Brum-Zorrilla & Postiglioni 1980). In the present work, the identification of all chromosome pairs was possible in *P. pythagoricus*. Taking into account that the pattern of pachytene chromomeres resembles that of G-bands on the same chromosome (Sumner 2003), it would be interesting to perform a

comparative analysis of the chromomere pattern of the three species in order to know if the scarcity and absence of G-bands in *P. punctulatus* and *P. rapidus* respectively is due to structural differences or to technical procedures.

The three species of *Polybetes* here analyzed are easily distinguished by morphological characters, but they are very conservative karyotypically. This fact could be useful in future for the delimitation of genera in a systematic revision of the family.

ACKNOWLEDGMENTS

The present study was supported by grants from the National University of Buenos Aires (UBA) (Ex 317 to Drs. L. Poggio and L. Mola) and from the National Council of Scientific and Technological Research (CONICET) (PIP 02296 and 05927 to Drs. L. Poggio and L. Mola and PIP 02202 and 05654 to Drs. A. González and C. Scioscia). The authors wish to thank to Mr. Hernán Dinapoli for technical assistance, to Lic. Pablo Rebagliati, Lic. Mariana López and the student Luis Piacentini for collecting some of the specimens, and to Dr. María Inés Pigozzi for critical reading of the manuscript. The authors wish to dedicate this paper to the memory of Dr. Carlos A. Naranjo.

LITERATURE CITED

Appels, R., R. Morris, B.S. Gill & C.E. May. 1998. Chromosome Biology. Kluwer Academic Publishers, Boston, Massachusetts. 401 pp.

Baldanza, F., L. Gaudio & G. Viggiani. 1999. Cytotaxonomic studies of *Encarsia* Förster (Hymenoptera: Aphelinidae). Bulletin of Entomological Research 89:209–215.

Benavente, R. & R. Wettstein. 1978. Ultrastructural cytogenetics of the sex determination mechanisms of araneids. Revista de Microscopía Electrónica 5:320–321.

Bole-Gowda, B.N. 1952. Studies on the chromosomes and the sex-determining system in four hunting spiders (Sparassidae). Proceedings of the Zoological Society, Bengal 5:51–70.

Brum-Zorrilla, N. & A. Postiglioni. 1980. Kariological studies on Uruguayan spiders. I. Banding pattern in chromosomes of *Lycosa* species (Araneae-Lycosidae). Genetica 54:149–153.

Brum-Zorrilla, N. & A.M. Cazenave. 1974. Heterochromatin localization in the chromosomes of *Lycosa malitiosa* (Arachnida). Experientia 30: 94–95.

Datta, S.N. & K. Chatterjee. 1983. Chromosome number and sex-determining system in fifty-two species of spiders for north-east India. Chromosome Information Service 35:6–8.

Datta, S.N. & K. Chatterjee. 1988. Chromosome and sex determination in 13 araneid spiders of North-Eastern India. Genetica 76:91–99.

De Araujo, D., D.M. Cella & A.D. Brescovit. 2005a. Cytogenetic analysis of the neotropical spider *Nephilengys cruentata* (Araneomorphae, Tetragnathidae): Standard staining, Nors, C-Bands and base-specific fluorochromes. Brazilian Journal of Biology 65:193–202.

De Araujo, D., D.M. Cella & A.D. Brescovit. 2005b. Variabilidade cromossômica em três espécies de *Scytodes* Latreille, 1804 (Araneae, Haplogynae, Scytodidae). Primer Congreso Latinoamericano de Aracnología (Minas, Uruguay) Actas: 107.

Díaz, M. & F.A. Sáez. 1966a. Karyotypes of South American Araneida. Memórias do Instituto Butantán Commemorativo 33:153–154.

Díaz, M. & F.A. Sáez. 1966b. Investigaciones citogenéticas sobre algunas especies de araneidos uruguayos. Anales (II) Congreso Latinoamericano de Zoología (San Pablo):3–9.

Gorlova, O.Y., J.P. Gorlov, E. Nevo & D.V. Logunov. 1997. Cytogenetic studies on seventeen spider species from Israel. Bulletin of the British Arachnological Society 10:249–252.

Hackman, W. 1948. Chromosomenstudien an Araneen mit besonderer Berücksichtigung der Gechlechtschromosomen. Acta Zoologica Fennica 54: 1–101.

Hancock, A.J. & D.M. Rowell. 1995. A chromosomal hybrid zone in the Australian huntsman spider, *Delena cancerides* (Araneae: Sparassidae). Evidence for a hybrid zone near Canberra, Australia. Australian Journal of Zoology 43:173–180.

Howell, W.M. & D.A. Black. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36:1014–1015.

Lorite, P., E. Chica & T. Palomeque. 1996. G-banding and chromosome condensation in the ant, *Tapinoma nigerrimum*. Chromosome Research 4:77–79.

Mittal, O.P. 1961. Chromosome number and sex mechanism in twenty-one species of the Indian spiders. Research Bulletin (N. S.) of the Panjab University 12:271–273.

Mittal, O.P. 1966. Karyological studies on Indian spiders. IV. Chromosomes in relation to taxonomy in Eusprassidae, Selenopidae and Thomisidae. Genetica 37:225–234.

Olivera, G. 1978. Estudio citogenetico en *Polybetes pythagoricus* (Araneomorphae-Sparassidae). XX. Semana Univisitaria Gaucha de Debates Biológicos, Porto Allegre 24(Abstracts):251–253.

Parida, B.B. & G.P. Sharma. 1986. Karyotype and

spermatogonia in an Indian hunting spider, *Sparassus* sp. (Sparassidae: Arachnida) with multiple sex chromosomes. Chromosome Information Service 40:28–30.

Parida, B.B. & G.P. Sharma. 1987. Chromosome number, sex mechanism and genome size in twenty seven species of Indian spiders. Chromosome Information Service 43:11–13.

Platnick, N.I. 2006. The World Spider Catalog, version 7.0. American Museum of Natural History, New York. Online at http://research.amnh.org/entomology/spiders/catalog/index.html.

Reeves, A. & J. Tear. 2000. Micromeasure for Windows. Version 3.3 free program distributed by the author from http://www.colostate.edu/Depts/Biology/Micromeasure.

Rodríguez-Gil, S.G. 2006. Citogenética de arañas sinantrópicas de Argentina (Arachnida: Araneae). Ph. D. Thesis. Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. 155 pp.

Rowell, D.M. 1985. Complex sex-linked fusion heterozygosity in the Australian huntsman spider *Delena cancerides* (Araneae: Sparassidae). Chromosoma (Berlin) 93:169–176.

Rowell, D.M. 1990. Fixed fusion heterozygosity in *Delena cancerides* Walck. (Araneae: Sparassidae): an alternative to speciation by monobranchial fusion. Genetica 80:139–157.

Rowell, D.M. 1991a. Chromosomal fusion and meiotic behaviour in *Delena cancerides* (Araneae: Sparassidae). I. Pairing behaviour and X-chromosome segregation. Genome 34:561–566.

Rowell, D.M. 1991b. Chromosomal fusion and meiotic behaviour in *Delena cancerides* (Araneae: Sparassidae). II. Chiasma position and its implications for speciation. Genome 34:567–573.

Silva, R.W., D.D.R. Klisiowicz, D.M. Cella, O.C. Mangili & I.J. Sbalqueiro. 2002. Differential distribution of constitutive heterochromatin in two species of brown spider: *Loxosceles intermedia* and *L. laeta* (Araneae, Sicariidae), from the metropolitan region of Curitiba, PR (Brazil). Acta Biológica Paranaense, Curitiba 31:123–136.

Srivastava, S.C. & S. Shukla. 1986. Chromosome number and sex determining mechanism in forty-seven species of Indian spiders. Chromosome Information Service 41:23–26.

Sumner, A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75:304–305.

Sumner, A.T. 2003. Chromosomes: Organization and Function. Blackwell Publishing, Oxford, UK. 287 pp.

Suzuki, S. 1950. Sex determining mechanism and karyotypes in spiders. Zoological Magazine (Tokyo) 59:31–32.

Suzuki, S. 1952. Cytological Studies in spiders II. Chromosomal investigation in the twenty two species of spiders belonging to the four families, Clubionidae, Sparassidae, Thomisidae, and Oxyopidae, which constitute Clubionoidea, with special reference to sex chromosomes. Journal of Science of the Hiroshima University, Series B, Division. 1 13:1–52.

Suzuki, S. & A. Okada. 1950. A study on the chromosomes of a spider, *Heteropoda venatoria*, with special reference to X1-, X2- and X3- chromosomes. Journal of Science of the Hiroshima University, Series B, Division 1. 11:1–47.

Manuscript received 2 August 2005, revised 19 January 2007.

2007. The Journal of Arachnology 35:238–277

A REVIEW OF SOME AUSTRALASIAN CHERNETIDAE: *SUNDOCHERNES*, *TROGLOCHERNES* AND A NEW GENUS (PSEUDOSCORPIONES)

Mark S. Harvey: Department of Terrestrial Invertebrates, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia. E-mail: mark.harvey@museum.wa.gov.au

Erich S. Volschenk: Department of Zoology, James Cook University, Townsville, Queensland 4811, Australia. Current address: Department of Terrestrial Invertebrates, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia

ABSTRACT. A systematic review of some Australasian species previously allocated to the chernetid genus *Sundochernes* Beier 1932 reveals numerous discrepancies from the type species, *S. modiglianii* (Ellingsen 1911). Three of these species are removed to the genus *Troglochernes* Beier 1969, previously known from only a single troglobitic species, and a fourth is removed to a new genus. *Troglochernes* contains six species: the type species *T. imitans* Beier 1969 from caves on the Nullarbor Plain, Western Australia; three species newly transferred from *Sundochernes*, *T. guanophilus* (Beier 1967) new combination, from Fig Tree Cave, New South Wales, *T. dewae* (Beier 1967) new combination, from bird nests in New South Wales, Queensland and Western Australia, *T. novaeguineae* (Beier 1965) new combination, from central Papua New Guinea; and two new species, *T. cruciatus* Volschenk, new species from Rope Ladder Cave, North East Queensland and *T. omorgus* Harvey & Volschenk, new species from a beetle in Queensland. The Lord Howe Island endemic pseudoscorpion *Sundochernes grayi* Beier 1975 is transferred to a new genus, *Satrapanus* Harvey & Volschenk, as it lacks the diagnostic features of *Sundochernes*. Problems with the generic allocation of species currently placed within *Sundochernes* are discussed and the female genitalia of *Nesochernes gracilis* Beier 1932 and *Paraustrochernes victorianus* Beier 1966 are illustrated for the first time. *Troglochernes imitans* is one of the most highly modified troglobitic members of the Chernetidae, displaying extremely elongate pedipalps and legs suggesting an extended period of isolation from ancestral epigean populations. The remaining cave-dwelling species, *T. cruciatus* and *T. guanophilus*, are less modified and show fewer morphological modifications which may suggest more recent colonization of the cave environments.

Keywords: Taxonomy, morphology, new species, *Nesochernes*, caves, bird nests

The Australasian chernetid fauna is moderately well developed, with representatives of 36 genera currently described. Few genera, however, contain more than a handful of species, and many are inadequately defined with crucial details, such as the internal female genitalia, often unknown. The genus *Sundochernes* Beier 1932 is one of the larger genera of the region with 10 named species ranging from tropical south-eastern Asia to temperate southern Australia. A further species, doubtfully referred to the genus, was named from Brazil (Beier 1974a). The type species *Chelifer modiglianii* Ellingsen 1911 was originally named from specimens collected in Sumatra (Ellingsen 1911), and later recorded from Malaysia (Beier 1967a). It was briefly redescribed, and illustrated for the first time, by Beier (1932). Our examination of the syntypes revealed that some species attributed to *Sundochernes* were clearly not congeneric with *S. modiglianii*. The study presented here demonstrates that four species originally attributed to *Sundochernes* lack the diagnostic features of that genus, and alternative taxonomic arrangements should be sought. Through the study of a range of specimens of *Troglochernes imitans* Beier 1969, we have been able to ascertain that three of these species, along with two other newly described Australian species, can be attributed to *Troglochernes* Beier 1969 rather than *Sundochernes*. We

have also been able to demonstrate that *Sundochernes grayi* Beier 1976 belongs to a separate genus, here named *Satrapanus*, largely based upon the distinctive morphology of the female internal genitalia. The morphology of the spermathecae, which has been shown to be of significant value at the generic level within the Chernetidae (e.g., Vachon 1938; Muchmore 1974, 1975; Mahnert 1978), provides support for the recognition of distinct genera.

METHODS

The specimens examined for this study are lodged in the following institutions: Australian Museum, Sydney, Australia (AM); American Museum of Natural History, New York, USA (AMNH); Australian National Insect Collection, Canberra, Australia (ANIC); Museum of Natural History, London, UK (BMNH); Bishop Museum, Honolulu, Hawaii, USA (BPBM); Field Museum of Natural History, Chicago, Illinois, USA (FMNH); Museo Civico di Storia Naturale "Giacomo Doria," Genova, Italy (MCSNG); Muséum d'Histoire Naturelle, Genève, Switzerland (MHNG); Muséum National d'Histoire Naturelle, Paris (MNHN); Museum of Tropical Queensland, Townsville, Australia (MTQ); Naturhistorisches Museum, Wien, Austria (NHMW); Museum Victoria, Melbourne, Australia (NMV); Queensland Museum, Brisbane, Australia (QM); South Australian Museum, Adelaide, Australia (SAM); United States National Museum, Smithsonian Institution, Washington, DC, USA (USNM); Western Australian Museum, Perth, Australia (WAM); and Universitets Lund, Lund, Sweden (ZMLU). Some specimens of *Satrapanus grayi* were collected by staff from the Australian Museum's Centre for Biodiversity and Conservation Research, abbreviated to CBCR.

In addition to the specimens detailed below, we also examined the internal genitalia of females of two Australasian chernetids. *Nesochernes gracilis norfolkensis* Beier 1976: 2 males, 2 females, 1 tritonymph, 1 deutonymph, 1 protonymph from Filmy Fern Walk, 29°01′S, 167°57′E, Norfolk Island National Park, Norfolk Island, Australia, 30 November 1984, litter under *Araucaria heterophylla*, T.A. Weir (WAM T68620). *Paraustrochernes victorianus* Beier 1966: 1 female from Cumberland Falls, Victoria, Australia, 37°34′S, 145°53′E, 27 May 1991, under bark of *Eucalyptus regnans*, M.S. Harvey & M.E. Blosfelds (WAM T66536).

Terminology and mensuration mostly follows Chamberlin (1931), with the exception of the nomenclature of the pedipalps and legs, and with some minor modifications to the terminology of the trichobothria (Harvey 1992b). The specimens were studied using three techniques. Temporary slide mounts were prepared by immersion of specimens in concentrated lactic acid at room temperature for several days, and mounting them on microscope slides with 10 or 12 mm coverslips supported by small sections of 0.25, 0.35 or 0.50 mm diameter nylon fishing line or with small slivers of broken coverslips. After study, the specimens were returned to 75% ethanol with the dissected portions placed in 12 x 3 mm glass genitalia microvials (BioQuip Products, Inc.). Permanent slide mounts were prepared by removing the pedipalps, the chelicera, left leg I, and left leg IV from specimens with the use of eye-scissors or small needles, and clearing overnight with 10% potassium hydroxide at room temperature. The specimens were then washed in several rinses of water and 5% acetic acid (to neutralise the potassium hydroxide), and dehydrated through a graded ethanol series. They were then transferred to Euparal Essence overnight at room temperature, prior to mounting in Euparal on microscope slides using 10 or 12 mm coverslips supported by small sections of 0.25, 0.35 or 0.50 mm diameter nylon fishing line. All specimens were studied using an Olympus BH-2 compound microscope and illustrated with the aid of a drawing tube. Measurements were taken at the highest possible magnification using an ocular graticule.

The maps were produced with the computer program ArcView 3.2 after the relevant locality data were stored in an Access database. Coordinates were obtained from various sources, including the Geoscience Australia Place Names Search website (http://www.ga.gov.au/maps/names) and the GeoNet Names Server (http://earth-info.nga.mil/gns/html/) produced by the National Geospatial-Intelligence Agency. Recently collected specimens were usually provided with GPS coordinates taken at the collecting site. Coordinates obtained from indirect sources (such as gazetteers) are listed below within parentheses.

FAMILY CHERNETIDAE MENGE 1855
SUBFAMILY CHERNETINAE MENGE 1855
Genus *Sundochernes* Beier 1932

Sundochernes Beier 1932:162; Beier 1933:531; Beier 1976:225; Harvey 1991:635.

Type species.—*Chelifer modiglianii* Ellingsen 1911, by original designation.

Type material.—*Sundochernes modiglianii* (Ellingsen 1911): Syntypes: 1 male, 7 females, 1 nymph, Sirambas (as Si-Rambé), Sumatra, Indonesia [0°49′N, 99°32′E], no date, E. Modigliani (MCSNG), examined.

Sundochernes australiensis Beier 1954b: Holotype female, Denmark, near mouth of Denmark River, Western Australia, Australia [34°58′N, 117°22′E], karri [*Eucalyptus diversicolor*] forest, 26 January 1952, T. Gislén (ZMLU), not examined.

Sundochernes brasiliensis Beier 1974a: Holotype male, Nova Teutônia, Santa Catarina, Brazil [27°03′S, 52°24′W], 300–500 m, F. Plaumann (MHNG), examined.

Sundochernes dubius Beier 1954b: Holotype female, Augusta, Western Australia, Australia [34°19′S, 115°09′E], 12 December 1952, T. Gislén (ZMLU), not examined.

Sundochernes gressitti Beier 1957: Holotype female, Ngaremeskang, Babelthuap, Palau [07°31′N, 134°33′E], 30 m, 21 December 1952, J. L. Gressitt (USNM 2262), examined. Paratype: 1 female, Ngercheu Islands [as Garakayo Island], "Pelew" Islands [= Palau] [07°05′N, 134°16′E], 8 August 1945, H. S. Dybas (FMNH), examined.

Sundochernes malayanus Beier 1963: Holotype male, Rantau Panjang, 5 mi N of Klang, Selangor, Malayasia [03°25′N, 101°28′E], from nest of Olive Bulbul, *Microscelis olivacea*, 28 June 1961 (BPBM), examined. Paratype: 1 tritonymph, same data as holotype, from nest of Yellow-vented Bulbul, *Pycnonotus goiavier*, 7 June 1961 (BPBM), examined.

Sundochernes queenslandicus Beier 1975: Holotype male, Marburg, Queensland, Australia [27°34′S, 152°35′E], litter and soil, 16 May 1966, K. E. Lee (SAM N197761), examined.

Diagnosis.—*Sundochernes* differs from all other chernetid genera by the following combination of characters: flagellum with 3 blades; spermathecae with 2 thickened tubes with rounded terminal bulbs; legs without tactile setae; 1 pair of eyespots present; vestitural setae generally small, dentate and clavate.

Remarks.—Beier (1932, 1933) recorded three blades in the cheliceral flagellum in *Chelifer modiglianii*, and accordingly placed his new genus *Sundochernes* in the tribe Chernetini which was based primarily upon the number of cheliceral blades. Descriptions of species subsequently attributed to *Sundochernes* have either reiterated the possession of three cheliceral blades or have omitted flagellal blade counts.

MSH examined the syntypes of *C. modiglianii* during 1986 while identifying Indonesian specimens of pseudoscorpions (Harvey 1988), and made observations on the chelicerae and female genitalia. Drawings made at the time have been subsequently mislaid, and the syntypes have not been available to us again, precluding the provision of illustrations of generically important features. Nevertheless, notes made at the time of study indicate that the cheliceral flagellum consists of 3 blades, as stated by Beier (1932, 1933), and that the female genitalic region consists of spermathecae with 2 thickened tubes with rounded terminal bulbs.

Examination of the type or other material of most *Sundochernes* species (listed above) indicates that although some species possess three blades (e.g., *S. modiglianii*, *S. australiensis*, *S. dubius*, *S. brasiliensis*, *S. queenslandicus*, *S. gressitti*, and *S. malayanus*), others possess four blades. As noted above, this basic distinction in flagellar blade number has long been used to separate chernetid taxa, commencing with Beier (1932, 1933) who used flagellar number to diagnose the tribes Chernetini and Hesperochernetini within the Chernetinae. Although these tribes are no longer recognized, the number of flagellar setae is still given considerable significance in chernetid taxonomy (e.g., Muchmore 1974). Aside from flagellar blade number, the species with four blades have been found by us to possess fundamentally different spermathecal morphology from that found in *S. modiglianii* which is afforded high value in chernetid taxonomy. Our study suggests that these four species can be referred to two different genera with *S. guanophilus*, *S. dewae*, and *S. novaeguineae* placed in *Troglochernes*, and *S. grayi* in a new genus, here named *Satrapanus*. This makes *Sundochernes* a slightly more coherent

genus but the situation is further complicated by species such as *S. queenslandicus* which is clearly distinct from both *S. modiglianii*, *Troglochernes* and *Satrapanus*, suggesting that a further new genus will be required to accommodate it. The systematic position of the remaining species of *Sundochernes* can only be determined when detailed examination of each species is completed, with particular reference to spermathecal morphology as highlighted for other chernetids by Vachon (1938), Muchmore (1974, 1975) and Mahnert (1978).

Genus *Troglochernes* Beier 1969

Troglochernes Beier 1969:185; Harvey 1991:638.

Type species.—*Troglochernes imitans* Beier 1969, by original designation.

Diagnosis.—*Troglochernes* differs from all other chernetid genera by the following combination of characters: flagellum with 4 blades, or possibly 3 blades in one species; spermathecae with 2 thickened and slightly curved tubes fused basally; legs without tactile setae; carapace unicolored and with two transverse furrows; eyes or eyespots absent; vestitural setae generally small, dentate and clavate.

Description.—*Adults:* Vestitural setae mostly short, slightly curved, and dentate.

Pedipalps: with most surfaces finely to heavily granulate. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria; *esb* closer to *eb* than to *est*; *isb* approximately midway between *it* and *ist*; *it* situated in distal third of fixed finger; *sb* closer to *b* than to *st*. Marginal teeth of chela all closely spaced; both chelal fingers with external and internal rows of accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating midway between *t* and *st*, or adjacent to *st*.

Chelicera: with 6 or 7 setae on hand; *ls* and *is* acuminate, *sbs*, *bs'*, *bs''* and *bs'''* (when present) dentate, *es* either acuminate (most species) or dentate (*T. omorgus*); movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures; lamina exterior present; movable finger with 1 dorsal tooth; galea long and slender with 5–6 rami; flagellum composed of 4 blades, or possibly 3 blades in one species; two anterior blades dentate along distal anterior half, two shorter blades smooth, or in case of species with 3 blades, anterior blade dentate and others smooth.

Cephalothorax: carapace with eyes or eyespots absent; unicolored; with two transverse furrows; posterior margin straight or nearly so. Median maxillary lyrifissure present and sub-medially situated; posterior maxillary lyrifissure present.

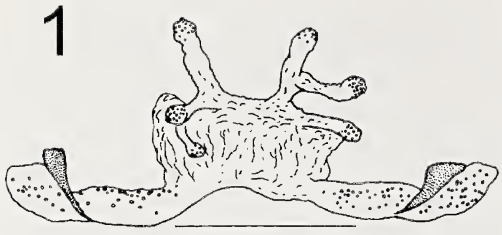
Abdomen: tergites and sternites generally divided. Pleural membrane wrinkled striate for entire length, without setae, but females of two species with setae. Each stigmatic sclerite with 1 or more setae. Spiracles simple, with spiracular helix.

Genitalia: male genitalia of typical chernetid form; female spermathecae with 2 thickened and curved tubes fused basally.

Legs: legs I and II with an oblique junction between femur and patella; legs III and IV without tactile setae on tibiae or tarsi; metatarsus and tarsus fused into single segment (tarsus); tarsi with single raised slit sensillum; subterminal tarsal seta curved and acuminate; tarsal claws simple; arolium slightly shorter than claws.

Nymphs: Much like adults, but trichobothrial patterns as follows: tritonymph with 7 on fixed finger and 3 on movable finger; deutonymph with 6 on fixed finger and 2 on movable finger; and protonymph with 3 on fixed finger and 1 on movable finger. Chelicera of protonymph lacking seta *gs*. Tarsi of protonymph without single raised slit sensillum.

Remarks.—Beier (1969) proposed the new genus *Troglochernes* for *T. imitans*, a highly troglomorphic species from caves on the Nullarbor Plain, Western Australia. He treated the genus as a member of the Hesperochernetini as it possessed four flagellal blades, and distinguished it from most other genera by the elongate pedipalps and legs, and lack of a tactile seta on the posterior tarsi. Our examination of the female spermathecae of *T. imitans* (Fig. 14) reveals a form unlike that documented for any other Australasian chernetid genus and we consider the genus distinct from all other previously described chernetid genera on the basis of this character. Other Australasian species with extremely similar spermathecae have been detected, and despite the lack of extreme troglomorphisms, we include them here in *Troglochernes* and extend the diagnosis of the genus to include less highly troglomorphic species than *T. imitans*. Three of these species which are here transferred to *Troglochernes* were previously placed in the



Figures 1, 2.—Spermathecae, ventral: 1. *Nesochernes gracilis norfolkensis* Beier, female from Norfolk Island, Australia (WAM T68620); 2. *Paraustrochernes victorianus* Beier, female from Cumberland Falls, Victoria, Australia (WAM T66536). Scale lines = 0.1 mm.

genus *Sundochernes* by Beier (1965, 1967b), but we cannot agree with this placement as they are clearly not congeneric with the type species, *S. modiglianii* (Ellingsen). Some other species currently included within *Sundochernes* are not congeneric with *S. modiglianii*, and we discuss these problems in more detail under that genus. In addition, we have found that all species here attributed to *Troglochernes* lack eye-spots, a feature that further distinguishes it from *Sundochernes*.

Species of *Troglochernes* differ from the other Australasian chernetid genera with four blades in the flagellum as follows: from *Austrochernes* Beier 1932 by the lack of tactile setae on tarsus IV [present in *Austrochernes*, see With (1905) and Beier (1932)]; from *Paraustrochernes* Beier 1966 by the unicolored carapace [bicolored metazone in *Paraustrochernes*; see Beier (1966)], and the presence of only a single pair of spermathecae in the female genitalia [two pairs of spermathecae in *P. victorianus* (Fig. 2)]; and from *Maracherne*s Harvey 1992a by the general shape of the chelal hand (which is not much wider than the base of the fingers in *Maracherne*s) and the lack of an internobasal mound bearing accessory teeth on the male movable chelal finger (Harvey 1992a, 1994). It differs from *Satrapanus* by the lack of eye-spots, which are present in *Satrapanus*, by the morphology of the female genitalia in which the spermathecae are usually lightly curved, and by the color of the carapace which is uniformly unicolored in *Troglochernes*, but is distinctly bicolored in *Satrapanus*, with the metazone paler than the remaining carapace.

Apart from these Australian genera, only 12 other genera, mostly from the northern hemisphere, have been reported as lacking tactile setae on the posterior tarsi and possessing four blades in the flagellum [Muchmore (1974) reported that species of *Chernes* mostly have a four-bladed flagellum, but Dr. V. Mahnert (in litt.) informs me that three-bladed specimens are equally abundant]. *Troglochernes* differs from these genera as follows:

The spermathecal morphology of two thickened and curved tubes that are fused basally segregates *Troglochernes* from *Chelodamus* R.V. Chamberlin 1925 (from Central America), *Chernes* Menge 1855 (from Europe, North Africa, Asia and North America), *Hesperochernes* Chamberlin 1924 (from North America and Japan), *Chelanops* Gervais 1849 (from South America), *Semeiochernes* Beier 1932 (from South America), and *Illinichernes* Hoff 1949 (from North America), which all possess long, slender spermathecae (Benedict & Malcolm 1982; Chamberlin 1952; Mahnert 1978, 1987; Muchmore 1974, 1975, 1984, 1999), and from *Gigantochernes* Beier 1932 (from South America) and *Cocinachernes* Hentschel & Muchmore 1989 (from Mexico), which have four (*Cocinachernes*) or apparently five (*Gigantochernes*) short spermathecal tubes (Hentschel & Muchmore 1989; Vitali-di Castri 1972).

The spermathecal morphology of *Atherochernes* Beier 1954a (from Venezuela), *Eumecochernes* Beier 1932 (from Hawaii) and *Nesochernes* Beier 1932 (from New Zealand and Norfolk Island) are unknown but each can be readily separated from *Troglochernes*. *Atherochernes* differs by the presence of 5 setae on the cheliceral hand (6 or more setae in *Troglochernes*), and by the presence of accessory teeth only on the movable chelal finger (accessory teeth on both chelal fingers in *Troglochernes*) (Beier 1954a). *Eumecochernes* has

trichobothrium *isb* situated basally to *est* (Beier 1932), whereas it is situated opposite or slightly distal to *est* in *Troglochernes*. Specimens of *Nesochernes gracilis norfolkensis* have spermathecae with three pairs of ducts each distally with small pores (Fig. 1); this arrangement is quite different to that of *Troglochernes*.

While most species of *Ceriochernes* Beier 1937, including the type species, *C. detritus* Beier 1937 from the Philippines, *C. foliaceosetosus* Beier 1974a from Brazil and *C. vestitus* Beier 1974b from Nepal and Pakistan, possess three flagellar blades (Beier 1937, 1974b, 1974a; Dashdamirov 2005), the Brazilian species *C. amazonicus* Mahnert 1985 possesses four blades (Mahnert 1985). The number of flagellar blades has not been reported for the remaining species currently included in the genus—*C. besucheti* Beier 1973 from Sri Lanka, *C. nepalensis* Beier 1974b and *C. martensi* Beier 1974b from Nepal, and *C. brasiliensis* Beier 1974a from Brazil (Beier 1973, 1974b, 1974a). Lack of knowledge of the morphology of the spermathecae for most species of *Ceriochernes* is severely hampering our understanding of this widespread and undoubtedly paraphyletic genus (Dashdamirov 2005). The sole member of *Ceriochernes* that has four flagellar blades and lacks tactile setae on the posterior tarsi, *C. amazonicus*, has highly unusual spermathecae in which there are numerous spermathecal bulbs, each circular on long thin stalks, leading from a central atrium (Mahnert 1985).

Ecology.—Although habitat preferences are unknown for *T. novaeguineae*, the remaining five species of *Troglochernes* occur in caves or are intimately associated with other animals. *Troglochernes guanophilus*, *T. cruciatus* and the highly troglomorphic *T. imitans* are known from caves where they inhabit guano deposits or reside under nearby rocks or leaf litter lying on the floor of the cave. *Troglochernes dewae* has been collected solely from bird nests, including that of the Galah (*Cacatua roseicapilla*), Sulphur-Crested Cockatoo (*C. galerita*), Carnaby's Cockatoo (*Calyptorhynchus latirostris*) and Rufous Treecreeper (*Climacteris rufa*). The sole specimen of *T. omorgus* found attached phoretically to the beetle *Omorgus costatus* (Trogidae), individuals of which are known to occur in caves where they live and breed in bat guano (Scholtz 1986).

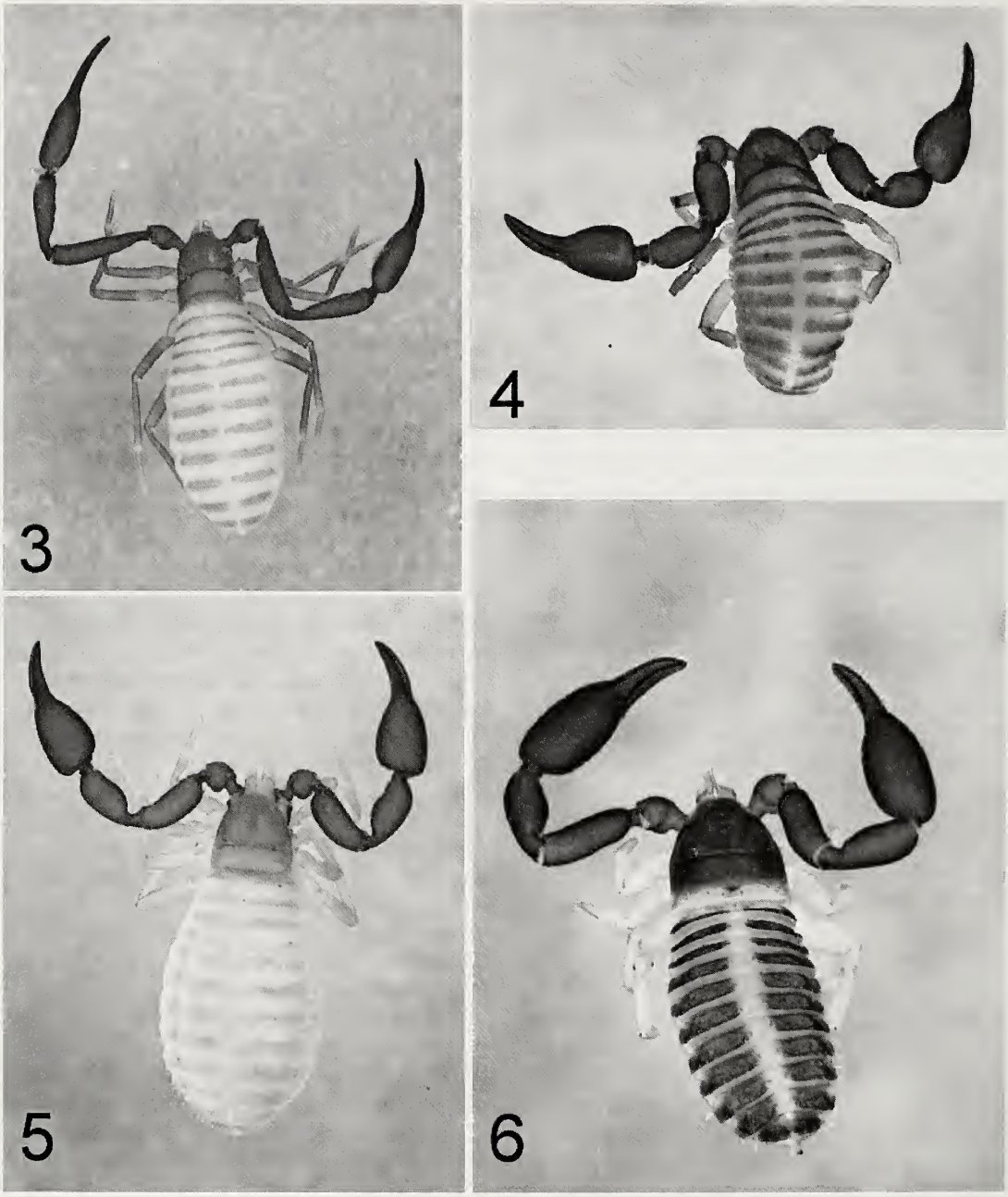
KEY TO SPECIES OF *TROGLOCHERNES*

1. Large species with long, slender pedipalps, e.g., chela (with pedicel) 2.036–2.408 (♂), 1.992–2.528 (♀) mm long and 4.98–5.39 (♂), 4.58–5.49 (♀) times longer than wide .... *Troglochernes imitans*
   Small species with short, robust pedipalps, e.g., chela (with pedicel) 1.00–1.34 (♂), 1.05–1.61 (♀) mm long and 2.50–3.00 (♂), 2.45–2.99 (♀) times longer than wide 2
2. Posterior margin of carapace with 25 setae; tergites generally with more than 30 setae ... *Troglochernes omorgus*
   Posterior margin of carapace with less than 20 setae; tergites generally with less than 30 setae 3
3. Cheliceral seta *es* dentate; posterior margin of carapace with 16–20 setae ..... *Troglochernes dewae*
   Cheliceral seta *es* acuminate; posterior margin of carapace with 8–16 setae 4
4. Posterior margin of carapace with 8 setae ..... *Troglochernes novaeguineae*
   Posterior margin of carapace with 10 or more setae 5
5. Posterior margin of carapace with 10–12 setae ..... *Troglochernes guanophilus*
   Posterior margin of carapace with 14–16 setae ..... *Troglochernes cruciatus*

Troglochernes imitans Beier 1969
Figs. 3, 7–14, 71

Troglochernes imitans Beier 1969:185–187, fig. 1; Richards 1971:19, 24, 25, 27, 28, 30, 43; Beier 1975:203; Harvey 1981:247; Harvey 1985:136; Harvey 1991:638; Moulds 2004:12.

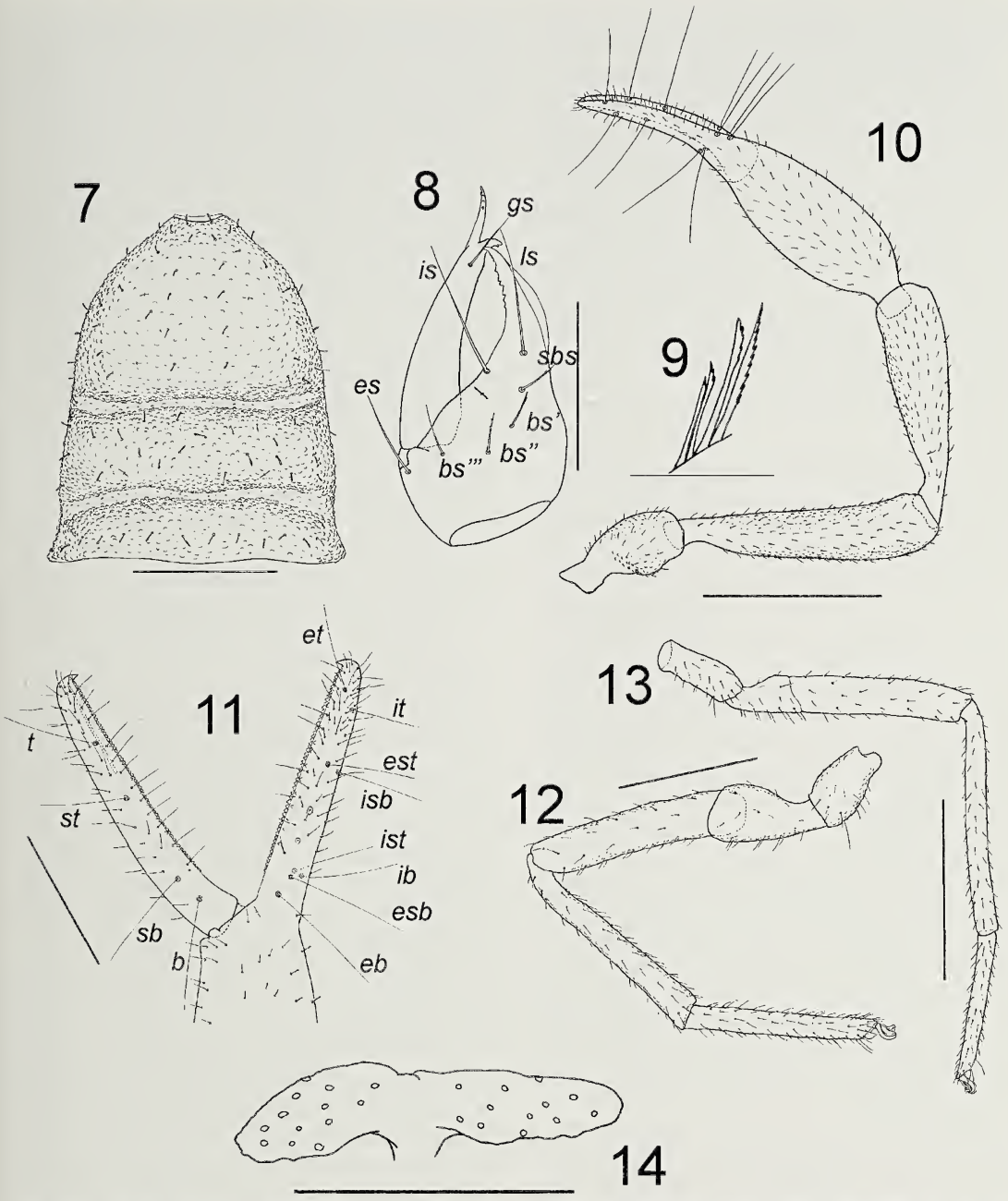
Type material examined.—AUSTRALIA: *Western Australia:* Holotype male, Dingo Cave [6N-160], Nullarbor Plain, Western Australia, Australia [31°51′S, 126°44′E], near entrance, 28 October 1968, J. Lowry (SAM N1980192). Allotype female, same data as ho-



Figures 3–6.—3. *Troglochernes imitans* Beier, male from Scudd Cave, Western Australia (WAM 98/1508); 4. *Troglochernes dewae* (Beier), female from Gingin Shire, Western Australia (WAM T48341); 5. *Troglochernes cruciatus*, sp. nov., female paratype from Rope Ladder Cave, Queensland (WAM T68621); 6. *Satrapanus grayi* (Beier), female from Lord Howe Island (AM).

lotype (SAM N1980193). Paratypes: 2 females, same data as holotype (NHMW).

Other material examined.—AUSTRALIA: *Western Australia:* 1 ♀, Murra-El-Elevyn Cave [6N-47] [32°02′S, 126°02′E], on dry guano, 21 April 1973, K. Williamson (WAM 74/361); 1 ♀, Murra-El-Elevyn Cave [6N-47] [32°02′S, 126°02′E], under mineral crusts, 21 April 1973, P.J. Bridge (WAM 74/362); 1 ♂, Tiggas Lair, 21.9 km E of Nurina [ca. 30°59′S, 126°53′E], 1 December 1985, C.E. Brown, S.J. Elliott, T.A. Smith (WAM 98/1515); 2 ♂,



Figures 7–14.—*Troglochernes imitans* Beier, female from Murra-El-Elevyn Cave, Western Australia (WAM 74/361), unless stated otherwise: 7. Carapace; 8. Left chelicera; 9. Flagellum; 10. Right pedipalp, dorsal (note that *est* is absent); 11. Left chelal fingers, lateral; 12. Left leg I; 13. Left leg IV; 14. Spermathecae, ventral, female from Scudd Cave (WAM 98/1511). Scale lines = 1.00 mm (Figs. 10, 13), 0.50 mm (Figs. 7, 11, 12), 0.20 mm (Fig. 8), 0.1 mm (9, 14). See Methods for abbreviations.

2 ♀, Scudd Cave [N-520], Kybo Station [31°10′S, 126°29′E], 13 April 1989, A. Baynes (WAM 98/1511–1514); 3 ♂, Phyllistine Flattener Cave, 6N-194 [31°32′54″S, 127°18′40″E], Madura Station, under rocks, dark zone, 6 January 1997, N. Poulter (WAM 98/1508–1510).

Diagnosis.—*Troglochernes imitans* differs

from all other species of the genus by the extremely elongate pedipalps and legs.

Description.—*Adults:* Pedipalps and carapace dark reddish-brown; legs reddish-brown; abdomen pale yellow-brown in color. Vestitural setae short, slightly curved, and dentate; most sternal setae acicular with few dentate.

Pedipalps (Fig. 10): all segments extremely elongate, with trochanter 1.95–2.20 (♂), 1.94–2.44 (♀), femur 4.89–5.27 (♂), 4.58–5.23 (♀), patella 3.85–4.16 (♂), 3.90–4.28 (♀), chela (with pedicel) 4.98–5.39 (♂), 4.58–5.49 (♀), chela (without pedicel) 4.66–5.09 (♂), 4.30–4.53 (♀), hand 2.37–2.91 (♂), 2.19–2.24 (♀) times longer than wide; movable finger 0.84–1.04 (♂), 1.00–1.18 (♀) times as long as hand. Surfaces of trochanter and femur moderately granulate, of patella and chelal hand finely granulate, chelal fingers smooth. Fixed finger with ca. 68 (♂), 67 (♀) marginal teeth, plus 12 (♂), 13 (♀) external accessory teeth and 5 (♂, ♀) internal accessory teeth; movable finger with ca. 75 (♂), 72 (♀), marginal teeth, plus 11 (♂), 10 (♀) external accessory teeth and 6 (♂), 5 (♀) internal accessory teeth. Pedipalpal setae generally slender and clavate-dentate, except on fingers where they are acuminate. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 11); *esb* closer to *eb* than to *est*; *est* closer to *et* than to *esb*; *isb* inserted dorsally, rather than internally or externally, and closer to *it* than to *ist*; *ist* closer to *ib* than to *isb*; *est* absent from the right pedipalp of 1 female (Fig. 10); *sb* closer to *b* than to *st*; *st* closer to *t* than to *sb*. Venom apparatus present in movable finger with nodus ramosus terminating midway between *t* and *st* (Fig. 11). External margin of fixed chelal finger with 2–3 "sense spots."

Chelicera (Fig. 8): with 6–7 setae on hand; *ls, is* and *es* acuminate, *sbs, bs′, bs″* and *bs‴* (when present) dentate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures. Movable finger with single dorsal tooth. Galea long and slender with 3 distal and 2 sub-distal to medial rami. Flagellum (Fig. 9) composed of 4 blades; each blade dentate along the distal anterior half. Serrula exterior with 20 (♂), 18 (♀) lamellae.

Cephalothorax: carapace (Fig. 7) 1.11–1.25 (♂), 1.07–1.18 (♀) times as long as broad; unicolored; eyes absent; with 6 (♂, ♀) setae on anterior margin, with 10–11 (♂, ♀) setae on posterior margin; posterior half with two moderately incised transverse furrows, anterior furrow crosses the carapace at ca. 0.55 of its length, posterior furrow crosses at ca. 0.80 of carapace length; entirely granulate with exception of transverse furrows; posterior margin gently undulate. Manducatory process with 1 long distal and 1 long sub-distal seta, with sub-oral seta; remainder of maxilla with 40 (♂), 42 (♀) setae. Chaetotaxy of coxae I–IV: 13: 16: 20: ca. 45 (♂), 13: 14: 21: ca. 55 (♀).

Abdomen: tergites I–X and sternites IV–X divided. Tergal chaetotaxy: ♂, 16: 14: 14: 16: 19: 23: 21: 22: 22: 18: 22: 2; ♀, 12: 13: 14: 18: 19: 18: 22: 21: 21: 17: 18: 2; setae usually restricted to posterior and lateral tergal margins. Sternal chaetotaxy: ♂, ca. 110: (3) 41 [12] (3): (4) 12 (4): 16: 16: 16: 17: 15: 18: 14: 2; ♀, ca. 60: (3) 15 (3): (4) 9 (4): 17: 15: 16: 16: 15: 16: 13: 2; posterior segments without tactile setae. Pleural membrane wrinkled and somewhat longitudinally striate for entire length, without setae.

Male genital opercula with numerous setae that are long and curved; anterior operculum with one pair of large slit sensilla; posterior operculum with 8 smaller sensilla. Female genital opercula: anterior operculum with numerous setae and 2 slit sensilla. Male genitalia of typical chernetid form (Vachon 1938). Female spermathecae with 2 thickened, slightly curved, and laterally directed tubes fusing near the genital operculum (Fig. 14).

Legs: legs I and II with an oblique junction between femur and patella (Fig. 12). Leg IV (Fig. 13) with femur + patella 6.56 (♂), 6.97 (♀) times longer than wide. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Male from Scudd Cave, Western Australia (WAM 98/1513), with other specimens in parentheses, where appropriate:* Body length 3.49 (3.23–4.50). Pedipalps: trochanter 0.710/0.364 (0.638–0.727/0.292–0.338), femur 1.664/0.320 (1.356–1.566/0.275–0.320), patella 1.421/0.346 (1.181–1.381/0.301–0.340), chela (with pedicel) 2.408/0.447 (2.036–2.384/0.389–0.460), chela (without pedicel) 2.208 (1.952–2.260), hand length 1.150 (0.960–1.189), movable finger length 1.200 (0.995–1.134). Chelicera 0.384/0.211, movable finger length

0.289. Carapace 1.336/1.128 (1.088–1.285/0.946–1.085). Leg I: femur 0.496/0.218, patella 0.830/0.168, tibia 0.928/0.115, tarsus 0.736/0.082. Leg IV: femur + patella 1.429/0.218, tibia 1.352/0.114, tarsus 0.864/0.098.

Female from Scudd Cave, Western Australia (WAM 98/1511), with other specimens in parentheses, where appropriate: Body length 4.74 (3.94–4.25). Pedipalps: trochanter 0.784/0.395 (0.662–0.760/0.290–0.356), femur 1.696/0.351 (1.344–1.580/0.280–0.315), patella 1.474/0.364 (1.202–1.400/0.290–0.329), chela (with pedicel) 2.528/0.525 (1.992–2.390/0.385–0.469), chela (without pedicel) 2.376 (1.872–2.112), hand length 1.184 (0.952–1.049), movable finger length 1.200 (0.947–1.175). Chelicera 0.390/0.185, movable finger length 0.294. Carapace 1.376/1.288 (1.072–1.179/0.952–1.040). Leg I: femur 0.493/0.232, patella 0.864/0.172, tibia 0.910/0.116, tarsus 0.752/0.083. Leg IV: femur + patella 1.523/0.218, tibia 1.342/0.126, tarsus 0.880/0.096.

Remarks.—This highly troglomorphic species differs from all other species of the genus by the elongate pedipalps and legs (Figs. 3, 10, 12, 13). *Troglochernes imitans* has been found in seven caves situated on the western edge of the Nullarbor Plain (Fig. 71): Dingo Cave (6N-160) (Beier 1969; Richards 1971), Cocklebiddy Cave (6N-48, 31°57′S, 125°55′E), Pannikin Plain Cave (6N-49, 32°02′S, 126°11′E), Murra-El-Elevyn Cave (6N-47) (Beier 1975), Tiggas Lair, Scudd Cave (N-520) and Phyllistine Flattener Cave (6N-194) (this study). Accurate habitat data are lacking for most specimens but individuals have been found under stones or mineral crusts, or on dry guano, presumably produced by the Chocolate Wattled Bat, *Chalinolobus morio* (Gray). Richards (1971) recorded *T. imitans* from bat guano and decaying vegetation in the dark zone of Dingo Cave.

Troglochernes guanophilus (Beier 1967) new combination

Figs. 15–23, 71

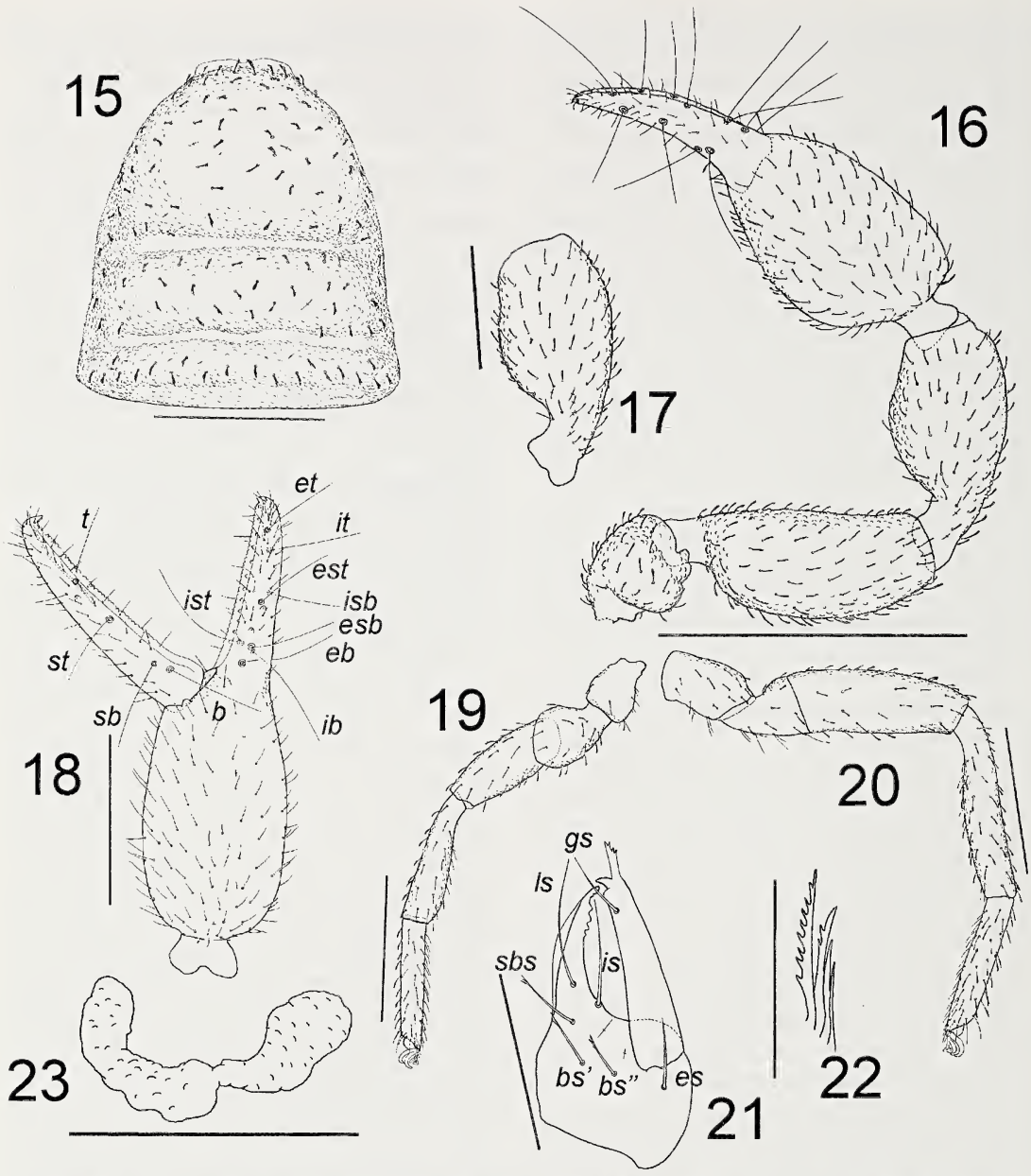
Sundochernes guanophilus Beier 1967b:202–203, fig. 3; Dew 1968:35; Harvey 1981:247; Harvey 1985:136; Harvey 1991:635; Moulds 2004:12.

Type material.—AUSTRALIA: *New South Wales:* Holotype male, Fig Tree Cave [2W-148], near Wombeyan [34°20′S, 149°55′E], in guano, 19 February 1963, B. Dew (SAM N1966163). Allotype female, same data as holotype (SAM N1966164). Paratype: 1 female, same data as holotype (NHMW).

Diagnosis.—*Troglochernes guanophilus* differs from *T. imitans* in the lack of long slender appendages, from *T. cruciatus* in the number of setae on the posterior margin of the carapace (10–12 in *T. guanophilus* and 14–16 in *T. cruciatus*), from *T. dewae* by the morphology of the male pedipalpal patella (expanded internal margin in *T. dewae*, not so expanded in *T. guanophilus*), and from *T. novaeguineae* by the more robust pedipalpal patella (2.2–2.3 times longer than wide in *T. guanophilus*, and 2.62 times in *T. novaeguineae*).

Description.—*Adults:* Pedipalps and carapace dark reddish-brown; abdomen and legs deep red-brown in color. Vestitural setae short, slightly curved, and dentate; most sternal setae acicular with few dentate, especially on posterior sternites.

Pedipalps (Figs. 16, 17): robust, with trochanter 1.63 (♂), 1.83 (♀), femur 2.64 (♂), 2.64 (♀), patella 2.24 (♂), 2.32 (♀), chela (with pedicel) 2.93 (♂), 2.99 (♀), chela (without pedicel) 2.72 (♂), 2.83 (♀), hand 1.40 (♂), 1.41 (♀) times longer than wide; movable finger 0.96 (♂), 1.02 (♀) times as long as hand. Most surfaces of pedipalp finely to heavily granulate with exception of dorsal and ventral surfaces of chelal hand and finger, and entire movable finger. Fixed finger with 36 (♂, ♀) marginal teeth, plus 12 external accessory teeth and 5 internal accessory teeth; movable finger with 36 (♂, ♀) marginal teeth, plus 6 external accessory teeth and 5 internal accessory teeth. Pedipalpal setae clavate-dentate, long and slender, except on fingers where they are acuminate. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 18); *esb* closer to *eb* than to *est*; *est* closer to *esb* than to *et,* which is situated in distal quarter of fixed finger; *isb* slightly closer to *ist* than to *it*; *ist* closer to *ib* than to *isb*; *sb* closer to *b* than to *st*; *st* slightly closer to *t* than to *sb.* Venom apparatus present in movable finger with nodus ramosus terminating midway between *t* and *st* (Fig. 18). External margin of fixed chelal finger with 1 "sense spot," internal margin of fixed chelal finger with 1 "sense spot," and external margin of movable chelal finger with 1 "sense spot."



Figures 15–23.—*Troglochernes guanophilus* (Beier), male holotype (SAM N1966163) and female allotype (SAM N1966164): 15. Carapace, dorsal, male; 16. Right pedipalp, dorsal, male; 17. Right pedipalpal patella, female; 18. Left chela, lateral, male; 19. Left leg I, male; 20. Left leg IV, male; 21. Right chelicera, male; 22. Left flagellum, female; 23. Spermathecae, female. Scale lines = 1.00 mm (Figs. 16–18), 0.50 mm (Figs. 15, 19, 20), 0.20 mm (Fig. 21), 0.10 mm (22, 23). See Methods for abbreviations.

Chelicera (Fig. 21): with 6 setae on hand; *ls, is* and *es* acuminate, *sbs, bs′* and *bs″* dentate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures. Movable finger with single dorsal tooth. Galea long and slender, with 3 small distal and 2 small subdistal rami. Flagellum (Fig. 22) composed of 4 blades; distal blades dentate along the distal anterior half, subdistal blade with 2 serrations, other blades smooth. Serrula exterior with 17 (♂, ♀) lamellae.

Cephalothorax: carapace (Fig. 15): 1.10

(♂), 1.07 (♀) times as long as broad; unicolored; eyes absent; with 6 setae on anterior margin, and 20 setae on posterior margin; posterior half with two moderately incised transverse furrows, anterior furrow crosses the carapace at ca. 0.54 (♂), 0.53 (♀) of its length, posterior furrow crosses at ca. 0.85 (♂, ♀) of carapace length; entirely granulate with exception of transverse furrows; posterior margin straight. Manducatory process with 1 long distal and 1 long sub-distal seta, with sub-oral seta; remainder of maxilla with 28 (♂), 29 (♀) setae. Chaetotaxy of coxae I–IV: 11: 12: 19: 34 (♂), 15: 18: 19: 47 (♀).

Abdomen: tergites I–X and sternites IV–X divided, tergite XI and sternite XI partially divided. Tergal chaetotaxy: holotype ♂, 22: 22: 19: 21: 25: 31: 30: 26: 29: 25: 18: 2, allotype ♀, 25: 24: 21: 25: 30: 31: 30: 28: 30: 24: 16: 2; setae usually restricted to posterior and lateral tergal margins; without abdominal tactile setae. Sternal chaetotaxy: holotype ♂, 79: (2) 27 [4+4] (3): (4) 10 (3): 19: 21: 19: 21: 22: 21: 18: 2, allotype ♀, 55: (3) 14 (3): (1) 9 (1): 16: 20: 21: 21: 22: 25: 14: 2. Pleural membrane wrinkled striate for entire length, without setae.

Male genital anterior operculum with long, curved setae (some reaching the genital opening); one pair of slit sensilla on anterior and posterior opercula, posterior operculum with smaller sensilla. Female genital opercula: anterior operculum with numerous setae and 3 slit sensilla, apparently formed by duplication of sensillum on left side. Male genitalia of typical chernetid form (Vachon 1938). Female spermathecae with 2 thickened and slightly curved tubes fusing near the genital operculum (Fig. 23).

Legs: legs I and II with an oblique junction between femur and patella (Fig. 19). Leg IV (Fig. 20) with femur + patella 4.02 (♂), 4.22 (♀) times longer than wide. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Male holotype (SAM N1966163):* Body length 3.36. Pedipalps: trochanter 0.512/0.314, femur 0.915/0.347, patella 0.830/0.371, chela (with pedicel) 1.424/0.486, chela (without pedicel) 1.322, hand length 0.680, movable finger length 0.656. Chelicera 0.314/0.170, movable finger length 0.218. Carapace 0.944/0.862. Leg I: femur 0.264/0.198, patella 0.424/0.164, tibia 0.468/0.114, tarsus 0.438/0.083. Leg IV: femur + patella 0.848/0.211, tibia 0.669/0.127, tarsus 0.506/0.096.

Female allotype (SAM N1966164): Body length 3.97. Pedipalps: trochanter 0.528/0.289, femur 0.850/0.322, patella 0.829/0.358, chela (with pedicel) 1.456/0.487, chela (without pedicel) 1.379, hand length 0.688, movable finger length 0.704. Chelicera 0.309/0.148, movable finger length 0.223. Carapace 0.952/0.888. Leg I: femur 0.262/0.187, patella 0.429/0.160, tibia 0.461/0.127, tarsus 0.554/0.081. Leg IV: femur + patella 0.866/0.205, tibia 0.668/0.128, tarsus 0.517/0.096.

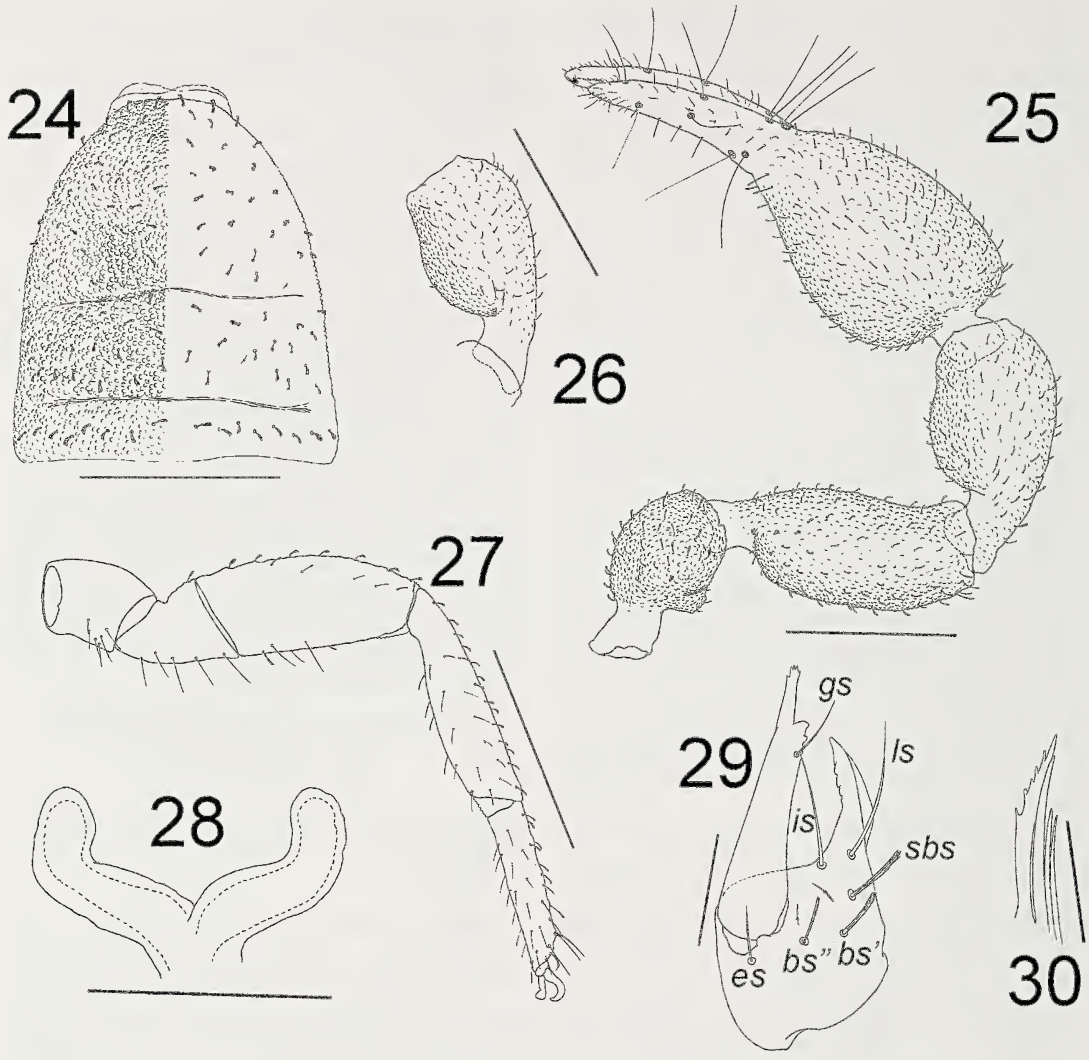
Remarks.—*Sundochernes guanophilus* has four blades in the flagellum and spermathecal morphology that demonstrates that this species cannot be retained in *Sundochernes*, and is here transferred to *Troglochernes*. Thus far, *T. guanophilus* is known only from Fig Tree Cave, located near Wombeyan in south-eastern New South Wales (Fig. 71).

Troglochernes dewae (Beier 1967)
new combination
Figs. 4, 24–33, 71

Sundochernes dewae Beier 1967b:200–202, fig. 2; Harvey 1981:247; Harvey 1985:135; Harvey 1991:635.

Type material.—AUSTRALIA: *New South Wales:* Holotype male, Brewarrina [29°58′S, 146°52′E], from nest of Galah (*Cacatua roseicapilla*) in hollow tree, June 1964, B. Dew (AM KS5867). Paratypes: 1 female, 2 tritonymphs, 2 deutonymphs, 1 protonymph, same data as holotype (AM KS5868); 1 male, 2 female, 1 nymph, same data as holotype (NHMW).

Other material examined.—AUSTRALIA: *Queensland:* 1 ♂, Fringe Dwellers, Iron Range [ca. 12°38′S, 143°05′E], 9 October 1998, nest of *Cacatua galerita* [Sulphur-Crested Cockatoo], S. Legge, R. Heinsohn (WAM T66299); *Western Australia:* 1 ♀, 1 tritonymph, Gingin Shire at 30°59′S, 115°45′E, 20 November 1998, ex *Calyptorhynchus latirostris* nest, nest 84, P. Mawson (WAM T48341); 1 ♂, Shire of Moora at 30°35′S, 116°01′E, 20 November 1998, ex *Cacatua latirostris* nest in healthy hollow of *Eucalyptus salmonophloia*, P. Mawson (WAM T66300); 1 ♂, 1 ♀, 2 tritonymphs, 1 deutonymph, Yilliminning Agricultural Region at



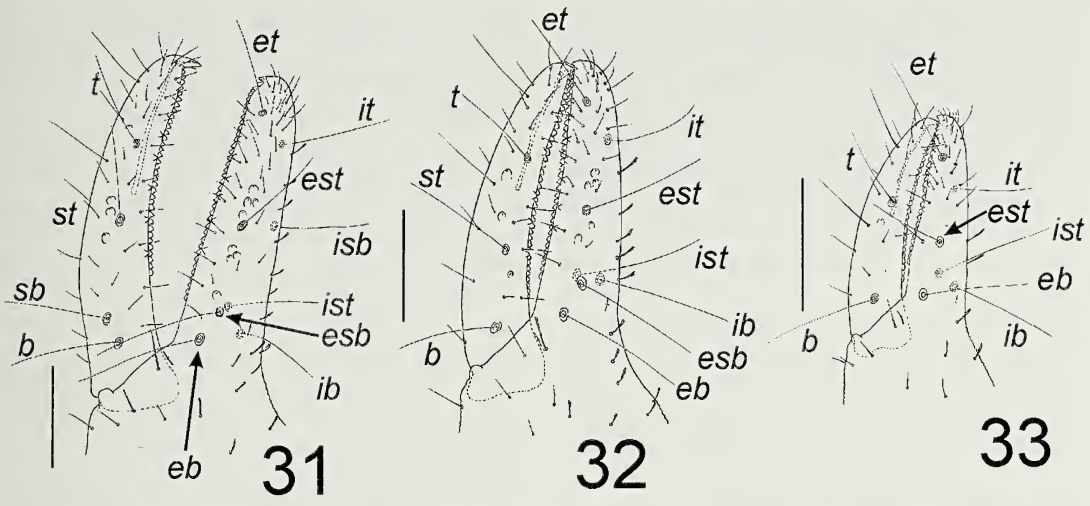
Figures 24–30.—*Troglochernes dewae* (Beier), specimens from Yilliminning Agricultural Region, Western Australia (WAM T66301): 24. Carapace, dorsal, male; 25. Right pedipalp, dorsal, female; 26. Right pedipalpal patella, dorsal, male; 27. Left leg IV, male; 28. Spermathecae, female; 29. Left chelicera, male; 30. Left flagellum, male. Scale lines = 0.50 mm (Figs. 24–27), 0.20 mm (Fig. 29), 0.10 mm (Fig. 28), 0.05 mm (Fig. 30). See Methods for abbreviations.

32°56′S, 117°25′E, 6 March 1999, ex *Climacteris rufa* [Rufous Treecreeper] nest, nest 206, G. Luck (WAM T66301).

Diagnosis.—*Troglochernes dewae* differs from all other members of the genus by the acuminate cheliceral seta *es,* the presence of 16–20 setae on the posterior margin of the carapace, and the strongly convex medial margin of the pedipalpal patella.

Description.—*Adults:* Pedipalps and carapace deep reddish-brown; abdomen and legs light yellowish brown in color (Fig. 4). Vestitural setae short, slightly curved, and dentate; most sternal setae acicular with few dentate.

Pedipalps (Figs. 25, 26): robust, with trochanter 1.48–1.60 (♂), 1.57–1.68 (♀), femur 2.22–2.55 (♂), 2.45–2.55 (♀), patella 2.04–2.14 (♂), 2.10–2.16 (♀), chela (with pedicel) 2.81–2.90 (♂), 2.80–2.85 (♀), chela (without pedicel) 2.67–2.71 (♂), 2.65–2.72 (♀) and hand 1.25–1.41 (♂), 1.30–1.33 (♀) times longer than wide; movable finger 1.08–1.17 (♂), 1.04–1.16 (♀) times longer than hand. All



Figures 31–33.—*Troglochernes dewae* (Beier), specimens from Yilliminning Agricultural Region, Western Australia (WAM T66301): 31. Left chelal fingers, lateral, female; 32. Left chelal fingers, lateral, tritonymph; 33. Left chelal fingers, lateral, deutonymph. Scale lines = 0.20 mm. See Methods for abbreviations.

surfaces of pedipalp finely to heavily granulate with exception of distal half of fixed chelal finger, and entire movable finger. Patella with mesal margin inflated and rounded. Fixed finger with 35 (♂), 36 (♀) marginal teeth, plus 13 (♂), 9 (♀) external accessory teeth and 9 (♂), 8 (♀) internal accessory teeth; movable finger with 41 (♂, ♀) marginal teeth, plus 12 (♂), 8 (♀) teeth external accessory teeth and 6 (♂), 5 (♀) internal accessory teeth. Pedipalpal setae stout and clavate-dentate, except on fingers where they are stout and acuminate on the entire movable finger and all but base of the fixed finger. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 31); *eb* adjacent to *esb*; *est* slightly closer to *esb* than to *et*; *isb* inserted sub-dorsally, rather than internally or externally, and mid-way between *it* and *ist*; *ist* adjacent to *ib*; *sb* adjacent to *b*; *st* slightly closer to *t* than to *sb*. Venom apparatus present in movable finger with nodus ramosus terminating near *st* (Fig. 31). External margin of fixed chelal finger with 8–9 "sense spots," internal margin of fixed chelal finger with 1 "sense spot," and external margin of movable chelal finger with 2 "sense spots."

Chelicera (Fig. 29): with 6 setae on hand; *ls, is* and *es* acuminate, *sbs, bs′* and *bs″* dentate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures. Movable finger with single dorsal tooth. Galea long and slender with 5 small distal rami. Flagellum (Fig. 30) composed of 4 blades; anterior blade dentate along the anterior margin; remaining blades smooth. Serrula exterior with 16 (♂), 18 (♀) lamellae.

Cephalothorax: carapace (Fig. 24): 0.99–1.12 (♂), 0.96–1.06 (♀) times longer than wide; unicolored; eyes absent; with 4–6 (♂), 6 (♀) setae on anterior margin, with 16–20 (♂), 20 (♀) setae on posterior margin; posterior half with two narrow transverse furrows, anterior furrow crosses carapace at ca. 0.52 (♂), 0.58 (♀) of its length, posterior furrow crosses at ca. 0.83 (♂), 0.84 (♀) of carapace length; entirely granulate with exception of transverse furrows; posterior margin straight. Manducatory process with 1 long distal and 1 long sub-distal seta, with sub-oral seta; remainder of maxilla with 31 (♂), 42 (♀) setae. Chaetotaxy of coxae I–IV: 10: 10: 15: 28 (♂), 11: 16: 23: ca. 52 (♀).

Abdomen: tergites I and XI partially divided, tergites II–X (Fig. 4) and sternites IV–X fully divided. Tergal chaetotaxy: ♂, 17: 16: 19: 22: 24: 22: 23: 26: 24: 22: 20: 2; ♀, 18: 20: 19: 24: 25: 27: 28: 26: 28: 26: 22: 2; setae usually restricted to posterior and lateral tergal margins. Sternal chaetotaxy: ♂ ca. 60: (3) 24 [3+3] (3): (3) 7 (3): 13: 16: 16: 15: 16: 16: 14: 2; ♀, 60: (3) 8 (3): (4) 10 (3): 16: 18: 19: 21: 21: 13: 13: 2. Pleural membrane wrinkled plicate for entire length, without setae.

Genital region: male anterior genital operculum with setae long and curved (some reaching genital opening); one pair of slit sensilla on anterior and posterior opercula. Female genital opercula: anterior operculum with ca. 60 setae and 2 slit sensilla. Male genitalia of typical chernetid form (Vachon 1938). Female spermathecae with 2 thickened and curved tubes fusing near the genital operculum (Fig. 28).

Legs: legs I and II with an oblique junction between femur and patella. Leg IV (Fig. 27) with femur + patella 3.54 (♂), 3.13 (♀) times longer than broad. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Male from Yilliminning Agricultural Region, Western Australia (WAM T66301), with other specimens in parentheses, where appropriate:* Body length 2.78 (2.34–2.81). Pedipalps: trochanter 0.499/0.314 (0.424–0.520/0.265–0.352), femur 0.766/0.300 (0.656–0.846/0.295–0.347), patella 0.712/0.333 (0.642–0.780/0.314–0.378), chela (with pedicel) 1.408/0.485 (1.158–1.480/0.403–0.526), chela (without pedicel) 1.314 (1.088–1.406), hand length 0.614 (0.568–0.658), movable finger length 0.720 (0.614–0.768). Chelicera 0.300/0.174, movable finger length 0.214. Carapace 0.893/0.794 (0.800–0.904/0.736–0.912). Leg I: femur 0.268/0.175, patella 0.403/0.157, tibia 0.397/0.111, tarsus 0.365/0.074. Leg IV: femur + patella 0.726/0.205, tibia 0.557/0.125, tarsus 0.403/0.086.

Female from Yilliminning Agricultural Region, Western Australia (WAM T66301), with female from Gin Gin Shire (WAM T48341) in parentheses, where appropriate: Body length 3.05 (2.51). Pedipalps: trochanter 0.538/0.320 (0.511/0.326), femur 0.800/0.327 (0.768/0.301), patella 0.755/0.360 (0.720/0.333), chela (with pedicel) 1.418/0.507 (1.371/0.481), chela (without pedicel) 1.344 (1.307), hand length 0.661 (0.640), movable finger length 0.768 (0.664). Chelicera 0.320/0.159, movable finger length 0.243. Carapace 0.978/0.922 (0.862/0.896). Leg I: femur 0.269/0.192, patella 0.430/0.180, tibia 0.422/0.087, tarsus 0.371/0.083. Leg IV: femur + patella 0.736/0.235, tibia 0.582/0.134, tarsus 0.426/0.096.

Tritonymphs: Morphology generally as in adults. Pedipalps, carapace, pedipalpal coxae and anterior half of coxa I red-brown, remainder of body pale red-yellow.

Chelicera: with 6 setae on hand, *ls, is* and *es* acuminate, *sbs, bs′* and *bs″* dentate; movable finger with 1 sub-distal seta (*gs*). Movable finger with single dorsal tooth. Galea long and slender with 5 small distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 1.90, femur 2.35, patella 1.86, chela (with pedicel) 2.76, chela (without pedicel) 2.58, hand 1.35 times longer than wide, movable finger 0.96 times longer than hand. Fixed chelal finger with 7 trichobothria, movable chelal finger with 3 trichobothria (Fig. 32): *isb* and *sb* absent; *esb* situated near *eb*; *est* slightly closer to *esb* than to *et*; *ist* adjacent to *ib*; *st* closer to *sb* than to *t*, which is situated in distal third of movable finger. Venom apparatus present in movable finger with nodus ramosus terminating basal to *t* (Fig. 32). Chelal teeth: fixed finger with 33 marginal teeth, plus 9 external accessory teeth and 3 internal accessory teeth; movable finger with 37 marginal teeth, plus 7 external accessory teeth and 3 internal accessory teeth. External margin of fixed chelal finger with 3 "sense spots," internal margin of fixed chelal finger with 0 "sense spots," and external margin of movable chelal finger with 1 "sense spot."

Cephalothorax: carapace 1.03 times longer than wide; without eyes; with 4 setae on anterior margin and 15 setae on posterior margin; with two deep transverse furrows.

Abdomen: Tergites I–X and sternites III–X with medial suture line. Tergal chaetotaxy: 14: 16: 17: 20: 18: 19: 19: 20: 18: 17: 17: 2. Sternal chaetotaxy: 15: (1) 14 (1): (2) 6 (2): 10: 13: 14: 15: 15: 14: 15: 2. Pleural membrane uniformly wrinkled plicate, without setae.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Tritonymph from Yilliminning Agricultural Region, Western Australia (WAM T66301):* Body length 2.64. Pedipalps: trochanter 0.376/0.198, femur 0.664/0.282, patella 0.592/0.318, chela (with pedicel) 1.150/0.416, chela (without pedicel)

1.072, hand length 0.563, movable finger length 0.540. Carapace 0.816/0.792.

Deutonymphs: Morphology generally as in adults. Pedipalps and carapace lightly sclerotized, abdomen creamy yellow.

Chelicera: with 5 setae on hand, *ls, is, bs* and *es* acuminate, *sbs* dentate; movable finger with 1 sub-distal seta (*gs*). Movable finger with single dorsal tooth. Galea long and slender with 3 small distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 1.69, femur 2.83, patella 1.92, chela (with pedicel) 2.99, chela (without pedicel) 2.85, hand 1.41 times longer than wide, movable finger 1.02 times longer than hand. Fixed chelal finger with 6 trichobothria, movable chelal finger with 2 trichobothria (Fig. 33): *esb, isb, sb* and *st* absent; *est* closer to *eb* than to *et*; *ist* adjacent to *ib*. Venom apparatus present in movable finger with nodus ramosus terminating basal to *t* (Fig. 33). Chelal teeth: fixed finger with 22 marginal teeth, plus 5 external accessory teeth and 3 internal accessory teeth; movable finger with 28 marginal teeth, plus 3 external accessory teeth and 2 internal accessory teeth. External margin of fixed chelal finger with 1 "sense spot," internal margin of fixed chelal finger with 0 "sense spots," and external margin of movable chelal finger with 1 "sense spot."

Cephalothorax: carapace 1.16 times longer than wide; without eyes; with 4 setae on anterior margin and 10 setae on posterior margin; with two shallow furrows.

Abdomen: Tergites I–X and sternites II–X with medial suture line. Tergal chaetotaxy: 10: 9: 10: 10: 10: 10: 10: 10: 10: 10: 11: 2. Sternal chaetotaxy: 0: (1) 3 (1): (2) 4 (2): 8: 8: 9: 9: 8: 9: 7: 2. Pleural membrane uniformly wrinkled plicate, without setae.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Deutonymph from Yilliminning Agricultural Region, Western Australia (WAM T66301):* Body length 1.66. Pedipalps: trochanter 0.250/0.148, femur 0.438/0.155, patella 0.333/0.173, chela (with pedicel) 0.690/0.231, chela (without pedicel) 0.658, hand length 0.326, movable finger length 0.333. Carapace 0.544/0.467.

Remarks.—As discussed above, the morphology of the spermathecae and the presence of four flagellal blades indicates that this species cannot be retained in the genus *Sundochernes*. *Troglochernes dewae* has been collected solely from bird nests, including that of the Galah (*Cacatua roseicapilla*), Sulphur-Crested Cockatoo (*C. galerita*), Carnaby's Cockatoo (*Calyptorhynchus latirostris*) and Rufous Treecreeper (*Climacteris rufa*). It has been recorded from many different parts of Australia (Fig. 71), making it the most widespread of any member of the genus *Troglochernes*.

Troglochernes novaeguineae (Beier 1965)
new combination
Figs. 34–40, 71

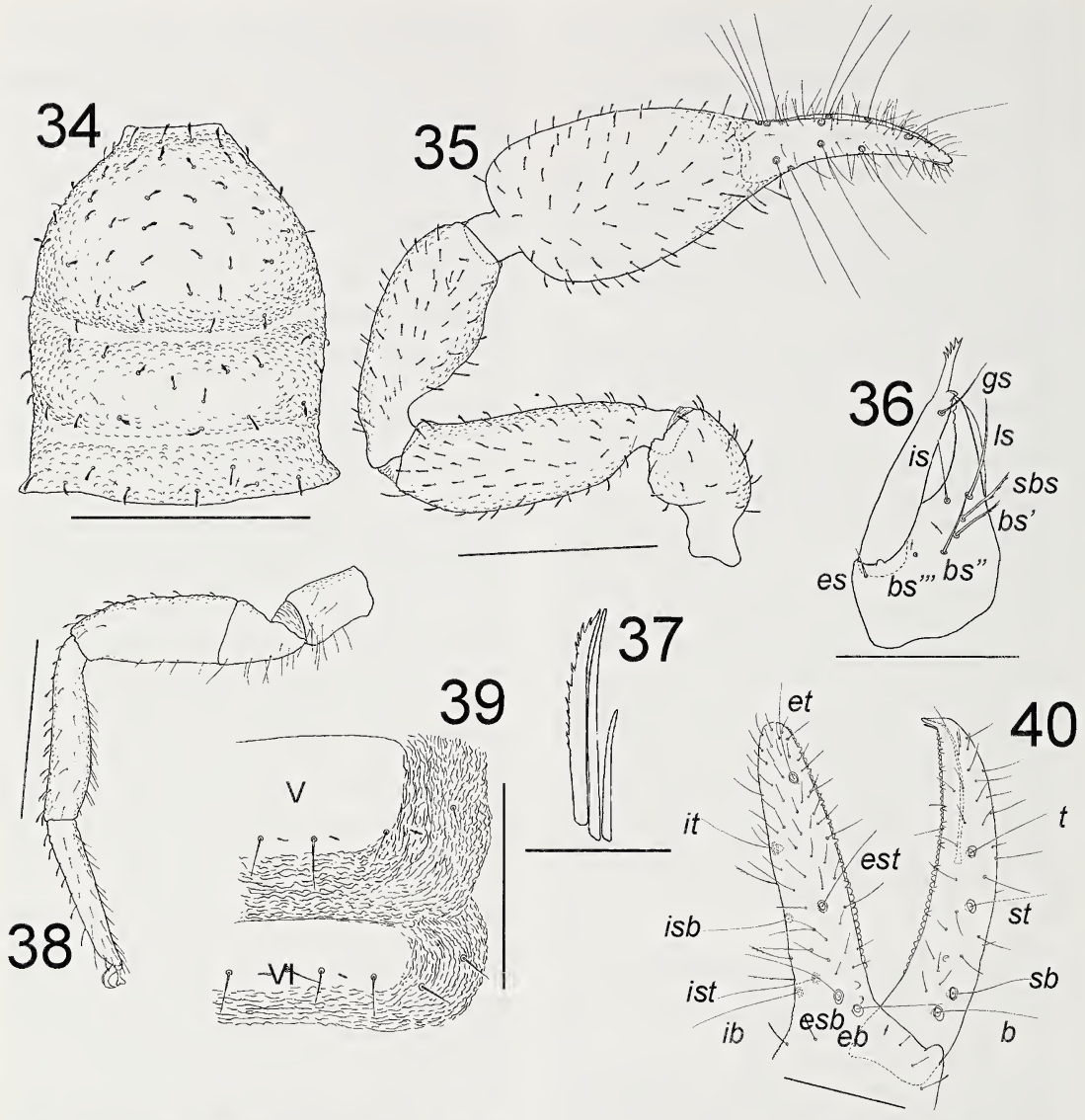
Sundochernes novaeguineae Beier 1965:779–780, fig. 19; Beier 1982:44; Tenorio & Muchmore 1982:381; Harvey 1991:626; Beron 2002:38.

Type material.—PAPUA NEW GUINEA: *Southern Highlands Province:* Holotype female, Mt. Giluwe [6°06′S, 143°54′E], 2550 m, 28 May 1963, Sedlacek (BPBM 6268).

Diagnosis.—*Troglochernes novaeguineae* lacks the long, elongate pedipalps and legs characteristic of *T. imitans*, and differs from all other members of the genus by possessing more slender pedipalpal segments, e.g., female chelal patella 2.50 times longer than wide in *T. novaguineae* and 2.06–2.36 times longer than wide in other females of the genus. It also differs from other species of the genus by the presence of 1 or 2 setae situated within the pleural membrane adjacent to sternites V-IX (see Remarks below).

Description.—*Adult female:* Pedipalps and carapace dark red-brown; abdomen and legs light red-brown in color. Vestitural setae short, curved and strongly dentate; most sternal setae acicular with few dentate.

Pedipalps (Fig. 35): robust and densely setose, with trochanter 1.67, femur 3.27, patella 2.50, chela (with pedicel) 3.04, chela (without pedicel) 2.90, and hand 1.57 times longer than wide; movable finger 1.17 times longer than hand. All surfaces of pedipalp finely to heavily granulate with exception of chelal fingers. Fixed finger with 39 marginal teeth, plus 7 external accessory teeth and 3 internal accessory teeth; movable finger with 43 marginal teeth, plus 9 external accessory teeth and 1 internal accessory tooth. Pedipalpal setae stout, clavate-dentate and curved, except on



Figures 34–40.—*Troglochernes novaeguineae*, sp. nov., female holotype (BPBM 6268): 34. Carapace, dorsal; 35. Left pedipalp, dorsal; 36. Left chelicera; 37. Left flagellum; 38. Left leg IV; 39. Left sternites V–VI; 40. Right chelal fingers, lateral. Scale lines = 0.50 mm (Figs. 34, 35, 38), 0.20 mm (Figs. 36, 39, 40), 0.05 mm (37). See Methods for abbreviations.

fingers where they are stout and acuminate on the entire movable finger and all but base of the fixed finger. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 40); *esb* closer to *eb* than to *est*; *est* closer to *esb* than to *et*; *isb* closer to *ist* than to *it*; *ist* closer to *ib* than to *isb*; *sb* closer to *b* than to *st*; *st* closer to *t* than to *sb*. Venom apparatus present in movable finger with nodus ramosus terminating adjacent to *st* (Fig. 40). External margin of fixed chelal finger with 2 "sense spots," internal margin of fixed chelal finger with 1 "sense spot," and external margin of movable chelal finger with 1 "sense spot."

Chelicera (Fig. 36): with 7 setae on hand; *es, ls* and *is* acuminate, *sbs, bs′* and *bs″* dentate, morphology of *bs‴* unknown (lost from each chelicera); movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures. Movable finger with single dorsal tooth. Galea long and slender with 6 small

distal to sub-distal rami. Flagellum (Fig. 37) apparently composed of 3 blades (see below); anterior blades dentate along the distal anterior half; other blades smooth. Serrula exterior with 17 lamellae.

Cephalothorax: carapace (Fig. 34): 1.26 times as long as broad; unicolored; eyes absent; with 5 setae on anterior margin, with 8 setae on posterior margin; posterior half with two moderately incised transverse furrows, anterior furrow crosses the carapace at ca. 0.54 of its length, posterior furrow crosses at ca. 0.86 of carapace length; entirely granulate with exception of transverse furrows, and parts of metazone; posterior margin slightly curved. Manducatory process with 1 long distal and 1 long sub-distal seta, with sub-oral seta; remainder of maxilla with 22 setae. Chaetotaxy of coxae I–IV: 12: 15: 21: ca. 42.

Abdomen: tergites I–X and sternites V–X divided. Tergal chaetotaxy: 10: 12: 13: 17: 15: 14: 14: 14: 12: 10: 10: 2; setae usually restricted to posterior and lateral tergal margins. Sternal chaetotaxy: 24: (3) 13 (3): (2) 18 (2): 18: 19: 19: 14: 12: ? : ? : 2. Pleural membrane longitudinally striate for entire length, with 1 or 2 setae within the pleural membrane adjacent to sternites V–IX.

Female genital opercula: anterior operculum with numerous setae and 2 slit sensilla. Spermathecae not visible.

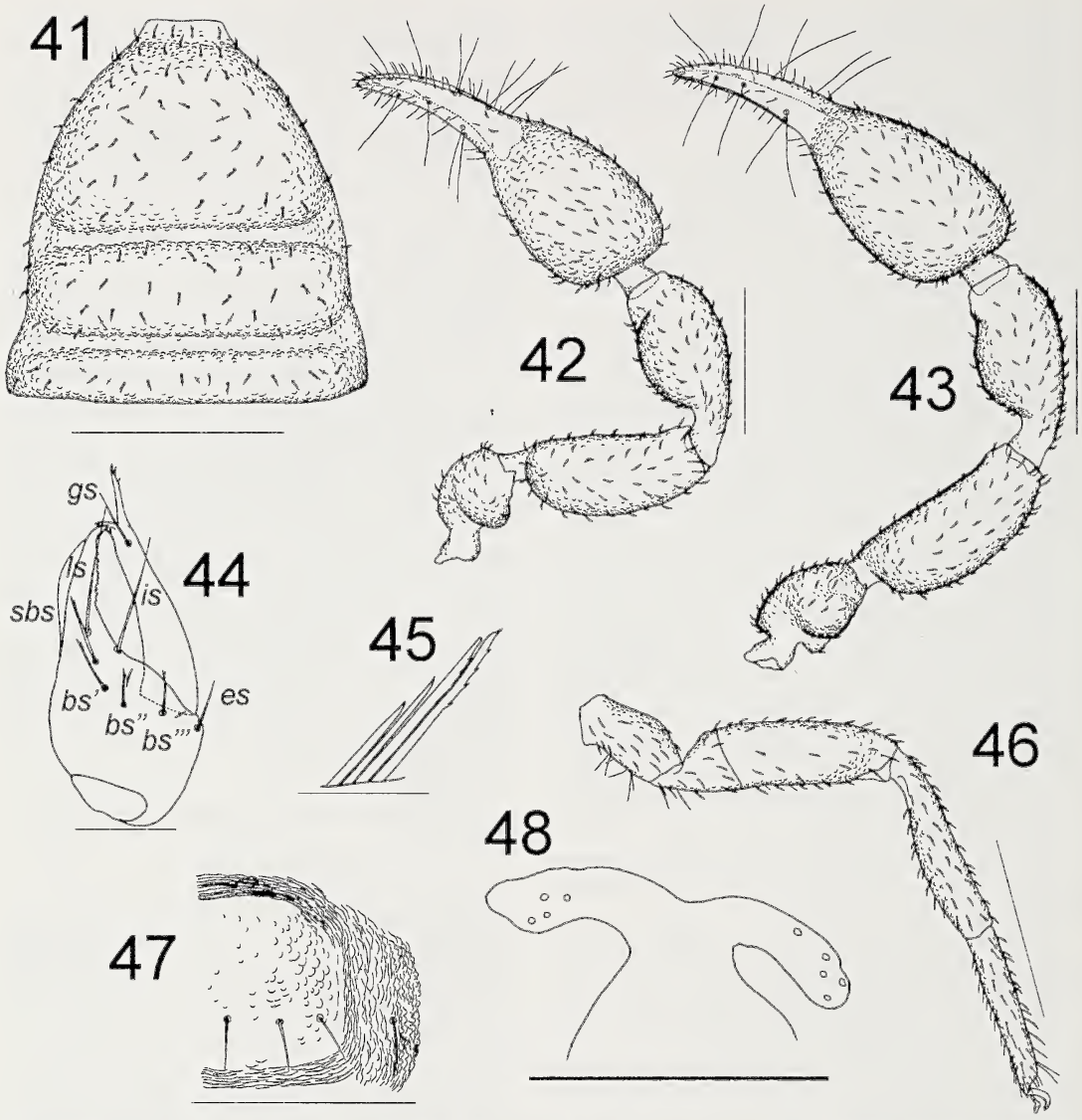
Legs: legs I and II with an oblique junction between femur and patella. Leg IV (Fig. 38) with femur + patella 5.08 times longer than wide. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Female holotype (BPBM 6268):* Body length 2.48. Pedipalps: trochanter 0.406/0.243, femur 0.752/0.230, patella 0.655/0.262, chela (with pedicel) 1.227/0.403, chela (without pedicel) 1.168, hand length 0.634, movable finger length 0.541. Chelicera 0.282/0.154, movable finger length 0.201. Carapace 0.786/0.624. Leg I: femur 0.217/0.144, patella 0.338/0.127, tibia 0.348/0.090, tarsus 0.381/0.067. Leg IV: femur + patella 0.665/0.131, tibia 0.529/0.114, tarsus 0.450/0.084.

Remarks.—Beier (1965) described *Sundochernes novaeguineae* from a single female specimen collected from central Papua New Guinea (Fig. 71) and later recorded *S. novaeguineae* based upon material taken from an unspecified Papuan locality (Beier 1982). Although we were able to only study the female holotype, we found that it possesses several features that suggest it is better placed in *Troglochernes* than in *Sundochernes*. It lacks eye-spots that are found in *Sundochernes*, and bears 1 or 2 setae within the pleural membrane adjacent to sternites V–IX. The only other species of *Troglochernes* with a similar condition is the female of *T. cruciatus* which bears a single seta adjacent to sternite V (Fig. 39). This feature may attest to a common ancestry between these two species which is supported by their geography as *T. novaeguineae* occurs in central Papua New Guinea and *T. cruciatus* in north-eastern Australia (Fig. 71). The permanent slide preparation of this specimen has obscured the morphology of the spermathecae, and the chelicerae are poorly positioned so that the number of flagellar blades is difficult to ascertain with any confidence. Our examination suggests that only three blades are present (Fig. 37) but additional specimens should be examined to obtain an accurate count.

Troglochernes cruciatus Volschenk
new species
Figs. 5, 41–52, 71

Type material.—AUSTRALIA: *Queensland:* Holotype female, back of Rope Ladder Cave, in dark zone, Fanning River Station, 19°45′S, 146°28′E, under rocks and in bat guano, 18 March 1995, E.S. Volschenk, D. Slaney (MTQ S105893). Paratypes: 10 males, 9 females, 8 tritonymphs, 6 deutonymphs, 2 protonymphs, collected with holotype (MTQ S105894–S105928); 12 males, 11 females, 10 tritonymphs, 8 deutonymphs, 2 protonymphs, collected with holotype (QM S74360–74402); 8 males, 10 females, 7 tritonymphs, 2 deutonymphs, 1 protonymph, collected with holotype (WAM T75430–75451); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (SAM PS1363–1367); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (AM KS96109–96113); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (NMV K-9870–9874); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (ANIC); 1 male, 1 fe-

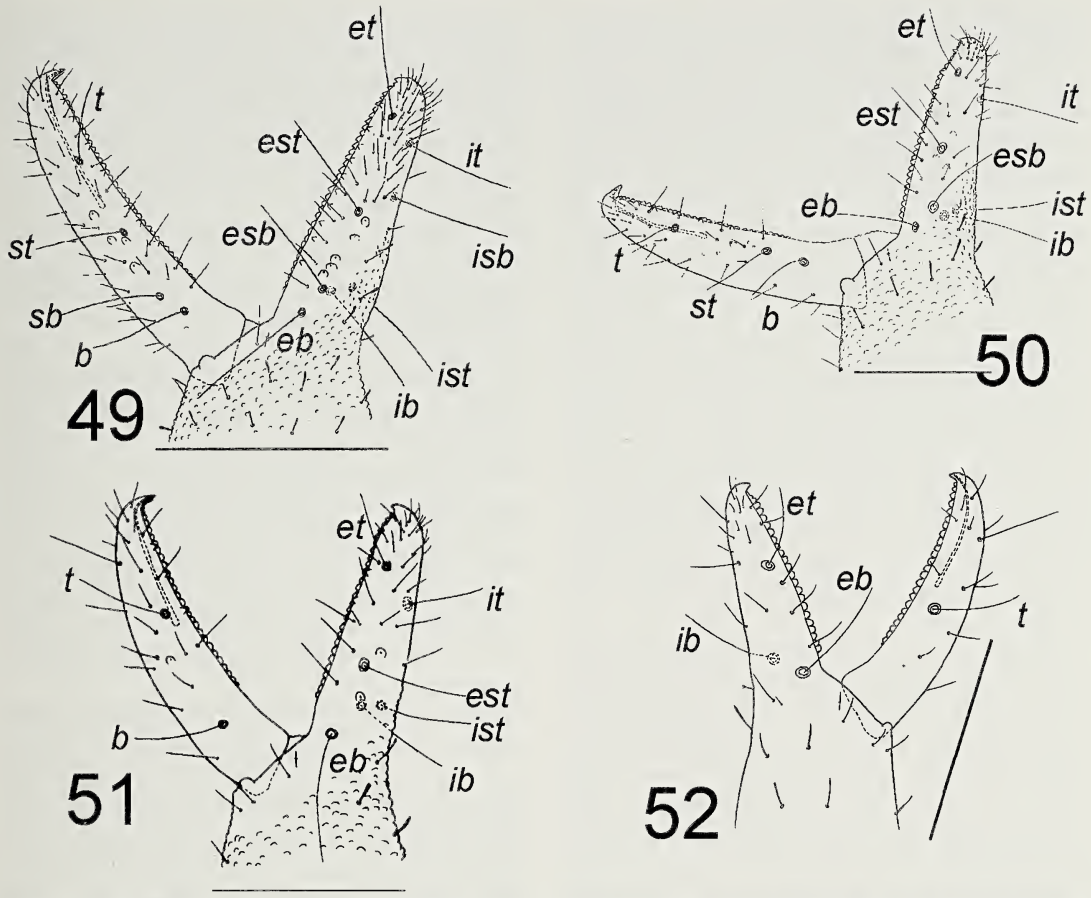


Figures 41–48.—*Troglochernes cruciatus*, sp. nov., female holotype (MTQ) unless stated otherwise: 41. Carapace; 42. Right pedipalp, dorsal; 43. Right pedipalp, dorsal male paratype (MTQ); 44. Right chelicera; 45. Left flagellum; 46. Left leg IV; 47. Left sternite V; 48. Spermathecae. Scale lines = 0.50 mm (Figs. 41–43, 46), 0.20 mm (Fig. 47), 0.10 mm (Figs. 44, 48), 0.05 mm (Fig. 45). See Methods for abbreviations.

male, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (AMNH); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (BMNH); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (MNHN); 1 male, 1 female, 1 tritonymph, 1 deutonymph, 1 protonymph, collected with holotype (MHNG); 1 male, 6 females, Rope Ladder Cave, 22 August 1993, P. Weinstein (WAM T68621).

Etymology.—The specific epithet *cruciatus*, is Latin for torture or torment, and is used in reference to the junior authors' honors year (1995), during which this species was discovered and studied.

Diagnosis.—*Troglochernes cruciatus* differs from *T. imitans* in the lack of long slender



Figures 49–52.—*Troglochernes cruciatus*, sp. nov., chelal fingers: 49. Left chelal fingers, lateral, female holotype (MTQ); 50. Left chelal fingers, lateral, tritonymph paratype (MTQ); 51. Left chelal fingers, lateral, deutonymph paratype (MTQ); 52. Right chelal fingers, lateral, protonymph paratype (MTQ). Scale lines = 0.50 mm (Fig. 52), 0.40 mm (Fig. 49), 0.20 mm (Figs. 50, 51). See Methods for abbreviations.

appendages, from *T. guanophilus*, *T. dewae* and *T. omorgus* in the number of setae on the posterior margin of the carapace (14–16 in *T. cruciatus* and 10–12 in *T. guanophilus*, 10 in *T. dewae* and 25 in *T. omorgus*), and from *T. novaeguineae* by the more robust pedipalpal patella (2.06–2.36 times longer than wide in female *T. cruciatus*, and 2.62 in *T. novaeguineae*). Females differ from all other species by the presence of a seta situated within the pleural membrane adjacent to sternite V; other species either lack such a seta (*T. imitans*, *T. guanophilus*, *T. dewae* and *T. omorgus*) or possess 1 or 2 similar setae adjacent to sternites V-IX (*T. novaeguineae*).

Description.—*Adults:* Pedipalps and carapace reddish-brown; abdomen and legs light yellowish brown in color. Vestitural setae short, slightly curved, and dentate; most sternal setae acicular with few dentate.

Pedipalps (Figs. 42, 43): robust, with trochanter 1.48–1.79 (♂), 1.41–1.81 (♀), femur 2.40–2.98 (♂), 2.43–2.92 (♀), patella 2.04–2.34 (♂), 2.06–2.36 (♀), chela (with pedicel) 2.66–3.00 (♂), 2.45–2.98 (♀), chela (without pedicel) 2.25–2.86 (♂), 2.39–3.10 (♀) times longer than wide; movable finger 0.35–0.55 (♂), 0.42–0.56 (♀) times the length of the chela (with pedicel). All surfaces of pedipalp finely to heavily granulate with exception of distal half of fixed chelal finger, and entire movable finger. Fixed finger with 33–37 (♂, ♀) marginal teeth, plus 9–12 external accessory teeth and 4–5 (♂, ♀) internal accessory teeth; movable finger with 34–39 (♂), 35–41 (♀), marginal teeth, plus 8–11 (♂), 7–11 (♀)

external accessory teeth and 3–4 internal accessory teeth. Pedipalpal setae stout and clavate-dentate, except on fingers where they are stout and acuminate on the entire movable finger and all but base of the fixed finger. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 49); *esb* closer to *eb* than to *est*; *est* closer to *esb* than to *et,* which is situated in distal quarter of fixed finger; *isb* inserted dorsally, rather than internally or externally, and closer to *it* than to *ist*; *ist* closer to *ib* than to *isb*; *sb* closer to *b* than to *st*; *st* closer to *sb* than to *t,* which is situated in distal third of movable finger. Venom apparatus present in movable finger with nodus ramosus terminating midway between *t* and *st* (Fig. 49). External margin of fixed chelal finger with 5 "sense spots," internal margin of fixed chelal finger with 3 "sense spots," and external margin of movable chelal finger with 3 "sense spots."

Chelicera (Fig. 44): with 6–7 setae on hand; *ls, is* and *es* acuminate, *sbs, bs′, bs″* and *bs‴* (when present) dentate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures. Movable finger with single dorsal tooth. Galea long and slender with 5–6 rami, extending past tip of movable finger by 0.21–0.32 (♂), 0.30–0.40 (♀) times length of movable finger. Flagellum (Fig. 45) composed of 4 blades; longest two blades dentate along the distal anterior half; two shorter blades smooth. Serrula exterior with 18 lamellae.

Cephalothorax: carapace (Fig. 41): 0.93–1.42 (♂), 0.87–1.23 (♀) times as long as broad; unicolored; eyes absent; with 4–6 setae on anterior margin, with 15–19 (♂), 15–19 (♀) setae on posterior margin; posterior half with two moderately incised transverse furrows, anterior furrow crosses the carapace at 0.56 of its length, posterior furrow crosses at 0.80 of carapace length; entirely granulate with exception of transverse furrows; posterior margin straight. Manducatory process with 1 long distal and 1 long sub-distal seta, with sub-oral seta; remainder of maxilla with 24 (♂), 29 (♀) setae. Chaetotaxy of coxae I–IV: 16: 20: 24: 44 (♂), 19: 24: 30: ca. 55 (♀).

Abdomen: tergites I–X (Fig. 5) and sternites IV–X (♂), III–X (♀) divided. Tergal chaetotaxy: ♂, 16–19: 17–20: 17–21: 22–24: 22–24: 22–25: 21–25: 22–25: 21–23: 19–22: 18–22: 2; ♀, 15–18: 16–19: 18–21: 23–26: 23–26: 22–26: 23–27: 21–26: 20–22: 18–22: 20–21: 2; setae usually restricted to posterior and lateral tergal margins. Sternal chaetotaxy: ♂, 53–67: (2–3) 23–29 [4–7] (2–3): (2–3) 14–16 (2–3): 18–21: 19–23: 18–21: 16–21: 16–19: 16–17: 10–15: 2; ♀ (excluding seta adjacent to sternite V), 37–58: (3) 15–21 (3): (3) 14–16 (3): 15–18: 18–20: 18–20: 18–20: 17–19: 15–17: 10–14: 2. Pleural membrane longitudinally striate for entire length with one seta adjacent to sternite V in ♀.

Genitalia: male genital opercula with setae on posterior region, long and curved (some reaching the genital opening), anterior setae are shorter and curved; one pair of slit sensilla on anterior and posterior operculum, posterior operculum with smaller sensilla. Female genital opercula: anterior operculum with numerous setae and 2 slit sensilla. Male genitalia of typical chernetid form (Vachon 1938). Female spermathecae with 2 thickened and slightly curved tubes fusing near the genital operculum (Fig. 48).

Legs: legs I and II with an oblique junction between femur and patella. Leg IV (Fig. 46) with femur + patella 3.77 (♂), 3.72 (♀) times longer than broad. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Male paratype from Rope Ladder Cave, Queensland (MTQ), with other specimens (n = 29) in parentheses, where appropriate:* Body length 2.60. Pedipalps: trochanter 0.497/0.256 (0.37–0.47/0.21–0.29), femur 0.784/0.282 (0.64–0.80/0.25–0.33), patella 0.693/0.303 (0.51–0.75/0.25–0.33), chela (with pedicel) 1.250/0.424 (1.02–1.34/0.38–0.49), chela (without pedicel) 1.192 (1.05–1.35), hand length 0.604, movable finger length 0.603 (0.42–0.63). Chelicera 0.267/0.140 (0.24–0.28/0.10–0.15), movable finger length 0.197 (0.14–0.20). Carapace 0.819/0.462 (0.80–0.93/0.57–0.91). Leg I: femur 0.237/0.121 (0.21–0.29/0.13–0.16), patella 0.371/0.131 (0.27–0.40/0.11–0.14), tibia 0.383/0.096 (0.28–0.42/0.08–0.10), tarsus 0.384/0.070 (0.30–0.40/0.06–0.08). Leg IV: femur + patella 0.648/0.172, femur 0.249/0.159 (0.20–0.26/0.13–0.17), patella 0.456/0.172 (0.35–0.51/0.14–0.18), tibia 0.522/0.113 (0.40–0.59/0.10–0.12), tarsus 0.447/0.077 (0.33–0.53/0.07–0.09).

Female holotype from Rope Ladder Cave,

Queensland (MTQ), with other specimens (n = 30) in parentheses, where appropriate: Body length 2.80. Pedipalps: trochanter 0.480/0.269 (0.31–0.49/0.22–0.30), femur 0.800/0.293 (0.64–0.82/0.24–0.32), patella 0.746/0.328 (0.52–0.77/0.21–0.35), chela (with pedicel) 1.349/0.461 (1.05–1.40/0.37–0.49), chela (without pedicel) 1.292 (0.92–1.29), hand length 0.656, movable finger length 0.640 (0.48–0.69). Chelicera 0.283/0.116 (0.22–0.30/0.12–0.16), movable finger length 0.211 (0.12–0.20). Carapace 0.902/0.861 (0.80–0.95/0.60–0.95). Leg I: femur 0.230/0.149 (0.19–0.30/0.12–0.17), patella 0.342/0.130 (0.28–0.40/0.11–0.14), tibia 0.378/0.093 (0.37–0.44/0.08–0.11), tarsus 0.384/0.071 (0.30–0.43/0.06–0.08). Leg IV: femur + patella 0.692/0.186, femur 0.261/0.171 (0.20–0.27/0.13–0.17), patella 0.500/0.186 (0.35–0.53/0.15–0.19), tibia 0.557/0.110 (0.47–0.60/0.09–0.12), tarsus 0.469/0.078 (0.40–0.49/0.07–0.09).

Tritonymphs: Morphology generally as in adults. Pedipalps, carapace, pedipalpal coxae and anterior half of coxa I red-brown, remainder of body pale red-yellow.

Chelicera: with 6 setae on hand, *ls, is* and *es* acuminate, *sbs, bs′* and *bs″* dentate; movable finger with 1 sub-distal seta (*gs*). Movable finger with single dorsal tooth. Galea long and slender with 4–5 small distal to subdistal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 1.45–1.71, femur 2.30–2.60, patella 1.91–2.18, chela (with pedicel) 2.63–3.23, chela (without pedicel) 2.41–3.00 times longer than wide. Fixed chelal finger with 7 trichobothria, movable chelal finger with 3 trichobothria (Fig. 50): *isb* and *sb* absent; *esb* situated near *eb*; *est* slightly closer to *esb* than to *et*; *ist* adjacent to *ib*; *st* closer to *sb* than to *t,* which is situated in distal third of movable finger. Chelal teeth: fixed finger with 32 marginal teeth, plus 5 external accessory teeth and 2 internal accessory teeth; movable finger with 31 marginal teeth, plus 5 external accessory teeth and 2 internal accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating basal to *t* (Fig. 50). External margin of fixed chelal finger with 2 "sense spots," internal margin of fixed chelal finger with 1 "sense spot," and external margin of movable chelal finger with 3 "sense spots."

Cephalothorax: carapace 0.98–1.34 times longer than wide; without eyes; with 4 setae on anterior margin and 12 setae on posterior margin; with two deep furrows.

Abdomen: Tergites I–X and sternites III–X with medial suture line. Tergal chaetotaxy: 13: 12: 12: 14: 15: 17: 16: 17: 16: 13: 12: 2. Sternal chaetotaxy: 12: (2) 8 (2): (3) 8 (3): 10: 13: 13: 13: 10: 10: 8: 2. Pleural membrane uniformly wrinkled plicate, with 1 setae situated at junction of sternite V and pleural membrane.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Tritonymphs (n = 30) from Rope Ladder Cave, Queensland (MTQ):* Body length 1.96. Pedipalps: trochanter 0.29–0.37/0.19–0.24, femur 0.50–0.58/0.20–0.24, patella 0.45–0.55/0.21–0.27, chela (with pedicel) 0.86–1.00/0.29–0.37, chela (without pedicel) 0.79–0.94, movable finger length 0.38–0.47. Carapace 0.62–0.75/0.47–0.75.

Deutonymphs: Morphology generally as in adults. Pedipalps and carapace lightly sclerotized, abdomen creamy white.

Chelicera: with 5 setae on hand, *ls, is* and *es* acuminate, *sbs* and *bs* dentate; movable finger with 1 sub-distal seta (*gs*). Movable finger with single dorsal tooth. Galea long and slender with 3 small distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 1.47–1.89, femur 2.28–2.71, patella 1.88–1.05, chela (with pedicel) 2.73–3.31, chela (without pedicel) 2.73–3.31 times longer than wide. Fixed chelal finger with 6 trichobothria, movable chelal finger with 2 trichobothria (Fig. 51): *esb, isb, sb* and *st* absent; *est* closer to *eb* than to *et*; *ist* adjacent to *ib.* Chelal teeth: fixed finger with 26 marginal teeth, plus 3 external accessory teeth and 2 internal accessory teeth; movable finger with 26 marginal teeth, plus 3 external accessory teeth and 1 internal accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating basal to *t* (Fig. 51). External margin of fixed chelal finger with 2 "sense spots," internal margin of fixed chelal finger with 0 "sense spots," and external margin of movable chelal finger with 2 "sense spots."

Cephalothorax: carapace 0.81–1.30 times longer than wide; without eyes; with 4 setae

on anterior margin and 7 setae on posterior margin; with two shallow furrows.

Abdomen: Tergites I–X and sternites II–X with medial suture line. Tergal chaetotaxy: 10: 10: 10: 10: 10: 10: 11: 11: 10: 10: 10: 2. Sternal chaetotaxy: 0: (1) 5 (1): (2) 6 (2): 10: 9: 10: 10: 10: 10: 6: 2. Pleural membrane uniformly wrinkled plicate, with 1 setae situated at junction of sternite V and pleural membrane.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Deutonymphs (n = 26) from Rope Ladder Cave, Queensland (MTQ):* Body length 1.41. Pedipalps: trochanter 0.21–0.26/0.13–0.16, femur 0.34–0.41/0.14–0.16, patella 0.32–0.37/0.16–0.18, chela (with pedicel) 0.61–0.71/0.20–0.23, chela (without pedicel) 0.58–0.66, movable finger length 0.24–0.33. Carapace 0.45–0.57/0.34–0.55.

Protonymphs: Morphology generally as in adults. Pedipalps and carapace pale yellow, abdomen white.

Chelicera: with 4 setae on hand, *ls, is* and *es* acuminate, *bs* dentate; movable finger without seta. Movable finger apparently without dorsal tooth. Galea long and slender with 2 small distal and 1 small sub-distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 1.54–1.81, femur 2.02–2.60, patella 1.49–2.08, chela (with pedicel) 2.84–3.34, chela (without pedicel) 2.60–3.14 times longer than wide. Fixed chelal finger with 3 trichobothria, movable chelal finger with 1 trichobothrium (Fig. 52): *esb, est, isb, ist, it, b, sb* and *st* absent; *et* situated sub-distally, *eb* and *ib* situated basally, and *t* situated medially. Chelal teeth: fixed finger with 23 marginal teeth; movable finger with 25 marginal teeth; both fingers without external accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating distal to *t* (Fig. 52). External margin of chelal fingers without "sense spots."

Cephalothorax: carapace 0.83–1.22 times longer than wide; without eyes; with 2 setae on anterior margin and 6 setae on posterior margin; with two very shallow furrows.

Abdomen: Tergites I–X and sternites II–X with medial suture line. Tergal chaetotaxy: 6: 6: 6: 7: 6: 6: 6: 6: 6: 6: 2. Sternal chaetotaxy: 0: (1) 2 (1): (1) 4 (1): 6: 6: 6: 6: 6: 6: 4: 2. Pleural membrane uniformly wrinkled plicate, with 1 setae situated at junction of sternite V and pleural membrane.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi without single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

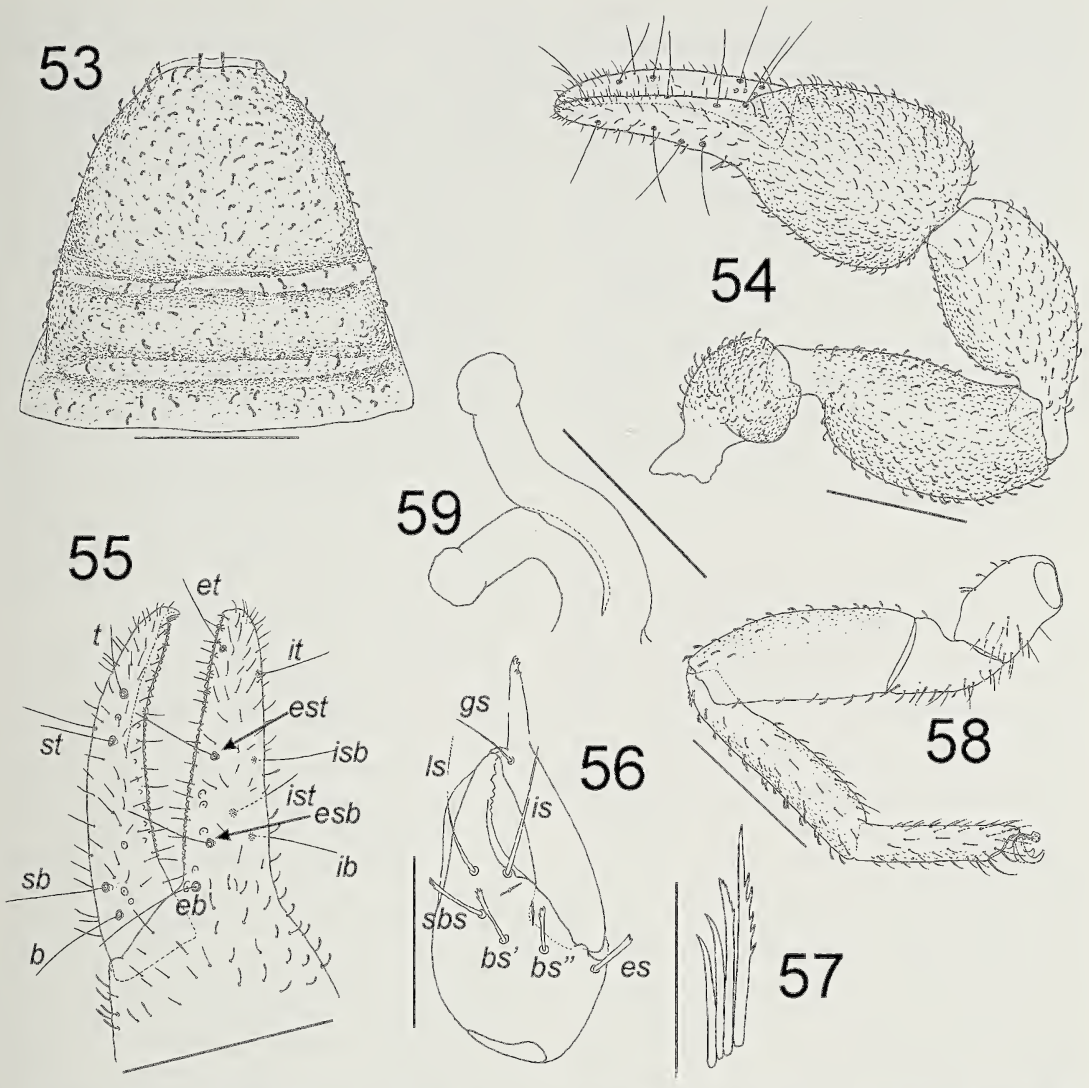
Dimensions (mm).—*Protonymphs (n = 15) from Rope Ladder Cave, Queensland (MTQ):* Body length 1.26. Pedipalps: trochanter 0.16–0.20/0.10–0.12, femur 0.21–0.29/0.10–0.12, patella 0.21–0.25/0.11–0.14, chela (with pedicel) 0.45–0.51/0.15–0.18, chela (without pedicel) 0.42–0.48, movable finger length 0.20–0.24. Carapace 0.31–0.44/0.36–0.49.

Remarks.—The type and only known locality of *T. cruciatus* is Rope Ladder Cave, on Fanning River Station, about 70 km southwest of Townsville, northern Queensland (Fig. 71). The Fanning River karst contains numerous caves, all of which are dry limestone solution caves. The surface ranges from open karst with no cover to karst covered in thick vine thicket. With the exception of Maternity Cave, which was inaccessible owing to its treacherous nature, all the local caves were thoroughly searched but only Rope Ladder Cave was found to support *T. cruciatus*.

While having an extremely limited range, the species is very abundant and specimens were found in the dark zone (sensu Howarth 1988) under moist rocks, in bat guano and in leaf litter inside the cave (Weinstein & Slaney 1995). The caves are inhabited by Common Bent-Wing Bats, *Miniopterus schreibersii* (Kuhl), and Little Bent-Wing Bats, *M. australis* (Tomes), which excrete large quantities of droppings that accumulate into guano deposits.

Troglochernes omorgus
Harvey & Volschenk new species
Figs. 53–59, 71

Type material.—AUSTRALIA: *Queensland:* Holotype female, Carnarvon National Park, Mt. Moffatt ranger's house [25°03′S, 148°03′E], from beetle [Trogidae, *Omorgus costatus* (Wiedemann)] at light, 24 November 1999, J.T. Jennings (QM S74354).



Figures 53–59.—*Troglochernes omorgus*, sp. nov., female holotype (QM): 53. Carapace, dorsal; 54. Right pedipalp, dorsal; 55. Left chelal fingers, lateral; 56. Right chelicera; 57. Right flagellum; 58. Left leg IV; 59. Spermathecae. Scale lines = 0.50 mm (Figs. 53, 54, 58), 0.20 mm (Fig. 56), 0.10 mm (Figs. 57, 59). See Methods for abbreviations.

Etymology.—The specific epithet refers to the beetle genus *Omorgus*, from which the holotype was found to be associated.

Diagnosis.—*Troglochernes omorgus* differs from other species of the genus by the greater number of setae on the carapace and tergites.

Description.—*Adult female:* Pedipalps and carapace dark red-brown; abdomen and legs light red-brown in color. Vestitural setae short, curved and strongly dentate; most sternal setae acicular with few dentate.

Pedipalps (Fig. 54): very robust and densely setose, with trochanter 1.61, femur 2.41, patella 2.07, chela (with pedicel) 2.67, chela (without pedicel) 2.48, and hand 1.09 times longer than wide; movable finger 1.27 times longer than hand. All surfaces of pedipalp finely to heavily granulate with exception of distal half of fixed chelal finger, and entire movable finger. Fixed finger with 42 marginal teeth, plus 15 external accessory teeth and 8 internal accessory teeth; movable finger with 46 marginal teeth, plus 15 external accessory

teeth and 6 internal accessory teeth. Pedipalpal setae stout, clavate-dentate and curved, except on fingers where they are stout and acuminate on the entire movable finger and all but base of the fixed finger. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 55); *esb* closer to *eb* than to *est*; *est* closer to *esb* than to *et*; *isb* closer to *ist* than to *it*; *ist* closer to *ib* than to *isb*; *sb* closer to *b* than to *st*; *st* closer to *t* than to *sb*. Venom apparatus present in movable finger with nodus ramosus terminating adjacent to *st* (Fig. 55). External margin of fixed chelal finger with 10 "sense spots," internal margin of fixed chelal finger with 18 "sense spots" (mostly placed basal to *ib* and *ist*), and external margin of movable chelal finger with 4 "sense spots."

Chelicera (Fig. 56): with 6 setae on hand; *ls* and *is* acuminate, *es, sbs, bs'* and *bs"* dentate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures. Movable finger with single dorsal tooth. Galea long and slender with 6 small distal rami. Flagellum (Fig. 57) composed of 4 blades; longest two blades dentate along the distal anterior half; two shorter blades smooth. Serrula exterior with 21 lamellae.

Cephalothorax: carapace (Fig. 53): 1.08 times as long as broad; unicolored; eyes absent; with 7 setae on anterior margin, with 25 setae on posterior margin; posterior half with two moderately incised transverse furrows, anterior furrow crosses the carapace at ca. 0.6 of its length, posterior furrow crosses at ca. 0.85 of carapace length; entirely granulate with exception of transverse furrows; posterior margin slightly curved. Manducatory process with 1 long distal and 1 long sub-distal seta, with sub-oral seta; remainder of maxilla with ca. 70 setae. Chaetotaxy of coxae I–IV: 24: 29: 32: 64.

Abdomen: tergites II–X and sternites III–X divided, and tergite XI partially divided. Tergal chaetotaxy: 30: 32: 34: 38: 43: 46: 41: 43: 40: 33: 27: 2; setae usually restricted to posterior and lateral tergal margins. Sternal chaetotaxy: 62: (3) 27 (4): (4) 13 (4): 23: 28: 30: 29: 28: 30: 17: 2. Pleural membrane longitudinally striate for entire length, without setae.

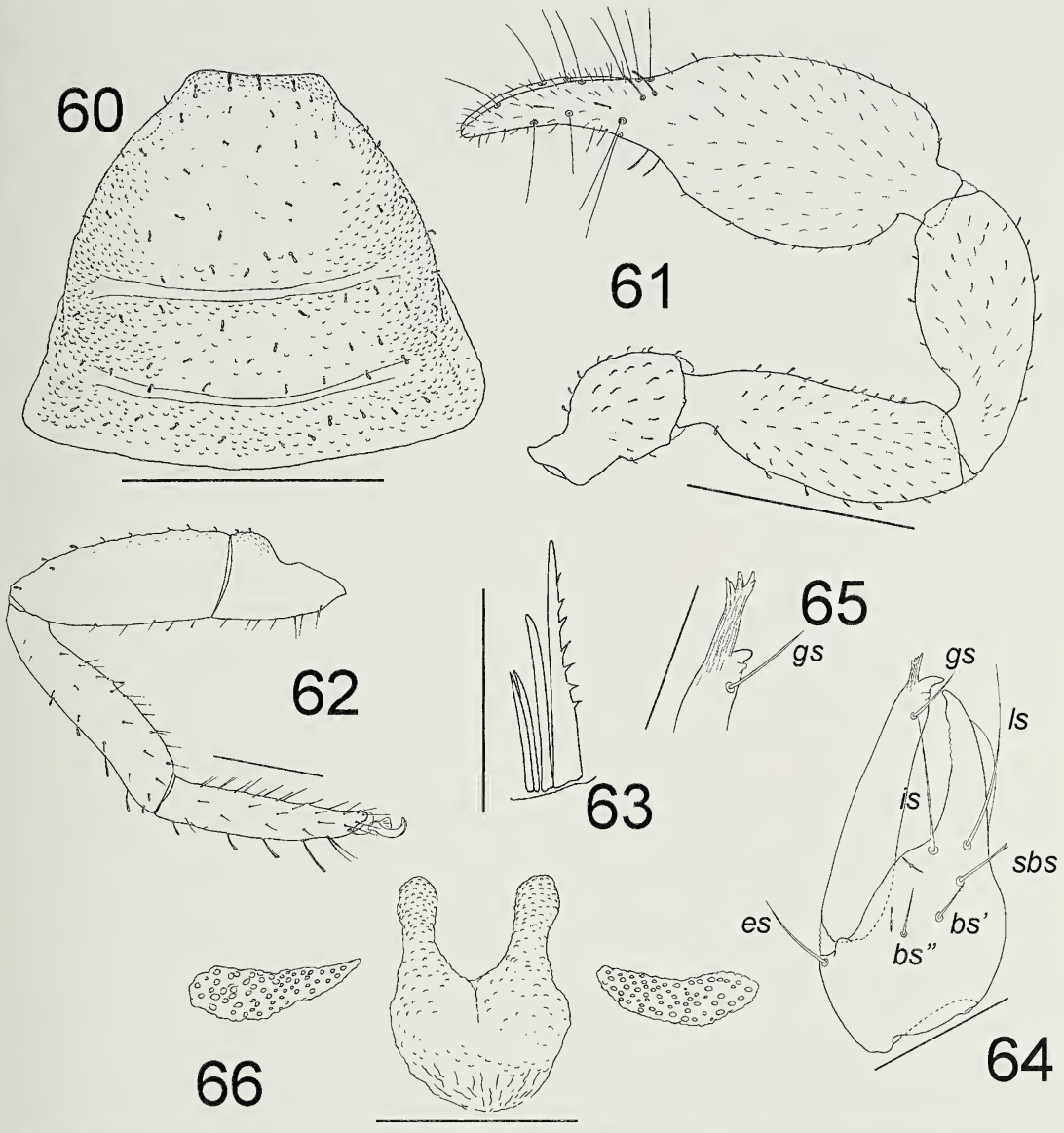
Female genital opercula: anterior operculum with numerous setae and 2 slit sensilla. Spermathecae with 2 long, thickened and slightly curved tubes fusing near the genital operculum and slightly thickened distally (Fig. 59).

Legs: legs I and II with an oblique junction between femur and patella. Leg IV (Fig. 58) with femur + patella 3.44 times longer than wide. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Female holotype (QM):* Body length 3.74. Pedipalps: trochanter 0.614/0.381, femur 1.066/0.442, patella 0.996/0.467, chela (with pedicel) 1.606/0.602, chela (without pedicel) 1.491, hand length 0.656, movable finger length 0.832. Chelicera 0.354/0.206, movable finger length 0.266. Carapace 1.136/1.054. Leg I: femur 0.323/0.225, patella 0.538/0.210, tibia 0.531/0.141, tarsus 0.434/0.099. Leg IV: femur + patella 1.005/0.292, tibia 0.798/0.173, tarsus 0.506/0.116.

Remarks.—*Troglochernes omorgus* is placed in the *Troglochernes* as it has four flagellar blades, lacks tactile setae on tarsi III and IV, lacks eyes or eyespots, has a unicolored carapace with two transverse furrows, and small, dentate, clavate vestitural setae. The spermathecae, however, are slightly different in morphology to those of other species of the genus, as they are oriented anteriorly (Fig. 59) rather than projecting laterally (Figs. 14, 23, 28, 48). While it is currently preferable to refer *T. omorgus* to *Troglochernes* rather than establish a separate monotypic genus, we suggest that the generic position of *T. omorgus* should be tested when further chernetids of this complex are discovered and analyzed.

The only known specimen of *T. omorgus* was found attached to a beetle of the family Trogidae, *Omorgus costatus* (Wiedemann) in south-eastern Queensland (Fig. 71). This beetle species is widely distributed throughout Australia, as well as Papua New Guinea, the Solomon Islands, Indonesia and the Asian mainland, and has been recorded from several different cave systems where it breeds in bat guano (Scholtz 1986). The presence of the pseudoscorpion on the beetle may simply be fortuitous, but may also signify that *T. omorgus* occurs in subterranean habitats like many of its congeners, and was collected while undertaking a phoretic journey after the beetle left the cave. Phoretic associations between pseudoscorpions and other animals, including



Figures 60–66.—*Satrapanus grayi* (Beier 1975), specimens from Lord Howe Island, female (AM) unless stated otherwise: 60. Carapace; 61. Right pedipalp, dorsal; 62. Left leg IV; 63. Left flagellum, male (AM); 64. Left chelicera, male (AM); 65. Detail of tip of movable cheliceral finger; 66. Spermathecae. Scale lines = 0.50 mm (Figs. 60, 61), 0.20 mm (Fig. 62), 0.10 mm (Figs. 64–66), 0.05 mm (Fig. 63). See Methods for abbreviations.

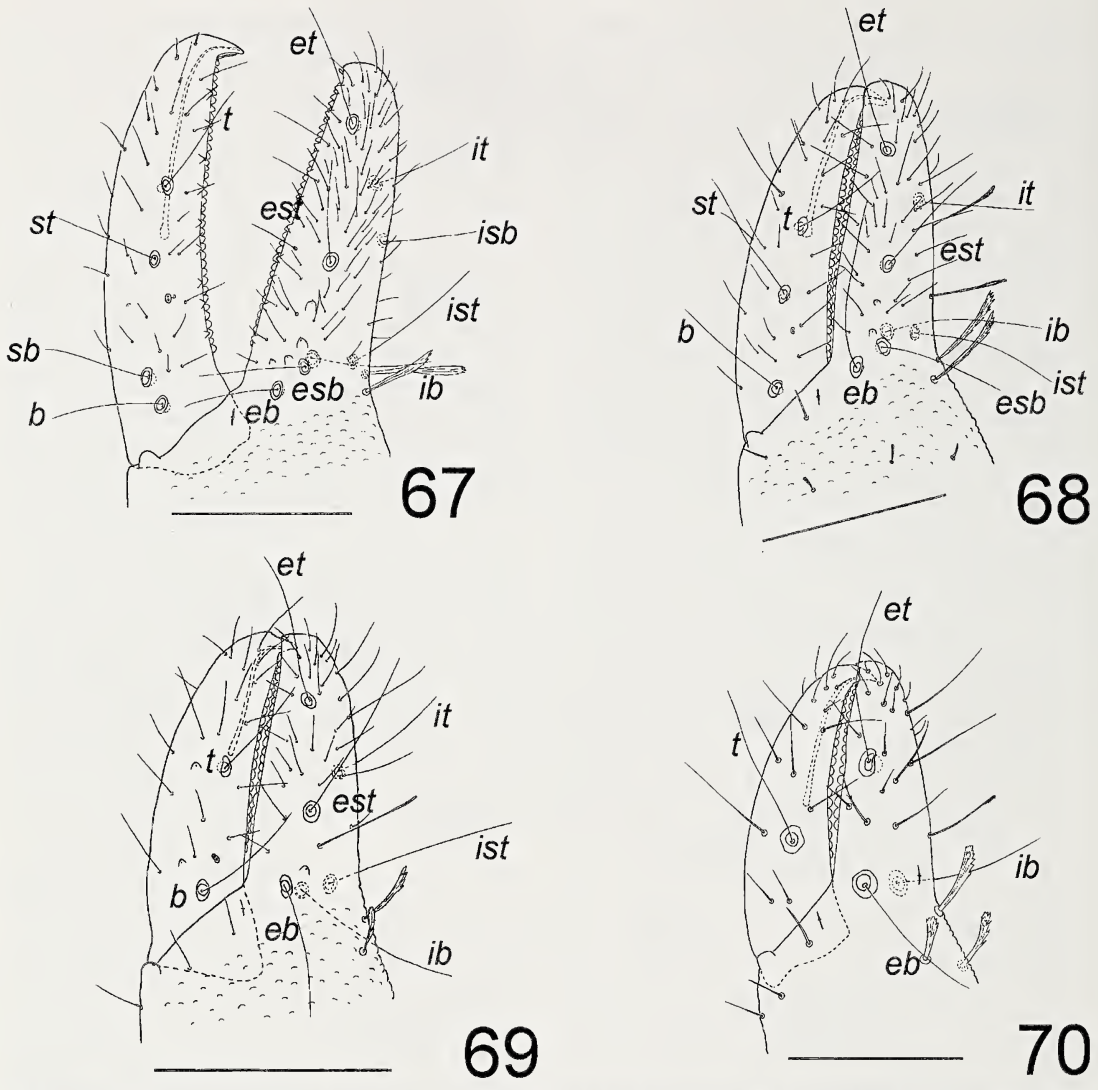
insects, are not uncommon (e.g., Beier 1948; Muchmore 1971; Poinar et al. 1998; Vachon 1940).

Genus *Satrapanus*
Harvey & Volschenk gen. nov.

Type species.—*Sundochernes grayi* Beier 1975.

Etymology.—The generic epithet refers to the presence of the only known species of the genus on Lord Howe Island (*satrapa,* Latin, governor of a province). The gender is masculine.

Diagnosis.—*Satrapanus* differs from all other chernetid genera by the following combination of characters: flagellum with 4 blades (Fig. 63); female genitalia with 2 thickened anteriorly-directed spermathecae fused basally



Figures 67–70.—*Satrapanus grayi* (Beier 1975) from Lord Howe Island, left chelal fingers, lateral: 67. Female (AM); 68. Tritonymph (AM); 69. Deutonymph (AM); 70. Protonymph (AM). Scale lines = 0.2 mm (Figs. 67–69), 0.1 mm (Fig. 70). See Methods for abbreviations.

into large bursa (Fig. 66); legs III and IV without tactile setae (Fig. 62); carapace dark colored except for pale metazone, and with two transverse furrows (Figs. 6, 60); single pair of eye-spots present (Fig. 60); vestitural setae generally short, slightly curved, dentate and clavate (Fig. 61); base of fixed chelal finger with long, clavate-dentate setae, 2 on dorsum and 2 on internal margin (Figs. 61, 67–70).

Description.—*Adults:* Vestitural setae mostly short, slightly curved, dentate and clavate, except for 4 setae at base of fixed chelal finger which are long and clavate-dentate setae, 2 on dorsum and 2 on internal margin (Figs. 61, 67).

Pedipalps: with most surfaces finely to heavily granulate; fingers generally smooth. Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Figs. 61, 67); *esb* closer to *eb* than to *est*; *isb* approximately midway between *it* and *ist*; *it* situated in distal third of fixed finger; *sb* closer to *b* than to *st*. Marginal teeth of chela all closely spaced; both chelal fingers with external row of accessory teeth, but without internal row of accessory teeth (Fig. 67). Venom apparatus

present in movable finger with nodus ramosus terminating slightly anterior to *st* (Fig. 67).

Chelicera (Figs. 63–65): with 6 setae on hand; *ls* and *is* long and acuminate, *sbs* and *bs'* short and dentate, *bs"* and *es* short and acuminate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures; lamina exterior present; movable finger with single dorsal tooth; galea long and slender with 4 small rami (♂) or 6 longer rami (♀); flagellum composed of 4 blades; distal blade dentate along anterior margin, remaining blades smooth.

Cephalothorax (Figs. 6, 60): carapace with 1 pair of eye-spots; yellow-brown or red-brown, with metazone pale yellow or creamy white, sometimes with a central darker patch; with two transverse furrows; posterior margin slightly angulate. Median maxillary lyrifissure present and sub-medially situated; posterior maxillary lyrifissure present.

Abdomen: tergites and sternites generally divided (Fig. 6). Pleural membrane wrinkled striate for entire length, without setae. Each stigmatic sclerite with 1 or more setae. Spiracles simple, with spiracular helix.

Genitalia: male genitalia of typical chernetid form; female genitalia with 2 thickened anteriorly-directed spermathecae fused basally into large bursa (Fig. 66).

Legs: legs I and II with an oblique junction between femur and patella; legs III and IV without tactile setae on tibiae or tarsi (Fig. 62); metatarsus and tarsus fused into single segment (tarsus); tarsi with single raised slit sensillum; subterminal tarsal seta curved and acuminate; tarsal claws simple; arolium slightly shorter than claws (Fig. 62).

Nymphs: Much like adults, but trichobothrial patterns as follows: tritonymph with 7 on fixed finger and 3 on movable finger (Fig. 68); deutonymph with 6 on fixed finger and 2 on movable finger (Fig. 69); and protonymph with 3 on fixed finger and 1 on movable finger (Fig. 70). Chelicera of protonymph lacking seta *gs*. Tarsi without single raised slit sensillum in protonymph.

Remarks.—The genus *Satrapanus* appears to be restricted to Lord Howe Island where a single species, *S. grayi*, occurs. As discussed above only a few genera of Chernetidae possess four blades in the cheliceral flagellum and also lack a tactile seta on tarsi of legs III and IV. The morphology of the female genitalia, with two short spermathecal ducts, distinguishes *Satrapanus* from *Chelodamus*, *Chernes*, *Hesperochernes*, *Chelanops*, *Semeiochernes*, *Illinichernes*, *Gigantochernes*, *Cocinachernes*, *Paraustrochernes* (Fig. 2) and *Nesochernes* (Fig. 1). The spermathecal morphology of *Atherochernes* and *Eumecochernes* are unknown but each can be readily separated from *Satrapanus*. *Atherochernes* has only 5 setae on the cheliceral hand (6 or 7 setae in *Satrapanus*), and by the presence of accessory teeth only on the movable chelal finger (accessory teeth on both chelal fingers in *Satrapanus*) (Beier 1954a). *Eumecochernes* has trichobothrium *isb* situated basally to *est* (Beier 1932), whereas it is situated slightly distal to *est* in *Satrapanus*. *Satrapanus* differs from *Troglochernes* by the presence of eye-spots, which are lacking in *Troglochernes*, by the morphology of the female genitalia in which the spermathecae are anteriorly directed and discharging from a large bursa, and by the color of the carapace in which the metazone is paler than the remaining carapace in *Satrapanus* but is uniformly unicolored in *Troglochernes*.

Satrapanus differs from the other Australian species of Chernetidae which possess four flagellar blades as follows: from *Austrochernes* by the lack of tactile setae on tarsus IV [present in *Austrochernes*, see With (1905); Beier (1932)]; from *Paraustrochernes* by the position of trichobothria *isb* and *it,* which are situated distal to *est* in *Satrapanus*, but are situated adjacent to *est* in *Paraustrochernes* [see Beier (1966)], and by the morphology of the female genitalia which consists of a single pair of spermathecae in *Satrapanus* (Fig. 66), and two pairs of spermathecae in *Paraustrochernes* (Fig. 2); and from *Marachernes* by the general shape of the chela (which is not much wider than the base of the fingers in *Marachernes*) and the lack of an internobasal mound bearing accessory teeth on the male movable chelal finger (Harvey 1992a, 1994).

Satrapanus grayi (Beier 1975)

new combination

Figs. 6, 60–71

Type material.—AUSTRALIA: *New South Wales:* Holotype male, near Old Settlement, Lord Howe Island [31°30′S, 159°03′E], 67 m, 30 January 1971, M. Gray (AM KS20).

Other material examined.—AUSTRA-

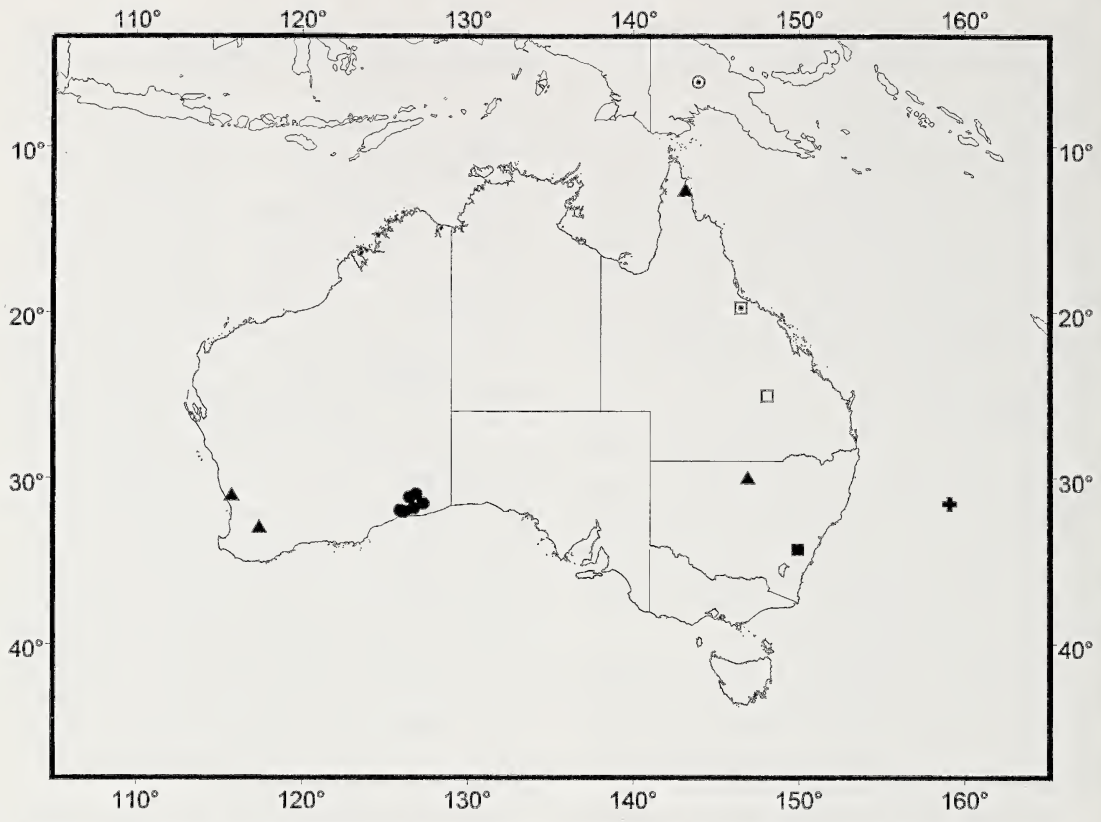


Figure 71.—Map showing recorded distributions of species of *Troglochernes* and *Satrapanus*: *T. cruciatus* new species (⊡), *T. dewae* (Beier) (▲), *T. guanophilus* (Beier) (■), *T. imitans* Beier (●), *T. novaeguineae* (Beier) (⊙), *T. omorgus* new species (□), and *Satrapanus grayi* (Beier) (✚).

LIA: *New South Wales:* Lord Howe Island: 1 ♀, N. end of Big Pocket at base of Razorback, Mt. Gower, IH030A, 31°35′15″S, 159°04′08″E, 26 April 2002, leaf litter, I. Hutton (AM KS96521); 1 ♂, S end of Big Pocket at base of Razorback, Mt. Gower, IH030B, 31°36′00″S, 159°04′11″E, 26 April 2002, leaf litter, I. Hutton (AM KS96527); 1 ♂, 4 ♀, 1 tritonymph, 2 deutonymphs, 16 protonymphs, eastern end of Boat Harbour beach, LHIS032L, 31°33′37″S, 159°05′53″E, 3 December 2000, leaf litter, CBCR (WAM T75452); 1 ♂, above trail to Boat Harbour, opp. turnoff to Mutton Bird Point, LHIS021/04, 31°32′57″S, 159°05′24″E, 26 November–3 December 2000, pit trap, CBCR (AM KS96566); 2 ♀, 7 protonymphs, approx. 25 m above above coastal trail to Boat Harbour, 750 m from start, LHIS030L, 31°32′51″S, 159°05′10″E, 3 December 2000, leaf litter, CBCR (AM KS96562); 1 deutonymph, on trail to The Clear Place, LHI/JT/05L, 31°31′51″S, 159°04′35″E, 21 February 2001, leaf litter, J. Tarnawski (AM KS96564); 1 ♂, 1 protonymph, eastern slope of Dawsons Point Ridge above old Settlement, LHIS014L, 31°31′15″S, 159°03′07″E, 1 December 2000, leaf litter, CBCR (AM KS96553); 1 ♂, western slope of Dawson's Point Ridge off North Beach Trail, LHIS012aL, 31°31′12″S, 159°02′27″E, 20 November 2000, leaf litter, CBCR (AM KS96555); 1 ♀, point where walking trial first enters Erskine Valley from coast, LHIS043L, 31°34′33″S, 159°04′17″E, 2 December 2000, leaf litter, CBCR (AM KS96524); 1 ♂, 3 tritonymphs, on walking track to Erskine Valley, adjacent to Salmon Beach, LHIS/GC/L18, 31°33′39″S, 159°04′31″E, 10 December 2000, leaf litter, G. Cassis (AM KS 96548); 1 deutonymph, walking trial through Erskine Valley, LHIS042L, 31°34′34″S, 159°04′31″E, 2 December 2000, leaf litter, CBCR (AM KS96517); 1 tritonymph, walking trial through Erskine Valley,

LHIS045L, 31°34′37″S, 159°04′33″E, 2 December 2000, leaf litter, CBCR (AM KS96528); 2 tritonymphs, "Get Up Place," trail to Mount Gower, LHIS048L, 31°34′58″S, 159°04′52″E, 2 December 2000, leaf litter, CBCR (AM KS96547); 2 tritonymphs, Goat House walking track, Intermediate Hill, LHI/JT/09L, 31°33′15″S, 159°04′57″E, 23 February 2001, leaf litter, J. Tamawski, M. Shea (AM KS 96529); 1 ♂, 1 protonymph, Goat House walking track, Intermediate Hill, LHI/JT/09L, 31°33′15″S, 159°04′56″E, 23 February 2001, leaf litter, J. Tamawski, M. Shea (AM KS96509); 3 tritonymphs, Goat House walking track, Intermediate Hill, LHI/JT/09L, 31°33′15″S, 159°04′56″E, 23 February 2001, leaf litter, J. Tamawski, M. Shea (AM KS96525); 1 ♀, 1 tritonymph, Goat House walking track, Intermediate Hill, LHI/GC/L07, 31°33′17″S, 159°05′05″E, 6 December 2000, leaf litter, G. Cassis (AM KS96506); 2 ♂, 1 ♀, 1 protonymph, ridge below Intermediate Hill, Boat Harbour walking trail, LHI/GC/L35, 31°32′59″S, 159°05′24″E, 12 December 2000, leaf litter, G. Cassis (AM KS96568); 1 ♂, 1 ♀, 1 tritonymph, Lagoon beach between rubbish tip and airstrip, LHIS022/04, 31°32′31″S, 159°04′31″E, 27 November–4 December 2000, pit trap, CBCR (AM KS96543); 1 deutonymph, 1 protonymph, Little Island, coastal track to Erskine Valley, LHIS/GC/L37, 31°34′10″S, 159°04′26″E, 13 December 2000, leaf litter, G. Cassis (AM KS96552); 1 ♂, 1 ♀, Little Island, coastal track to Erskine Valley, LHIS/GC/L38, 31°34′10″S, 159°04′26″E, 13 December 2000, leaf litter, G. Cassis (AM KS96516); 4 ♂, 4 ♀, Little Island, below Far Flats, IH021B, 31°34′08″S, 159°04′32″E, 10 August 2001, leaf litter, I. Hutton (WAM T75453); 2 ♂, 1 ♀, 2 tritonymphs, "Little Slope," LHIS051L, 31°35′12″S, 159°04′03″E, 30 November 2000, leaf litter, CBCR (AM KS96512); 2 ♂, 2 ♀, 2 tritonymphs, southern end of Little Slope, IH018A, 31°35′15″S, 159°04′04″E, 28 June 2001, leaf litter, I. Hutton (AM KS96515); 2 ♂, 1 tritonymph, Malabar Hill, on path to Kim's Lookout, LHIS004L, 31°30′54″S, 159°03′22″E, 24 November 2000, leaf litter, CBCR (AM KS96554); 6 ♀, 3 tritonymphs, 3 deutonymphs, 3 protonymphs, north-western slope of Malabar Hill, IH019A, 31°31′08″S, 159°03′42″E, 7 August 2001, leaf litter, I. Hutton (AM KS96558); 1 deutonymph, 20 m SE of walking track on Malabar Hill, half way to summit, IH022B, 31°31′00″S, 159°03′41″E, 10 August 2001, leaf litter, I. Hutton (AM KS96557); 1 ♂, 1 ♀, 2 tritonymphs, walking track on Malabar Hill, 50 m north of summit, IH022A, 31°30′50″S, 159°03′39″E, 10 August 2001, leaf litter, I. Hutton (AM KS96530); 1 ♀, 10 m NW Malabar Hill walking track in forest at beginning, IH022C, 31°31′10″S, 159°03′44″E, 10 August 2001, leaf litter, I. Hutton (AM KS96550); 1 ♀, western slope of Malabar Ridge, S. of Kim's lookout, LHIS007L, 31°30′57″S, 159°03′31″E, 24 November 2000, leaf litter, CBCR (AM KS96545); 1 protonymph, eastern slope of Malabar Ridge above Neds Beach, LHIS0011L, 31°31′03″S, 159°03′38″E, 19 November 2000, leaf litter, CBCR (AM KS96551); 1 tritonymph, approx. 50 m S of summit of Mt. Eliza on western face, LHIS005L, 31°30′57″S, 159°02′25″E, 20 November 2000, leaf litter, CBCR (AM KS96526); 1 ♀, 1 protonymph, approx. 50 m S of summit of Mt. Eliza on western face, LHIS005/05, 31°30′57″S, 159°02′25″E, 25 November–2 December 2000, pit trap, CBCR (AM KS96544); 2 deutonymphs, Mt. Gower, middle summit, IH024B, 31°35′15″S, 159°04′29″E, 28 August 2001, leaf litter, I. Hutton (AM KS96534); 1 ♀, N-face of Mt. Gower, IH013D, 31°34′58″S, 159°04′28″E, 26 May 2001, leaf litter, I. Hutton (AMKS96565); 1 tritonymph, eastern face of Mt. Lidgberg, LHIS039L, 31°34′27″S, 159°05′04″E, 3 December 2000, leaf litter, CBCR (AM KS96531); 1 tritonymph, 1 deutonymph, 2 protonymphs, Muttonbird point booby colony area, IH015B, 31°33′00″S, 159°05′24″E, 22 June 2001, leaf litter, I. Hutton (AM KS96542); 1 ♂, North Hummock (trail to Intermediate Hill), LHIS020/01, 31°32′54″S, 159°04′58″E, February 2001, pit trap (AM KS96504); 1 protonymph, just behind beach at "Old Gulch" on western footslopes, LHIS006/01, 31°30′53″S, 159°02′36″E, 2–11 December 2000, pit trap, CBCR (AM KS96533); 1 protonymph, just behind beach at "Old Gulch" on western footslopes, LHIS006/05, 31°30′53″S, 159°02′36″E, 25 November–2 December 2000, pit trap, CBCR (AM KS96549); 1 deutonymph, Peach Tree Ridge, just below summit of Intermediate Hill, LHIS023L, 31°33′01″S, 159°05′05″E, 3 December 2000, leaf litter (AM KS96563); 1 ♂, 1 protonymph, eastern slope of Philip Point

(North Head), LHIS015L, 31°31′20″S, 159° 02′29″E, 1 December 2000, leaf litter, CBCR (AM KS96519); 3 ♂, 2 ♀, 1 protonymph, eastern slope of Philip Point (North Head), LHIS015L, 31°31′20″S, 159°02′29″E, 1 December 2000, leaf litter, CBCR (AM KS96540); 4 ♂, 3 ♀, 7 deutonymphs, 1 protonymph, in forest behind Research Station, LHI/JT/08L, 31°31′37″S, 159°03′58″E, 22 February 2001, leaf litter, J. Tamawski (AM KS96514); 1 ♂, 1 ♀, Rocky Run Creek, where Intermediate Hill track crosses, IH031A, 31°33′22″S, 159°05′14″E, 18 May 2002, leaf litter, I. Hutton (AM KS96507); 1 tritonymph, 1 deutonymph, Rocky Run, east side of creek, IH023B, 31°31′10″S, 159°05′ 34″E, 25 August 2001, leaf litter, I. Hutton (AM KS96513); 1 ♀, Rocky Run, east side of creek, IH023B, 31°31′10″S, 159°05′34″E, 25 August 2001, leaf litter, I. Hutton (AM KS96518); 1 deutonymph, Rocky Run Creek, where Intermediate Hill track crosses, IH031C, 31°33′22″S, 159°05′14″E, 18 May 2002, leaf litter, I. Hutton (AM KS96539); 1 ♂, Rocky Run Creek, where Intermediate Hill track crosses, IH031D, 31°33′22″S, 159°05′ 14″E, 18 May 2002, leaf litter, I. Hutton (AM KS96508); 1 tritonymph, N. bank of Rocky Run Creek, at junction with coastal trail to Boat Harbour, LHIS024L, 31°33′19″S, 159° 05′33″E, 21 November 2000, leaf litter, CBCR (AM KS96511); 1 protonymph, base of 'Round Face', Mt. Lidgberg, Far Flats, LHIS036L, 31°34′09″S, 159°04′35″E, 27 November 2000, pit trap, CBCR (AM KS96532); 1 tritonymph, base of "Round Face," Mt. Lidgberg, Far Flats, LHIS036/04, 31°34′09″S, 159°04′35″E, 4–14 December 2000, pit trap, CBCR (AM KS96559); 1 ♀, base of "Round Face," Mt. Lidgberg, Far Flats, site LHIS036/ 04, 31°34′09″S, 159°04′35″E, 27 November–4 December 2000, pit trap, CBCR (AM KS96505); 1 tritonymph, base of "Round Face," Mt. Lidgberg, Far Flats, LHIS036/02, 31°34′09″S, 159°04′35″E, Feburary 2001, pit trap, CBCR (AM KS96567); 1 ♂, Stephens Reserve, New Settlement, IH025B, 31°31′ 33″S, 159°03′53″E, 30 September 2001, leaf litter, I. Hutton (AM KS96510); 1 ♂, 1 tritonymph, Stephens Reserve, New Settlement, IH025D, 31°31′33″S, 159°03′53″E, 30 September 2001, leaf litter, I. Hutton (AM KS96535); 1 protonymph, Stephens Reserve, New Settlement, LHIS059/01, 31°31′33″S, 159°03′53″E, 4–14 December 2000, pit trap, CBCR (AM KS96560); 1 ♂, Stephens Reserve, New Settlement, LHIS059/05, 31°31′ 33″S, 159°03′53″E, 4–14 December 2000, pit trap, CBCR (AM KS96520); 1 tritonymph, Stephens Reserve, New Settlement, LHIS058/ 02, 31°31′33″S, 159°03′53″E, 4–14 December 2000, pit trap, CBCR (AM KS96561); 2 ♂, Stephens Reserve, New Settlement, IH025A, 31°31′33″S, 159°03′53″E, 30 September 2001, leaf litter, I. Hutton (AM KS96536); 1 ♂, Stephens Reserve, New Settlement, LHIS059/02, 31°31′33″S, 159°03′53″E, 4–14 December 2000, pit trap, CBCR (AM KS96569); 1 ♂, 1 ♀, The Saddle, Erskine Valley, LHIS046L, 31°34′49″S, 159°04′58″E, 2 December 2000, leaf litter, CBCR (AM KS96537); 1 ♀, 2 protonymphs, eastern aspect of Transit Hill near summit, LHIS018L, 31°32′01″S, 159°04′43″E, 19 November 2000, leaf litter, CBCR (AM KS96541); 2 tritonymphs, 1 protonymph, south-eastern aspect of Transit Hill near summit, LHIS019/01, 31°32′13″S, 159°04′40″E, 1–11 December 2000, pit trap, CBCR (AM KS965122); 2 ♂, 1 protonymph, creek gully crossing Transit Hill walking track, IH020B, 31°31′51″S, 159°04′22″E, 9 August 2001, leaf litter, I. Hutton (AM KS96523); 1 ♀, 1 deutonymph, south-eastern aspect of Transit Hill near summit, LHIS019L, 31°32′13″S, 159° 04′40″E, 24 November 2000, leaf litter, CBCR (AM KS96546); 1 protonymph, site 58, 31° 31′33″S, 159°03′53″E, 12 December 2000, litter, *Ficus* (AM KS96538); 3 protonymphs, site 59, 31°31′33″S, 159°03′53″E, 13 December 2000, litter (AM KS96556).

Diagnosis.—As for genus.

Description.—*Adults:* Pedipalps, carapace, coxae and tergites deep reddish-brown; sternites and legs light red-brown in color. Vestitural setae short, slightly curved, and dentate; most sternal setae acicular with few dentate. Pleural membrane wrinkled plicate for entire length.

Pedipalps (Fig. 61): robust, with trochanter 1.31–1.81 (♂), 1.58–1.83 (♀), femur 2.95–3.84 (♂), 2.56–3.40 (♀), patella 2.18–2.69 (♂), 2.35–2.77 (♀), chela (with pedicel) 2.71–3.20 (♂), 2.55–3.08 (♀), chela (without pedicel) 2.53–3.05 (♂), 2.42–2.97 (♀), hand strongly rounded, 1.26–1.71 (♂), 1.29–1.74 (♀) times longer than wide; movable finger 0.41–0.48 (♂), 0.42–0.46 (♀) times the length of the chela (with pedicel). All surfaces of

pedipalp finely to heavily granulate with exception of chelal fingers, and entire movable finger. Fixed finger with 36 (♂, ♀) marginal teeth, plus 8 (♂), 10 (♀) external accessory teeth and 1 (♂, ♀) internal accessory tooth; movable finger with 39 (♂, ♀) marginal teeth, plus 7 (♂), 9 (♀) external accessory teeth and without internal accessory teeth. Pedipalpal setae short, stout and clavate-dentate, except on fingers where they are acuminate, and at base of fixed finger where 2 very long, stout, dentate setae are present on dorsum and 2 similar setae on internal margin (Fig. 61). Fixed finger with 8 trichobothria, movable chelal finger with 4 trichobothria (Fig. 67); *esb* closer to *eb* than to *est*; *est* slightly closer to *esb* than to *et,* which is situated sub-distally; *isb* inserted dorsally, rather than internally or externally, and closer to *it* than to *ist*; *ist* adjacent to *ib*; *sb* closer to *b* than to *st*; *st* slightly closer to *t* than to *sb,* which is situated in distal third of movable finger. Venom apparatus present in movable finger with nodus ramosus terminating midway between *t* and *st* (Fig. 67); fixed finger with vestigial venom apparatus. External margin of fixed chelal finger with 3–4 "sense spots," internal margin of fixed chelal finger with 8 (♂), 5 (♀) "sense spots," and external margin of movable chelal finger 2–3 "sense spots"; diploid sensillum situated between *sb* and *st.*

Chelicera (Fig. 64): with 6–7 setae on hand; *ls* and *is* long and acuminate, *sbs* and *bs′* short and dentate, *bs″* and *es* short and acuminate; movable finger with 1 acuminate seta (*gs*); with 2 dorsal and 1 ventral lyrifissures; lamina exterior present; movable finger with single dorsal tooth; galea long and slender with 4 small rami (♂) (Fig. 64) or 6 longer rami (♀) (Fig. 65); flagellum composed of 4 blades (Fig. 63); distal blade dentate along anterior margin, remaining blades smooth; third and fourth blades very closely adpressed. Serrula exterior with 16–17 lamellae.

Cephalothorax: carapace (Figs. 6, 60): 1.03–1.14 (♂), 0.85–1.15 (♀) times as long as broad; yellow-brown or red-brown, with metazone pale yellow or creamy white, sometimes with a central darker patch; with 1 pair of pale eye-spots; with 4–5 setae on anterior margin, with 8–10 setae on posterior margin; posterior half with two moderately incised transverse furrows, anterior furrow crosses the carapace at ca. 0.58 of its length, posterior furrow crosses at ca. 0.83 of carapace length; posterior margin slightly angulate; lightly granulate with exception of median area and transverse furrows. Manducatory process with 1 long distal and 1 shorter sub-distal seta, with sub-oral seta; remainder of maxilla with 26 (♂), 38 (♀) setae. Chaetotaxy of coxae I–IV: ♂, 18: 25: 27: 31; ♀, 19: 21: 27: ca. 42.

Abdomen: tergites I–X (Fig. 6) and sternites IV–X (♂, ♀) divided. Tergal chaetotaxy: ♂, 10: 10: 9: 11: 12: 12: 11: 11: 10: 8: 4: 2; ♀, 8: 10: 12: 12: 12: 12: 13: 12: 10: 9: 4: 2; setae restricted to posterior and lateral tergal margins. Sternal chaetotaxy: ♂, 26: (2) 18 [3+3] (2): (2) 12 (2): 16: 18: 17: 14: 14: 10: 4: 2; ♀, 19: (2) 16 (2): (2) 11 (2): 14: 18: 20: 17: 15: 11: 5: 2.

Genitalia: male genital opercula with setae generally long and curved; anterior operculum with 1 pair of slit sensilla and posterior operculum with 2 pairs. Female genital opercula with curved setae arranged approximately in inverted U; anterior and posterior opercula each with 1 pair of small slit sensilla. Male genitalia of typical chernetid form (Vachon 1938). Female genitalia with 2 thickened anteriorly-directed spermathecae fused basally into large bursa near genital operculum (Fig. 66); spermathecae covered with small structures that appear to be pores.

Legs: legs I and II with an oblique junction between femur and patella. Leg IV (Fig. 62) with femur + patella 3.41 (♂), 3.73 (♀) times longer than broad. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Male from Lord Howe Island (AM), with 9 other specimens (AM) in parentheses, where appropriate:* Body length 1.86 (1.68–2.03). Pedipalps: trochanter 0.357/0.210 (0.296–0.366/0.187–0.262), femur 0.626/0.212 (0.582–0.810/0.189–0.261), patella 0.557/0.245 (0.499–0.698/0.229–0.310), chela (with pedicel) 0.969/0.337 (0.876–1.186/0.322–0.385), chela (without pedicel) 0.915 (0.832–1.106), hand length 0.490 (0.426–0.593), movable finger length 0.456 (0.419–0.520). Chelicera 0.255/0.28, movable finger length 0.192. Carapace 0.735/0.717 (0.598–0.703/0.568–0.624). Leg I: femur 0.192/0.141, patella 0.275/0.114, tibia 0.285/0.084, tarsus 0.338/0.069. Leg IV: fe-

mur + patella 0.545/0.160, tibia 0.420/0.101, tarsus 0.361/0.077.

Female from Lord Howe Island (AM), with 9 other specimens (AM) in parentheses, where appropriate: Body length 2.17 (1.91–2.78). Pedipalps: trochanter 0.397/0.217 (0.338–0.426/0.206–0.248), femur 0.675/0.224 (0.603–1.009/0.206–0.297), patella 0.610/0.256 (0.567–0.905/0.214–0.331), chela (with pedicel) 1.101/0.398 (0.988–1.310/0.366–0.456), chela (without pedicel) 1.040 (0.938–1.260), hand length 0.557 (0.501–0.754), movable finger length 0.486 (0.418–0.591). Chelicera 0.273/0.144, movable finger length 0.196. Carapace 0.787/0.928 (0.743–0.851/0.647–0.848). Leg I: femur 0.224/0.156, patella 0.319/0.119, tibia 0.307/0.085, tarsus 0.326/0.065. Leg IV: femur + patella 0.615/0.165, tibia 0.462/0.101, tarsus 0.381/0.077.

Tritonymphs: Morphology generally as in adults. Pedipalps, carapace, coxae and legs pale red-brown, remainder of body pale red-yellow.

Chelicera: with 6 setae on hand, *ls* and *is* long and acuminate, *sbs* and *bs′* short and dentate, *bs″* and *es* short and acuminate; movable finger with 1 sub-distal seta (*gs*). Movable finger with single dorsal tooth. Galea long and slender with 4 small distal and 1 small sub-distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 2.84, femur 2.82, patella 2.22, chela (with pedicel) 2.74, chela (without pedicel) 2.58 times longer than wide. Fixed chelal finger with 7 trichobothria, movable chelal finger with 3 trichobothria (Fig. 68): *isb* and *sb* absent; *esb* situated near *eb*; *est* slightly closer to *esb* than to *et*; *ist* adjacent to *ib*; *st* midway between *sb* and *t*, the latter situated in distal third of movable finger. Two very long, stout, dentate setae present on dorsum of chelal hand at base of fixed finger (Fig. 68). Chelal teeth: fixed finger with 28 marginal teeth, plus 4 external accessory teeth and 1 internal accessory tooth; movable finger with 35 marginal teeth, plus 5 external accessory teeth and no internal accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating adjacent to *t* (Fig. 68). External margin of fixed chelal finger with 2 "sense spots," internal margin of fixed chelal finger with 1 "sense spot," and movable chelal finger without "sense spots."

Cephalothorax: carapace 1.01 times longer than broad; with 1 pair of very faint eye-spots; with 4 setae on anterior margin and 8 setae on posterior margin; with two deep furrows.

Abdomen: Pleural membrane uniformly wrinkled plicate. Tergites I–X and sternites II–X with medial suture line. Tergal chaetotaxy: 6: 9: 8: 8: 9: 10: 10: 10: 10: 8: 4: 2. Sternal chaetotaxy: 7: (1) 9 (1): (2) 8 (2): 14: 15: 13: 13: 11: 10: 4: 2.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Tritonymph from Lord Howe Island (AM):* Body length 1.82. Pedipalps: trochanter 0.317/0.178, femur 0.505/0.179, patella 0.457/0.206, chela (with pedicel) 0.846/0.308, chela (without pedicel) 0.796, movable finger length 0.352, hand length 0.443. Carapace 0.627/0.621.

Deutonymphs: Morphology generally as in adults. Pedipalps and carapace pale red-brown, abdomen pale yellow.

Chelicera: with 5 setae on hand, *ls, is, bs* and *es* acuminate, *sbs* dentate; movable finger with 1 sub-distal seta (*gs*). Movable finger with single dorsal tooth. Galea long and slender with 4 small distal to sub-distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 1.78, femur 2.67, patella 1.91, chela (with pedicel) 2.59, chela (without pedicel) 2.47 times longer than wide. Fixed chelal finger with 6 trichobothria, movable chelal finger with 2 trichobothria (Fig. 69): *esb, isb, sb* and *st* absent; *est* closer to *eb* than to *et*; *ist* adjacent to *ib*. Two very long, stout, dentate setae present on dorsum of chelal hand at base of fixed finger (Fig. 69). Chelal teeth: fixed finger with 21 marginal teeth, plus 1 internal accessory tooth; movable finger with 23 marginal teeth, with no accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating near *t* (Fig. 69). External margin of fixed chelal finger with 1 "sense spot," internal margin of fixed chelal finger with 2 "sense spots," and external margin of movable chelal finger with 2 "sense spots."

Cephalothorax: carapace 1.17 times longer than broad; with 1 pair of eye-spots; with 4 setae on anterior margin and 8 setae on posterior margin; with two shallow furrows.

Abdomen: Pleural membrane uniformly

wrinkled plicate. Tergites I–X and sternites II–X with medial suture line. Tergal chaetotaxy: 8: 8: 8: 8: 8: 8: 8: 8: 8: 6: 6: 2. Sternal chaetotaxy: 0: (1) 4 (1): (2) 6 (2): 10: 10: 10: 10: 10: 8: 6: 2.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi with single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Deutonymph from Lord Howe Island (AM):* Body length 1.48. Pedipalps: trochanter 0.257/0.146, femur 0.384/0.144, patella 0.352/0/184, chela (with pedicel) 0.675/0.261, chela (without pedicel) 0.646, movable finger length 0.319, hand length 0.384. Carapace 0.530/0.454.

Protonymphs: Morphology generally as in adults. Pedipalps and carapace pale yellow, abdomen white.

Chelicera: with 4 setae on hand, *ls, is, bs* and *es,* all acuminate; movable finger without seta. Movable finger with single dorsal tooth. Galea long and slender with 2 small distal and 1 small sub-distal rami. Flagellum composed of 4 blades.

Pedipalp: trochanter 2.66, femur 2.39, patella 1.81, chela (with pedicel) 2.81, chela (without pedicel) 2.65 times longer than wide. Fixed chelal finger with 3 trichobothria, movable chelal finger with 1 trichobothrium (Fig. 70): *esb, est, isb, ist, it, b, sb* and *st* absent; *et* situated sub-distally, *eb* and *ib* situated basally, and *t* situated medially. Three very long, stout, dentate setae present on dorsum of chelal hand at base of fixed finger (Fig. 70). Chelal teeth: fixed finger with 21 marginal teeth; movable finger with 21 marginal teeth; both fingers without accessory teeth. Venom apparatus present in movable finger with nodus ramosus terminating distal to *t* (Fig. 70). Chelal fingers without "sense spots."

Cephalothorax: carapace 1.66 times longer than broad; eye-spots not visible; with 4 setae on anterior margin and 6 setae on posterior margin; with two very shallow furrows.

Abdomen: Pleural membrane uniformly wrinkled plicate. Tergites II–X and sternites V–X with medial suture line. Tergal chaetotaxy: 6: 6: 6: 7: 6: 6: 6: 6: 6: 6: 4: 2. Sternal chaetotaxy: 0: (0) 2 (0): (1) 4 (1): 6: 6: 6: 6: 6: 6: 4: 2.

Legs: legs I and II with an oblique junction between femur and patella. Tibiae and tarsi of legs III and IV without tactile setae. Tarsi without single raised slit sensillum. Tarsal claws simple; arolium slightly shorter than claws.

Dimensions (mm).—*Protonymphs from Lord Howe Island (AM):* Body length 1.08. Pedipalps: trochanter 0.165/0.062, femur 0.227/0.095, patella 0.203/0.112, chela (with pedicel) 0.413/0.147, chela (without pedicel) 0.390, movable finger length 0.198, hand length 0.189. Carapace 0.416/0.250.

Remarks.—*Satrapanus grayi* has been found at a variety of locations on Lord Howe Island (Fig. 71) where it occurs in leaf litter and other ground habitats.

DISCUSSION

The Chernetidae are the largest pseudoscorpion family with 112 genera and more than 650 valid species. Harvey (1991) recorded 110 genera, and while six new genera have since been named (Harvey 1992a, 1995; Mahnert 1994, 2001; Muchmore 1997; Dashdamirov 2005), four genera have been synonymized (Muchmore 1992, 1996, 1999; Mahnert 1994). The Australasian fauna is poorly known with only 37 named genera (Table 1) and with many unnamed species represented in museum collections. Including the two new species named in this manuscript, there are only 36 indigenous species named from Australia, 36 from New Zealand, 4 from New Caledonia, 21 from Papua New Guinea, and 20 species from Indonesia. The present study has partially clarified the identity of the genus *Sundochernes* by removing some species originally included in that genus. Three of these species were transferred to *Troglochernes*, a genus originally named for a peculiar troglobite, *T. imitans*, found within caves situated in the Nullarbor karst of southern Australia. A third was removed to a new genus, *Satrapanus*, thus far only known from Lord Howe Island, and two new species were named, one from a cave in north-eastern Queensland and the other from a beetle in south-eastern Queensland.

The new definition of *Troglochernes* presented here avoids the problem of characterizing a genus principally by a suite of troglomorphic features. Nevertheless, *T. imitans* remains one of the most highly modified troglobitic members of the Chernetidae, with extremely attenuated pedipalps and legs (Fig. 3),

Table 1.—Genera of indigenous Chernetidae recorded from the Australasian region.

Genus	Author	Australia	New Zealand	New Caledonia
Acanthicochernes	Beier 1964a			
Allochernes	Beier 1932			
Americhernes	Muchmore 1976	×		
Apatochernes	Beier 1948	× (Norfolk Island)	×	
Austrochernes	Beier 1932	×		
Barbaraella	Harvey 1995	×		
Cacoxylus	Beier 1965			
Calymmachernes	Beier 1954b	×		
Chiridiochernes	Muchmore 1972			
Conicochernes	Beier 1932	×		
Cordylochernes	Beier 1932	×		
Cyclochernes	Beier 1970			
Gelachernes	Beier 1940			
Haplochernes	Beier 1932	×		
Hebridochernes	Beier 1940			×
Heterochernes	Beier 1932		×	
Maorichernes	Beier 1932		×	
Marachernes	Harvey 1992a	×		
Megachernes	Beier 1932	×		
Nesidiochernes	Beier 1957	×	×	×
Nesiotochernes	Beier 1976		×	
Nesochernes	Beier 1932	× (Norfolk Island)	×	
Ochrochernes	Beier 1932			
Opsochernes	Beier 1966		×	
Paracanthicochernes	Beier 1966			
Parachernes	Beier 1932	×		
Paraustrochernes	Beier 1966	×		
Phaulochernes	Beier 1976		×	
Reischekia	Beier 1948		×	
Satrapanus	Harvey & Volschenk, this paper	× (Lord Howe Island)		
Smeringochernes	Beier 1957		×	
Sundochernes	Beier 1932	×		
Sundowithius	Beier 1932			
Systellochernes	Beier 1964b		×	
Thalassochernes	Beier 1932		×	
Troglochernes	Beier 1969	×		
Verrucachernes	Chamberlin 1947			
TOTAL		17	12	2

and is perhaps only rivalled by some species of the Brazilian genus *Spelaeochernes* Mahnert 2001 (Mahnert 2001). The two other cave-dwelling species of *Troglochernes*, *T. guanophilus* and *T. cruciatus* (Fig. 5), show no clear troglomorphies but are paler in coloration than the other three species, *T. novaeguineae*, *T. dewae* (Fig. 4) and *T. omorgus*, suggesting modifications resulting from their cave-dwelling existence. The absence of more obvious morphological changes in *T. guanophilus* and *T. cruciatus* may suggest a more recent colonization of cave environments than *T. imitans*.

The Nullarbor karst is composed of Upper Eocene and Miocene limestones within the Eucla Basin that were deposited during periods when the sea level was up to 300 m above the current sea level (Lowry & Jennings 1974). Substantial uplifting during the late Miocene (10–11 mya) lifted the Eucla Basin above sea level (Lowry & Jennings 1974), thus exposing the karst to weathering and erosion. The entire plain is very flat with little

Table 1.—Extended.

Fiji	Vanuatu	Solomon Islands	Papua New Guinea	Indonesia	Extralimital
		×	×		—
				×	Palaearctic
					Pacific Islands, Americas
					—
					—
					—
		×	×		—
					—
				×	—
					—
					Central and South America, South Africa (doubtful)
		×			—
		×	×		—
×	×	×	×	×	Pacific Islands, Indian Ocean islands, Asia
	×	×	×	×	—
					—
					—
					—
			×	×	Asia
			×	×	Pacific Islands
					—
					—
				×	—
					—
		×			—
		×	×		Circum-tropical
			×		—
					—
				×	—
					—
		×	×	×	Pacific Islands
				×	South-east Asia, South America
			×	×	South-east Asia
					—
					—
			×		—
		×	×	×	Pacific Islands, Asia, Africa
1	2	10	13	12	

inclination. While a marine transgression covered the Roe Plain, which is situated on the southern flank of the Eucla Basin, during the Pleistocene, the remainder of the basin was unaffected. Much of the Nullarbor karst has cavernous spaces ranging from small solution pipes to enormous caves (Richards 1971; Lowry & Jennings 1974). Aspects of the biological attributes of the caves known at the time were documented by Richards (1971), who noted a number of unusual and highly modified troglobites. As well as *T. imitans*, the arachnid fauna of the caves currently consists of several blind spiders including the mygalomorph *Troglodiplura lowryi* Main 1969, the ctenid *Janusia muiri* Gray 1973 and four species of the stiphidiid genus *Tartarus* Gray 1973, *T. mullamullangensis* Gray 1973, *T. murdochensis* Gray 1992, *T. nurina* Gray 1992 and *T. thampannensis* Gray 1992 (Main 1969, 1993; Gray 1973, 1992), as well as a blind gnaphosid (V. Ovtsharenko, pers. comm.). Recently, the discovery and description of a large blind cryptopid centipede, *Cry-*

tops roeplainsensis Edgecombe 2005, with a body length of 4.6–7.8 cm, from two caves on the Roe Plains (Edgecombe 2005) highlights that further troglobites are to be expected from the region.

Troglochernes imitans was coded as a troglophile by Richards (1971), based upon an assessment of the preference for guano habitat and the lack of extremely pallid coloration. But the relatively high levels of morphological modification found in *T. imitans* suggest a long divergence time from the epigean populations from which it was originally derived. The karst has been exposed above sea levels and hence habitable by terrestrial organisms since the late Miocene, but the relative age of the caves themselves is somewhat conjectural.

The three cave-dwelling species of *Troglochernes* all appear to be guanophiles. *Troglochernes imitans* inhabits dry guano produced by the Chocolate Wattled Bat *Chalinolobus morio* (Gray) within caves on the western portion of the Nullarbor Plain (Fig. 71). *Troglochernes guanophilus* resides in bat guano within Fig Tree Cave in southeastern New South Wales; the caves in the Wombeyan region accommodates five bat species including the Common Bent-Wing Bat (*Miniopterus schreibersii*) and the Eastern Horseshoe Bat (*Rhinolophus megaphyllus* Gray) (Jones et al. 1998). Rope Ladder Cave in northeastern Queensland supports large populations of *T. cruciatus* living in or near guano derived from Common Bent-Wing Bats (*Miniopterus schreibersii*) and Little Bent-Wing Bats (*M. australis*). Although *T. imitans*, *T. guanophilus*, and *T. cruciatus* are all found in guano deposits within cave ecosystems, there is some indication that they are not each others closest relatives, suggesting that they have independently developed from surface dwelling ancestors. This evidence consists of the presence of setae within the pleural membrane adjacent to the sternites in the epigean *T. novaeguineae* (Fig. 39) and the cavernicolous *T. cruciatus* (Fig. 47) suggesting that they may represent sister-species. This relationship is, however, only tenuously supported and alternative scenarios may be possible.

The presence of large populations of *Troglochernes cruciatus* within Rope Ladder Cave enables us to document intraspecific variation within what is undoubtedly a single biological species. The meristic data, in particular, including the appendicular ratios presented above, highlight that extreme caution should be used when distinguishing species based solely upon size data when utilizing low numbers of specimens. For example, the pedipalpal femur of *T. cruciatus* was found to be 0.64–0.80 mm long and 2.40–2.98 times longer than broad in males (n = 30) and 0.64–0.82 mm long and 2.43–2.92 times longer than broad in females (n = 30). Similarly the pedipalpal chela (with pedicel) was 1.00–1.37 mm long and 2.66–3.00 times longer than broad in males (n = 30), and 1.05–1.40 mm long and 2.45–2.98 times longer than broad in females (n = 30). These ranges are greater than those cited to designate some newly described species of pseudoscorpions, and although it is not always possible to obtain large series of specimens for taxonomic research, suitable recognition should be given to alternative scenarios where allopatric outliers may not, in fact, represent distinct separate species.

ACKNOWLEDGMENTS

ESV wishes to thank Bronwen Scott and Phil Weinstein for their guidance and Peter Arnold (MTQ) for regular access to laboratory facilities. Chris and Ray Whitney are thanked for providing access to the caves located on their property. The following curators allowed access to the specimens examined during this study: Dr. G. Arbocco (MCSNG), Jürgen Gruber (NHMW), David Hirst and the late David Lee (SAM), John Kethley (FMNH), Graham Milledge (AM) and G.A. Samuelson (BPBM). We also wish to thank Gerry Cassis and Elizabeth Jeffery of the Australian Museum for access to the Lord Howe Island specimens examined during this study. We also thank Peter Mawson (Department of Conservation and Land Management, Perth), John Jennings (University of Adelaide), Alex Baynes (Western Australian Museum) and Norm Poulter (Western Australian Speleological Group) for providing some of the specimens used in this study, Karen Edward (Western Australian Museum) for assistance with some illustrations, and Brian Hanich (Western Australian Museum) who identified the trogid beetle from which *T. omorgus* was taken.

LITERATURE CITED

Beier, M. 1932. Pseudoscorpionidea II. Subord. C. Cheliferinea. Tierreich 58:i–xxi, 1–294.

Beier, M. 1933. Revision der Chernetidae (Pseudoscorp.). Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere 64:509–548.

Beier, M. 1937. Neue ostasiatische Pseudoscorpione aus dem Zoologischen Museum Berlin. Mitteilung aus dem Zoologischen Museum in Berlin 22:268–279.

Beier, M. 1948. Phoresie und Phagophilie bei Pseudoscorpionen. Österreichische Zoologische Zeitschrift 1:441–497.

Beier, M. 1954a. Eine Pseudoscorpioniden-Ausbeute aus Venezuela. Memorie del Museo Civico di Storia Naturale di Verona 4:131–142.

Beier, M. 1954b. Report from Prof. T. Gislén's expedition to Australia in 1951–1952. 7. Pseudoscorpionidea. Acta Universitatis Lundensis, nova series (2) 50:1–26.

Beier, M. 1957. Pseudoscorpionida. Insects of Micronesia 3:1–64.

Beier, M. 1963. Pseudoscorpione aus Vogelnestern von Malaya. Pacific Insects 5:507–511.

Beier, M. 1964a. Further records of Pseudoscorpionidea from the Solomon Islands. Pacific Insects 6:592–598.

Beier, M. 1964b. Insects of Campbell Island. Pseudoscorpionidea. Pacific Insects Monographs 7: 116–120.

Beier, M. 1965. Die Pseudoscorpioniden Neu-Guineas und der benachbarten Inseln. Pacific Insects 7:749–796.

Beier, M. 1966. On the Pseudoscorpionidea of Australia. Australian Journal of Zoology 14:275–303.

Beier, M. 1967a. Pseudoscorpione vom kontinentalen Südost-Asien. Pacific Insects 9:341–369.

Beier, M. 1967b. Some Pseudoscorpionidea from Australia, chiefly from caves. Australian Zoologist 14:199–205.

Beier, M. 1969. Neue Pseudoskorpione aus Australien. Annalen des Naturhistorischen Museums in Wien 73:171–187.

Beier, M. 1973. Pseudoscorpionidea von Ceylon. Entomologica Scandinavica, Supplement 4:39–55.

Beier, M. 1974a. Brasilianische Pseudoscorpione aus dem Museum in Genf. Revue Suisse de Zoologie 81:899–909.

Beier, M. 1974b. Pseudoscorpione aus Nepal. Senckenbergiana Biologica 55:261–280.

Beier, M. 1975. Neue Pseudoskorpione aus Australien und Neu-Guinea. Annalen des Naturhistorischen Museums in Wien 78:203–213.

Beier, M. 1976. The pseudoscorpions of New Zealand, Norfolk and Lord Howe. New Zealand Journal of Zoology 3:199–246.

Beier, M. 1982. Zoological results of the British Speleological Expedition to Papua New Guinea 1975. 9. Pseudoscorpionidea. Acta Zoologica Bulgarica 19:43–45.

Benedict, E.M. & D.R. Malcolm. 1982. Pseudoscorpions of the family Chernetidae newly identified from Oregon (Pseudoscorpionida, Cheliferoidea). Journal of Arachnology 10:97–109.

Beron, P. 2002. On the high altitude pseudoscorpions (Arachnida: Pseudoscorpionida) in the Old World. Historia Naturalis Bulgarica 14:29–44.

Chamberlin, J.C. 1924. *Hesperochernes laurae*, a new species of false scorpion from California inhabiting the nest of *Vespa*. Pan-Pacific Entomologist 1:89–92.

Chamberlin, J.C. 1931. The arachnid order Chelonethida. Stanford University Publications, Biological Sciences 7(1):1–284.

Chamberlin, J.C. 1947. Three new species of false scorpions from the islands of Guam (Arachnida, Chelonethida). Occasional Papers of the Bernice P. Bishop Museum 18(20):305–316.

Chamberlin, J.C. 1952. New and little-known false scorpions (Arachnida, Chelonethida) from Monterey County, California. Bulletin of the American Museum of Natural History 99:259–312.

Chamberlin, R.V. 1925. Diagnoses of new American Arachnida. Bulletin of the Museum of Comparative Zoology 67:211–248.

Dashdamirov, S. 2005. Pseudoscorpions from the mountains of northern Pakistan (Arachnida: Pseudoscorpiones). Arthropoda Selecta 13:225–261.

Dew, B.B. 1968. Bats and bat banding at Wombeyan 1961–1966. Journal of the Sydney University Speleological Society 7:28–35.

Edgecombe, G.D. 2005. A troglomorphic species of the centipede *Crytops* (*Trigonocryptops*) (Chilopoda: Scolopendromorpha) from Western Australia. Records of the Western Australian Museum 22:315–323.

Ellingsen, E. 1911. Pseudoscorpions from Sumatra. Annali del Museo Civico di Storia Naturale di Genova (3a) 5:34–40.

Gervais, P. 1849. Aracnidos. Pp. 517. *In* Historia Fisica y Politica de Chile, Zoologia. (C. Gay, ed.). Volume 4. Claudio Gay and Museo de Historia Naturel de Santiago, Paris and Santiago.

Gray, M.R. 1973. Cavernicolous spiders from the Nullarbor Plain and south-west Australia. Journal of the Australian Entomological Society 12:207–221.

Gray, M.R. 1992. The troglobitic spider genus *Tartarus* Gray with a cladistic analysis of *Tartarus* and *Baiami* Lehtinen (Araneae: Stiphidiidae). Proceedings of the Linnean Society of New South Wales 113:165–173.

Harvey, M.S. 1981. A checklist of the Australian Pseudoscorpionida. Bulletin of the British Arachnological Society 5:237–252.

Harvey, M.S. 1985. Pseudoscorpionida. Pp. 126–

155. *In* Zoological Catalogue of Australia. (D.W. Walton, ed.). Volume 3. Australian Government Publishing Service, Canberra, Australia.

Harvey, M.S. 1988. Pseudoscorpions from the Krakatau Islands and adjacent regions, Indonesia (Chelicerata: Pseudoscorpionida). Memoirs of the Museum of Victoria 49:309–353.

Harvey, M.S. 1991. Catalogue of the Pseudoscorpionida. Manchester University Press, Manchester, UK. 850 pp.

Harvey, M.S. 1992a. A new genus of myrmecophilous Chernetidae from southern Australia (Pseudoscorpionida). Records of the Western Australian Museum 15:763–775.

Harvey, M.S. 1992b. The phylogeny and classification of the Pseudoscorpionida (Chelicerata: Arachnida). Invertebrate Taxonomy 6:1373–1435.

Harvey, M.S. 1994. Redescription and the systematic position of the Brazilian genus *Xenochernes* Feio (Pseudoscorpionida: Chernetidae). Journal of Arachnology 22:131–137.

Harvey, M.S. 1995. *Barbaraella* gen. nov. and *Cacoxylus* Beier (Pseudoscorpionda: Chernetidae), two remarkable sexually dimorphic pseudoscorpions from Australasia. Records of the Western Australian Museum, Supplement 52:199–208.

Hentschel, E. & W.B. Muchmore. 1989. *Cocinachernes foliosus*, a new genus and species of pseudoscorpion (Chernetidae) from Mexico. Journal of Arachnology 17:345–349.

Hoff, C.C. 1949. The pseudoscorpions of Illinois. Bulletin of the Illinois Natural History Survey 24:407–498.

Howarth, F.G. 1988. Environmental ecology of north Queensland caves: or why are there so many troglobites in Australia? Pp. 76–84. *In* Preprints of Papers for the 17th Biennial Conference, Australian Speleological Federation Tropicon Conference, Lake Tinaroo, Far North Queensland. (L. Pearson, ed.). Australian Speleological Federation, Cairns, Australia.

Jones, K., E. Holland, M. Chalker & A. Ramsay. 1998. Wombeyan Karst Conservation Reserve. Draft management plan. Jenolan Caves Reserve Trust, Bathurst, Australia.

Lowry, D.C. & J.N. Jennings. 1974. The Nullarbor karst Australia. Zeitschrift für Geomorphologie 18:35–81.

Mahnert, V. 1978. Die Pseudskorpiongattung *Toxochernes* Beier, 1932. Pp. 309–315. *In* Arachnology: Seventh International Congress. Symposia of the Zoological Society of London. (P. Merrett, ed.). Volume 42. Academic Press, London.

Mahnert, V. 1985. Weitere Pseudoskorpione (Arachnida) aus dem zentralen Amazonasgebiet (Brasilien). Amazoniana 9:215–241.

Mahnert, V. 1987. Neue oder wenig bekannte, vorwiegend mit Insekten vergesellschaftete Pseudoskorpione (Arachnida) aus Südamerika. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 60:403–416.

Mahnert, V. 1994. New chernetid pseudoscorpions (Pseudoscorpionida: Chernetidae) from Venezuela and Brazil, with remarks on the genus *Ancalochernes* Beier. Revue Suisse de Zoologie 101: 829–838.

Mahnert, V. 2001. Cave-dwelling pseudoscorpions (Arachnida, Pseudoscorpiones) from Brazil. Revue Suisse de Zoologie 108:95–148.

Main, B.Y. 1969. A blind mygalomorph spider from a Nullarbor Plain Cave. Journal of the Royal Society of Western Australia 52:9–11.

Main, B.Y. 1993. Biogeographic significance of the Nullarbor cave mygalomorph spider *Troglodiplura* and its taxonomic affinities. Journal of the Royal Society of Western Australia 76:77–85.

Menge, A. 1855. Über die Scheerenspinnen, Chernetidae. Neueste Schriften der Naturforschenden Gesellschaft 5(2):1–43.

Moulds, T. 2004. Review of Australian cave guano ecosystems with a checklist of guano invertebrates. Proceedings of the Linnean Society of New South Wales 125:1–42.

Muchmore, W.B. 1971. Phoresy by North and Central American pseudoscorpions. Proceedings of the Rochester Academy of Science 12:79–97.

Muchmore, W.B. 1972. A remarkable pseudoscorpion from the hair of a rat (Pseudoscorpionida, Chernetidae). Proceedings of the Biological Society of Washington 85:427–432.

Muchmore, W.B. 1974. Clarification of the genera *Hesperochernes* and *Dinocheirus* (Pseudoscorpionida, Chernetidae). Journal of Arachnology 2: 25–36.

Muchmore, W.B. 1975. Use of the spermathecae in the taxonomy of chernetid pseudoscorpions. Pp. 17–20. *In* Proceedings of the 6th International Arachnological Congress. Vrije Universiteit of Amsterdam, Amsterdam.

Muchmore, W.B. 1984. The pseudoscorpions described by R.V. Chamberlin (Pseudoscorpionida, Olpiidae and Chernetidae). Journal of Arachnology 11:353–362.

Muchmore, W.B. 1992. Cavernicolous pseudoscorpions from Texas and New Mexico (Arachnida: Pseudoscorpionida). Texas Memorial Museum, Speleological Monographs 3:127–153.

Muchmore, W.B. 1996. On the occurrence of *Wyochernes* in Asia (Pseudoscorpionida: Chernetidae). Bulletin of the British Arachnological Society 10:215–217.

Muchmore, W.B. 1997. *Tuberochernes* (Pseudoscorpionida, Chernetidae), a new genus with species in caves in California and Arizona. Journal of Arachnology 25:206–212.

Muchmore, W.B. 1999. Redefinition of the genus

Chelanops Gervais (Pseudoscorpionida: Chernetidae). Pan-Pacific Entomologist 75:103–111.

Poinar, G.O., Jr, B.P.M. Curcic & J.C. Cokendolpher. 1998. Arthropod phoresy involving pseudoscorpions in the past and present. Acta Arachnologica 47:79–96.

Richards, A.M. 1971. An ecological study of the cavernicolous fauna of the Nullarbor Plain, southern Australia. Journal of Zoology, London 164:1–60.

Scholtz, C.H. 1986. Revision of the genus *Trox* Fabricius (Coleoptera: Trogidae) in the Australasian region. Australian Journal of Zoology, Supplementary Series 125:1–99.

Tenorio, J.M. & W.B. Muchmore. 1982. Catalog of entomological types in the Bishop Museum. Pseudoscorpionida. Pacific Insects 24:377–385.

Vachon, M. 1938. Recherches anatomiques et biologiques sur la réproduction et le développement des Pseudoscorpions. Annales des Sciences Naturelles, Zoologie (11) 1:1–207.

Vachon, M. 1940. Remarques sur la phóresie des Pseudoscorpions. Annales de la Société Entomologique de France 109:1–18.

Vitali-di Castri, V. 1972. El genero sudamericano *Gigantochernes* (Pseudoscorpionida, Chernetidae) con descripcion de dos nuevas especies. Physis, Buenos Aires 31:23–38.

Weinstein, P. & D. Slaney. 1995. Invertebrate faunal survey of Rope Ladder Cave, northern Queensland: a comparative study of sampling methods. Journal of the Australian Entomological Society 34:233–236.

With, C.J. 1905. On Chelonethi, chiefly from the Australian region, in the collection of the British Museum, with observations on the "coxal sac" and on some cases of abnormal segmentation. Annals and Magazine of Natural History (7) 15: 94–143, 328.

Manuscript received 28 June 2006, revised 28 December 2006.

2007. The Journal of Arachnology 35:278–292

THE UTILITY OF MOLECULAR MARKERS FROM NON-LETHAL DNA SAMPLES OF THE CITES II PROTECTED "TARANTULA" *BRACHYPELMA VAGANS* (ARANEAE, THERAPHOSIDAE)

Stuart J. Longhorn,[1, 2] **Martin Nicholas,**[3] **Julie Chuter**[3, 4] and **Alfried P. Vogler**[1, 2]: [1]Molecular Systematics Laboratory, Department of Entomology, The Natural History Museum, London, SW7 5BD, UK. E-mail: stul@pdx.edu; [2]Imperial College of Science, Technology and Medicine, London, SW7 2BX, UK; [3]St. Thomas House, Wells, Somerset, England, BA5 3LA, UK; [4]Sparsholt College Hampshire, Winchester, SO21 2NF, UK

ABSTRACT. Tarantula spiders of the genus *Brachypelma* Simon 1891 are the only complete genus of arachnids protected from international trade under CITES law. To better understand the genetic cohesion of spiders within this genus, we evaluated multiple genetic fragments (totalling about 2200 bp) for their ability to recover population sub-structure among wild-caught *Brachypelma vagans* (Ausserer 1875) from Belize. We used a novel non-lethal method of tissue sampling, by inducing autospasy of the medial leg. This method allowed us to release wild-caught individuals of this protected species after DNA sampling. We used arachnid specific PCR primers to amplify targeted regions of *B. vagans* DNA, testing various combinations for consistency. We compared mitochondrial fragments from two populations of *B. vagans* (~50 km apart) for variation in mitochondrial 16S lrRNA (plus 5′ ND1), CO1, and the nuclear ITS-2 spacer. Both lrRNA-ND1 and CO1 provided congruent estimates of population subdivision, and indicated that lrRNA-ND1 contained the greatest variation. The nuclear ITS-2 was surprisingly short (193 bp) and relatively invariant across *B. vagans*. While both mitochondrial fragments appear suitable to elucidate population subdivision and historical processes in *B. vagans*, we suggest that mitochondrial markers may overestimate population division in *B. vagans*. We conclude that along with valuable inferences from mitochondrial regions, the characterization of population sub-structure in tarantula spiders will be enhanced by other estimates from alternate nuclear fragments.

Keywords: Belize, forced autospasy, molecular divergence

The "tarantula" family Theraphosidae (Araneae, Mygalomorphae) includes some of the most impressive spiders alive today. In particular, members of the genus *Brachypelma* are conspicuous members of the Mesoamerican invertebrate fauna, but are threatened by habitat destruction, road building, and illegal pet-trade collection (Cleva 1998; Locht et al. 1999). Over-collection of the red-knee tarantula *Brachypelma smithi* (F.O. Pickard-Cambridge 1897) for the pet-trade was a major factor that led to the blanket protection of the entire genus under CITES (Convention on International Trade in Endangered Species). To date, *Brachypelma* is the only genus of arachnids completely protected under CITES (Anonymous 1998). The anthropogenic pressures on natural populations of *Brachypelma* are exacerbated by high juvenile mortality rates (Baerg 1958) and low population densities (Yáñez & Floater 2000).

Conservation efforts to protect threatened arthropods can be most effectively focused with clarification of the genetic affinities among individuals (Joyce & Pullin 2003; Paquin & Hedin 2004). Here, we use three genetic regions to evaluate population subdivision in the red-rump tarantula *Brachypelma vagans* (Ausserer 1875). Unlike most *Brachypelma* species with narrow geographic ranges, *B. vagans* is unusually widespread (Locht et al. 1999; Striffler & Graminske 2003; West 2005). This species is found across much of the Yucatan peninsula from Mexico to Guatemala and perhaps Honduras, with minor morphological variation across the range (Smith 1994). The wide distribution of *B. vagans* compared to its congeners, and its sur-

vival in a wide variety of natural habitats (from peri-humid forest to savannah ecotypes) suggests that the species is, in reality, a low risk species for conservation priorities. Furthermore, *B. vagans* exploits areas extensively modified by human activity (Nicholas 2002; M'Rabet et al. 2005), showing a broad habitat tolerance unlike most other members of the genus, where the ecology may be easily disrupted by human activity (Locht et al. 1999; Yáñez & Floater 2000). In this context, the widespread *B. vagans* might provide a low-risk model against which to evaluate the population dynamics of more vulnerable *Brachypelma* species.

Most fossorial tarantulas, including all *Brachypelma* spp., have similar ecologies to *B. vagans*, and hence share population dynamics that can result in clustered spatial distribution and over-dispersion (Reichling 1999, 2003; Yáñez & Floater 2000; Shillington & McEwen 2006). Long-range gene flow is mediated by mature males, which migrate long-distances between aggregated populations of females (Shillington & Verrell 1997; Janowski-Bell & Horner 1999; Yáñez & Floater 2000). Exceptionally in *B. vagans*, recently hatched siblings show an unusual "group-dispersal" behavior (Reichling 2000, 2003; Nicholas 2002; Shillington & McEwen 2006). This unusual behavior, unknown in other species of *Brachypelma* may add to the relative persistence and wide geographic range of *B. vagans* compared to its congeners and might allow new populations (i.e., colonies) to establish from sibling groups that dispersed in unison. However, to our knowledge, genetic affinities have not been evaluated in any natural populations of fossorial tarantulas to date.

A problem for genetic studies of threatened taxa is the potential need to sacrifice valuable specimens (Lushai et al. 2000). This is not defensible with tarantulas due to their small population sizes, slow growth, and low reproductive success. We therefore evaluated a non-lethal technique for DNA tissue sampling, by inducing leg autospasy from live specimens (Longhorn 2001, 2002). To clarify the population sub-structure and gene flow in *Brachypelma vagans*, we then evaluated three genetic fragments for their ability to distinguish individuals from two populations in Belize. The combination of geographic and genealogical data (phylo-geographic analyses) has been widely used to infer the historical distribution and range movements of araneomorph spiders (Hedin 1997a, b; Masta 2000a; Hormiga et al. 2003; Ayoub & Riechert 2004; Paquin & Hedin 2004; Arnedo & Gillespie 2006; Murphy et al. 2006). Few studies of this type have been conducted with mygalomorph spiders (but see Bond et al. 2001, 2006; Bond 2004; Hendrixson & Bond 2005; Woodman et al. 2006), none of which focused on the tarantula family Theraphosidae.

We suggest that estimates of gene flow and population sub-structure from the widespread species *B. vagans* can help clarify the geographic distribution of other threatened *Brachypelma* species. Due to sex-biased dispersal patterns in fossorial theraphosids, we predict that mitochondrial markers might provide suitable resolution for population-level studies of theraphosids, and plausibly reveal a high degree of population sub-structure as in other mygalomorphs (e.g., Bond et al. 2001). By tracking the maternal lineage, we might expect mitochondrial markers to show minimal genetic differentiation among spiders within localized (spatially aggregated) populations and much greater differentiation across neighboring populations. In contrast, nuclear markers might be expected to show a greater degree of homogeneity across different levels of geographic sampling, due to the greater degree of admixture and gene flow mediated by long-range migration of mature males.

Here, we compare genetic variability of two mitochondrial fragments (16S lrRNA-ND1, and CO1) and a nuclear region (ITS-2 spacer) from *B. vagans* to define the variation in different genetic regions and evaluate population subdivision. The genetic sub-structure of *B. vagans* populations may be useful to contrast against other allied species with narrower geographic distributions. Clarifying the genetic affinities of any natural populations of theraphosid spiders, especially the genus *Brachypelma*, should facilitate attempts to maintain viable populations of the most threatened species and help ensure their long-term survival.

METHODS

Non-Lethal Tissue Sampling.—As a novel source of DNA, we removed a single medial leg from tarantulas by autospasy, inducing the voluntary fracture at the coxa-trochanter joint (Breene 1998; Johnson & Jakob 1999; Brau-

tigam & Persons 2003). We chose to remove a medial limb (III) as anterior legs (I–II) are used in sensory behaviors such as prey detection, while posterior legs (IV) are used defensively in brushing urticating hairs (Smith 1994). Initial trials of autospasy were conducted on captive theraphosids prior to field trials, mostly on other species of *Brachypelma* (n = 30). This allowed an evaluation of their survivorship in a controlled environment. Field trials were conducted on two populations of *B. vagans* in Southern Belize (48.2 km apart). Field caught spiders were lured from burrows at dusk during the primary foraging period by vibrating a grass stalk near the burrow entrance to simulate prey movements (Longhorn 2002; Nicholas 2002). We sampled several individuals (n = 14) in a grass clearing within a broadleaf forest near the *Las Cuevas* Research Station in the Chiquibul forest, Southern Cayo District, Belize (16°44′08″N, 88°59′20″W). The second field sample (n = 8) was from an open grassy area near Pooks Hill Lodge, Northern Cayo District, Belize (17°09′25″N, 88°51′13″W). Captured spiders were restrained by a sponge in a clear plastic container, and the femur of leg III grasped using forceps. The leg was then gently manipulated until voluntary rupture of the coxal apodeme was achieved and the isolated limb placed in absolute ethanol (stored at −20° C). The natural wound seal at the coxal stump was artificially enhanced with cosmetic nail hardener during which time spiders were placed in open containers to allow air-circulation (after Breene 1998). Spiders were released back to intact burrows within a few hours of the procedure (after confirming wound sealing). Where possible, burrows were revisited over successive days to assess the status of their occupant. All tissue samples were collected by permits from the Belize forestry department (CD/60/3/01 and CD/72/2/01) and transported under CITES permits (Export 001214; UK Import 237532). Tissue is stored at −80° C at the Natural History Museum (NHM), London, UK. We also collected a single voucher specimen from each locality, both confirmed as *B. vagans* by morphological evaluation (by Mr. A. Smith). Specimens were deposited together in the NHM arachnid collection under accession BMNH (E) 2003-148.

DNA extractions.—Under sterile conditions, ~1 g of muscle was taken from isolated legs of 22 *B. vagans* (wild caught from Belize) and a mature male *B. angustum* Valerio 1980 (captive-bred stock). Tissue was vacuum dried, then incubated at 37° C in the presence of 5 μl of 20 mg/ml proteinase K, 20 μl of 10% SDS, and 200 μl of TEN buffer (200 μl of 0.1 M Tris-HCl at pH 8.0, 100 mM NaCl, 10 mM Na_2EDTA). DNA was extracted with phenol:chloroform:isoamyl alcohol (24:24:1) and then chloroform:isoamyl alcohol (24:1), before precipitation with absolute ethanol: NaAc, then rinsed with 70% ethanol, and resuspensed in ddH_2O.

PCR primers.—For each targeted genetic fragment, multiple primers were tested for reliable PCR amplification in 50 μl reactions containing 1–2 μl of $MgCl_2$ (50 mM), 1 μl of dNTPs (10 mM), 1 μl of each primer (10 pmol), 5 μl buffer (10 ×), 0.2 μl (1 U) of *Taq* (Bioline), each made to volume with dd H_2O.

To amplify ~1-Kb of the mitochondrial lrRNA (16S) to ND1 (NADH1) we used LR-N-13398 (CGCCTGTTTAACAAAAACAT) (Simon et al. 1994, aka 16Sar) with NI-J-12261 (TCRTAAGAAATTATTTGA) (Hedin 1997a, b). In other spiders [except Mesothelae], this fragment includes a single tRNA (Leucine; CUN) and a short spacer region (Fig. 1, top). Fragments were amplified using a thermal cycle of 95° (120 s); 5 cycles of 95° (30 s), 46° (30 s), 72° (120 s); then 35 cycles of 95° (30 s), 48° (30 s), 72° (120 s); and 72° (10 min). We used LR-N-12945 (CGACCTC GATGTTGAATTAA) (Hedin & Maddison 2001) as a third nested primer for internal sequencing of these amplifications. We tested the ability of other primers to amplify a shorter fragment (~650 bp) of the same region that could be sequenced in a single reaction. We compared amplifications from LR-N-12945 with NI-J-12261, plus LR-N-13398 with NI-J-12581 (CCTTTACGAATTTGAATATA) (Hedin & Maddison 2001) and Hb16S (TTA CGGAAGTGCACATATCG) with HbND1 (TGAGCTACTCTTCGAATAGC) (Masta 2000a).

To amplify ~1-Kb of the mitochondrial Cytochrome Oxidase (CO1) gene, we used primer C1-J-1751 (GAGCTCCTGATATAGCTTT TCC) with C1-N-2776 (GGATAATCAGAA TATCGTCGAGG) (Hedin & Maddison 2001). Fragments were amplified using a thermal cycle of 95° (120 s); 35 cycles of 95° (30

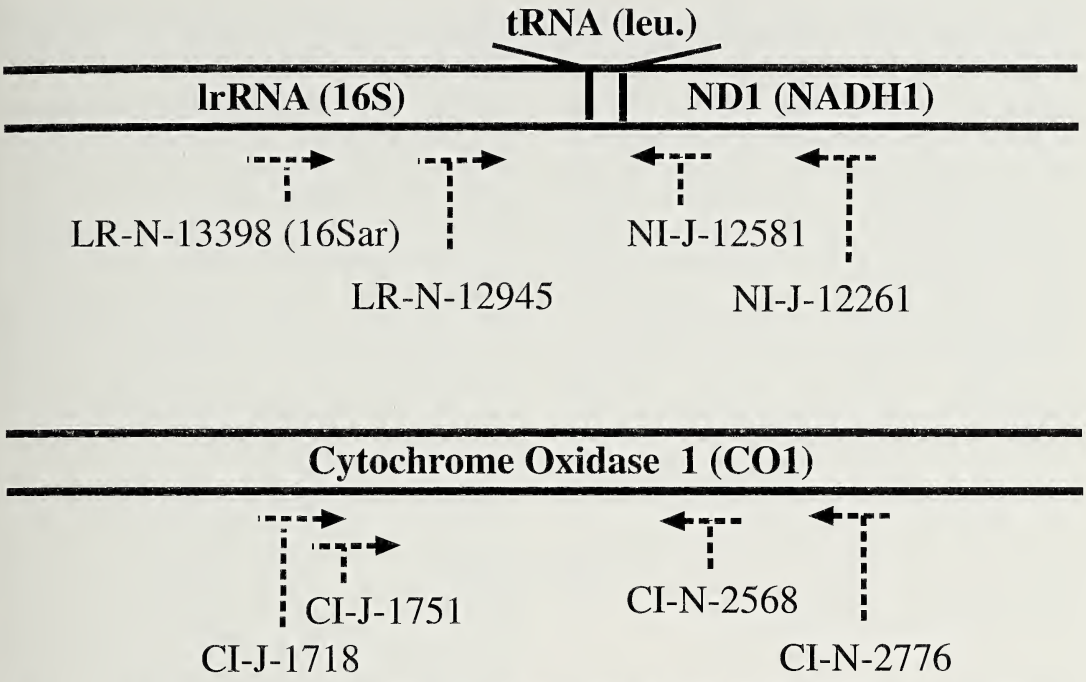


Fig. 1.—Top: The lrRNA-ND1 fragment of the mitochondrial genome sequenced in *Brachypelma vagans* (adapted from Masta 2000b). Bottom: The CO1 mitochondrial fragment sequenced in *B. vagans*.

s), 52° (30 s), 72° (110 s); and 72° (10 min), modified from Hedin (2001) and Hedin & Maddison (2001). We used CI-N-2568 (GCT ACAACATAATAAGTATCATG) (Hedin & Maddison 2001) as a nested primer for sequencing (See Fig. 1, bottom). Again, we tested other primer combinations for amplification consistency including C1-J-1751 with CI-N-2568 (GCTACAACATAATAAGTATC ATG) (Hedin & Maddison 2001) and CI-J-1718 (GGATCACCTGATATAGCATTCCC) (Simon et al. 1994) with CI-N-2776. Finally, to amplify ~350 bp of the nuclear (rRNA) internal transcribed spacer 2 (ITS-2), we used the primers 5.8S (GGGACGATGAAGAACG CAGC) with 28S (TCCTCCGCTTATTGAT ATGC) (Hedin 1997b). Reactions were run using 95° (120 s); 35 cycles of 95° (30 s), 48° (60 s), 72° (110 s); and 72° (10 min).

Purification and Sequencing.—PCR products were purified using a Gene-clean II kit (Bio 101), and run on an ABI PRISM® 377 automated sequencer (PE Applied Biosystems) using standard protocols. We sequenced both strands of the largest reliable amplification products for all specimens (except ITS-2 from Cuevas 10 and 11). Chromatograms were edited using Sequencher™ 4.1 (Gene Codes Corp, MI), and ambiguities scored with IUPAC coding. Sequences of *B. vagans* were deposited in GenBank as AJ585053–AJ585072 (ITS-2), AJ584615–AJ584636 (CO1) and AJ585387–AJ585408 (lrRNA-ND1). For *B. angustum*, the accessions are AJ585073 (ITS-2), AJ584637 (CO1), and AJ585409 (lrRNA-ND1).

Population diversity analyses.—Population diversity was calculated using DnaSP version 3.50 (Rozas & Rozas 1999) assuming that the lrRNA-ND1 and CO1 mitochondrial fragments show uni-parental inheritance and complete vegetative assortment, while nuclear ITS-2 is autosomal and diploid (though probably part of a repetitive family). For each fragment (lrRNA-ND1; CO1 and ITS-2) we evaluated variation within each population by the number of segregating nucleotides per sequence (S), and average nucleotide differences (κ) (Nei 1987). Values for S and κ were used to estimate the neutral parameter (θ) (Watterson 1975; Tajima 1983), and the D statistic of Tajima (1989). Under the finite-sites model, the differences between estimates of θ (from either S or κ) are expected to be zero where there is no selection and demographic conditions are at equilibrium. Population subdivi-

sion was described using the F_{ST} statistic (Wright 1951), which estimates the fraction of sequence diversity attributed to differences between populations (here, colonies). Using the pair-wise variance approach (Hudson et al. 1992), F_{ST} was estimated as 1 minus the ratio of within population heterozygosity (H_w) to between population heterozygosity (H_B). Slatkin & Maddison's (1989) coalescent approach provided estimates of the effective number of migrating individuals per generation (N_m), assuming the sampled genes are selectively equivalent, and that population structure approximates an island model. For each genetic fragment, haplotype relationships were generated using statistical parsimony (Templeton et al. 1995) using TCS version 1.13 (Clement et al. 2000). Other comparisons between sequences of *B. vagans* and other arachnids were made with Blast tools at http://www.ncbi.nlm.nih.gov/BLAST/.

RESULTS

Non-lethal DNA sampling in *Brachypelma*.—Trials of leg autospasy on several captive bred theraphosids (mostly *Brachypelma* spp.) showed that spiders regained normal behavior within 4–5 h, and accepted prey items within a few days (Longhorn 2001). These results matched our field experiences with *B. vagans* in Belize, where we successfully induced autospasy from all spiders, averaging between 30 s and 3 min per specimen. In many cases, juveniles were noticeably more willing to cast limbs than adults. In captive spiders, survival rates were high. At 3 mo post-autospasy, all captive specimens remained alive, although after 6 mo survivorship had decreased to 97% and fell to 94% after 1 yr. Though we did not maintain a separate control sample, this mortality rate is not much different from the standard survivorship of captive tarantulas. However, all surviving spiders in the captive group regenerated lost limb(s) over successive molts. Return observations of the Las Cuevas population during 2004 (by A. P. Vogler) showed a high density of *B. vagans* at this field site three years after autospasy was conducted. During the 2004 re-evaluation, large specimens of *B. vagans* (age > 5 yr) were found to occupy the same burrows where spiders had been returned after tissue sampling. In our opinion, the presence of these large spiders probably indicates the long-term survival of wild caught specimens following autospasy (for DNA), or at the least, re-colonization of empty burrows.

Survey of PCR primers for *Brachypelma*.—We generated ~2200 bp of DNA sequence from 22 *B. vagans*, for three genetic regions (except ITS-2 in Las Cuevas 10 and 11). For lrRNA-ND1, the most consistent PCR amplification was using primers LR-N-13398 and NI-J-12261 to give a 940 bp fragment. However, other primer combinations were quite effective in amplifying a shorter fragment of the same region, like LR-N-12945 with NI-J-12261 which readily yielded ~650 bp. Using the same thermal profile (on request), LR-N-13398 and NI-J-12581 amplified a similar size fragment (~650 bp) with far less consistency and lower product yield than other primer combinations. Primers Hb16S with HbND1 failed to give consistent amplifications in *B. vagans* using the thermal profile for Salticidae (Masta 2000b) or with profile variations. For the CO1, the primer C1-J-1751 worked best with CI-N-2776 to give a 960 bp fragment, but C1-J-1751 also worked well with CI-N-2568 yielding about 850 bp. Here, consistency was increased by adding 5 low stringency cycles of 95° (30 s), 50° (30 s), 72° (60 s) prior to 35 cycles as for CI-J-1751 with CI-N-2776. The same modified profile can also be used to amplify CO1 from other species of *Brachypelma* and more distantly related Theraphosidae (Longhorn 2001, unpublished data). Primer CI-J-1718 with CI-N-2776 failed to give strong amplifications for *B. vagans* under a variety of thermal conditions.

Characterization of the lrRNA-ND1.—The 940 bp fragment of lrRNA-ND1 from *B. vagans* gave the closest nucleotide match (by BlastN) to published mitochondrial sequences from jumping spiders (Araneae, Salticidae). Nucleotide identity was higher between *B. vagans* and several Salticidae (Top match 6e-44 to AY477266; Hedin & Maddison 2001, 2003) than another tarantula (6e-41) *Haplopelma huwenum* (Wang et al. 1993; Qiu et al. 2005) [In Genbank as *Ornithoctonus huwena* NC_005925, but recently transferred to *Haplopelma* (Araneae, Theraphosidae)]. However, measures of similarity alone often give misleading views of taxon affinities, while informative characters can be more useful. That said, similarity is often useful to identify func-

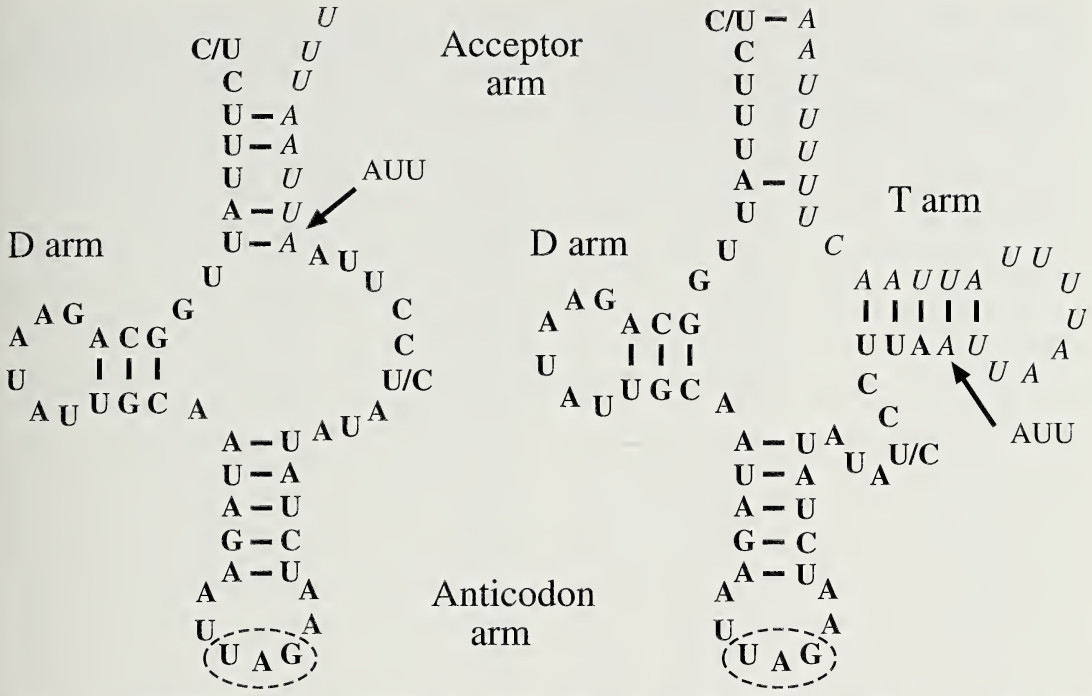


Fig. 2.—Putative secondary structures for tRNA leucine in the mygalomorph spider *B. vagans*. Left: Structure fitted to the truncated model from the araneomorph spider *Habronattus* (Masta & Boore 2004). Right: Fitted to yield a conventional "cloverleaf" structure with a functional TψC arm.

tionally important domains. Four regions of high identity were detected between lrRNA-ND1 of *B. vagans* and other spiders. The largest high identity segment (about 203 nucleotides) corresponded to the peptidyl transferase centre of the lrRNA, identified using the secondary structure map of the salticid *Habronattus oregonensis* (Peckham & Peckham 1888) (Masta 2000b). The next largest identity region (30/33 bp) was the DHU and anticodon arms of $tRNA^{LEU(CUN)}$. With the exception of two sites, the *B. vagans* tRNA sequences were identical across all 22 individuals, and had a higher AT composition (75.6%) than lrRNA (~68.5%). The $tRNA^{LEU(CUN)}$ in *H. oregonensis* folds to an unusual truncated structure, lacking the TψC arm (Masta 2000b; Masta & Boore 2004). According to this truncated model, the TψC arm is substituted by a TV-replacement loop. It is possible to fit a cloverleaf structure to this tRNA in *H. oregonensis*, but this model allows less complementary bases in the acceptor arm than the truncated model and more problematic overlap with ND1 (Masta 2000b; Masta & Boore 2004). The truncated model fits reasonably well to $tRNA^{LEU(CUN)}$ from *B. vagans*, with strong intra-molecular pairing (high degree of complementary) in the DHU-arm (Fig. 2, left). In the anticodon arm, the UAG anticodon was found as expected, supported by five paired stem bases as *H. oregonensis*. The cloverleaf structure fits well to the *B. vagans* data (Fig. 2, right), revealing the possibility of a conventional TψC arm. For our data, the cloverleaf model also requires greater mismatch in the acceptor stem than the truncated model plus more overlap with ND1 (Longhorn 2001). The start codon of *Brachypelma* ND1 appears to be an atypical ATT (AUU), as in other spiders (Masta 2000b). To accept the cloverleaf structure as the preferred model for *B. vagans* requires the first seven codons (18 b.p.) of ND1 to also function as part of the $tRNA^{LEU(CUN)}$. If allowed, our data suggest that the canonical cloverleaf model provides a better fit to *Brachypelma* $tRNA^{LEU(CUN)}$ than the truncated model, which fitted best for *H. oregonenesis* (Masta 2000b).

Characterization of ITS-2.—The 358 bp ITS-2 from *B. vagans* gave the closest nucleotide (BlastN) match to *Aphonopelma hentzi*

(Girard 1852) (AY210803, Mallatt et al. 2004). This is another tarantula closely allied to the *Brachypelma*, both in the subfamily Theraphosinae (Smith 1994). A single region of high identity (e-129) was found between these two genera (92%; 338/367). Across different *B. vagans*, much of the fragment at the 5.8S rRNA end is invariant (up to 120 bp here; 162 bp in *A. hentzi*). The small segment of 28S rRNA included was also invariant across *B. vagans*, with only two nucleotide differences from *A. hentzi*. In the actual ITS-2 spacer, there were size differences between *A. hentzi* (203 bp) and *B. vagans* (193 bp) and nineteen inter-specific nucleotide differences, most at the 3′. The available ITS-2 from other spiders matched *B. vagans* with much less identity, the next most significant (2e-50) was *Orsonwelles* spp. Hormiga 2002 (Araneae, Linyphiidae) (Hormiga et al. 2003). Sequences from other araneomorphs matched less well again, like Theridiidae (S.W. A'Hara, unpublished), Nesticidae (Hedin 1997b), Linyphiidae (Hormiga et al. 2003) and Salticidae (Arnedo & Gillespie 2006), many of which had a similar size (150–250 bp) to our ITS-2 (193 bp), considerably smaller than other arthropods.

Characterization of CO1.—The 960 bp fragment of *B. vagans* CO1 gave the closest nucleotide (BlastN) match to *Promyrmekiaphila* sp. Schenkel 1950 (Araneae, Mygalomorphae, Cyrtaucheniidae: AY621508; Bond 2004) with 84% identity (2e-151, 623/740 sites). Similarly, CO1 from other mygalomorphs also matched *B. vagans* with a high identity. For example, CO1 from *Sphodros abbotti* Walckenaer 1835 (Atypidae: AF303528; Hedin 2001) matched with 83% nucleotide identity (559/666 sites), while *Apomastus schlingeri* Bond & Opell 2002 (Cyrtaucheniidae: e.g., DQ388588; Bond et al. 2006), or *Antrodiaetus unicolor* (Hentz 1842) (Antrodiaetidae: e.g., AY896899; Hendrixson & Bond 2005) matched with higher identity (up to 85%) but over a shorter length (up to 534/622 sites). The most significant match (6e-124) to another member of the family Theraphosidae was with *H. huwenum* (Qiu et al. 2005; as *O. huwena*). Surprisingly, the level of CO1 identity between these tarantulas was almost identical to levels seen between *B. vagans* and other families of mygalomorph spiders (83%; 518/616 sites, versus above). This was surprising as *Brachypelma* and *Haplopelma* only currently warrant different subfamilies (both Theraphosidae, Theraphosinae and Ornithoctoninae, respectively).

Recently, a few CO1 sequences from other *Brachypelma* species have been published (Petersen et al. 2006). Each of these is quite short, only averaging around 300 bp, and severely limits the number of sites for comparison. The highest identity was between our *B. vagans* and *B. albopilosum* up to 97%; (DQ224243, 141/144 sites), followed by *B. angustum* (to 95%, DQ224245, 137/144). This result supports a close phylogenetic position of *B. vagans* with these Mesoamerican species, compared to other *Brachypelma* from the Pacific coast of Mexico, like *B. smithi* (as in Longhorn 2001; see also Petersen et al. 2006). Translated (BlastX) searches showed that *B. vagans* sequences also displayed greatest amino acid similarity with *H. huwenum* (85%; 268/312 sites). However, the next most significant matches were from araneomorph spiders of the Salticidae, probably due to convergence. Overall, the *B. vagans* CO1 showed similar nucleotide composition to other spiders except *Heptathela* (Table 3), which suggested that shared biases in composition were not a factor that led protein searches to identify high similarity with between salticids and *B. vagans* or the unexpectedly high genetic divergence between *Brachypelma* and *Haplopelma* CO1 sequences.

Population structure in *Brachypelma vagans*.—It was possible to join all 22 *B. vagans* sequences by parsimonious connections into a haplotype network for each fragment (Fig. 3), each identical with gaps were coded as missing or 5th state. The two mitochondrial fragments (lrRNA-ND1 and CO1) gave similar structure among individuals and separated the two populations (Pooks Hill and Las Cuevas). The lrRNA-ND1 (Fig. 3, top) revealed slightly more divergence between populations than CO1 (Fig. 3, middle) though both regions are size equivalent (~950 bp). Both mitochondrial fragments also revealed more unique haplotypes in the Las Cuevas population than at Pooks Hill, and both separated the outgroup *B. angustum*. These results contrast with the smaller ITS-2 (Fig. 3, bottom), which could not distinguish the two *B. vagans* populations, nor showed sufficient nucleotide differences to separate the outgroup. Across all fragments,

Table 1.—Specimens with GenBank accession numbers. Samples of *B. vagans* are listed by their collection location, either from P = Pooks Hill or C = Las Cuevas.

Sample (all *B. vagans* unless indicated)	Carapace Length (cm)	Fragment sequenced		
		ITS-2	CO1	lrRNA-ND1
P1	1.95	AJ585053	AJ584615	AJ585387
P2	2.20	AJ585054	AJ584616	AJ585388
P3	2.00	AJ585055	AJ584617	AJ585389
P4	2.50	AJ585056	AJ584618	AJ585390
P5	3.00	AJ585057	AJ584619	AJ585391
P6	2.70	AJ585058	AJ584620	AJ585392
P7	2.20	AJ585059	AJ584621	AJ585393
P8	2.85	AJ585060	AJ584622	AJ585394
C9	2.20	AJ585061	AJ584623	AJ585395
C10	3.00	No Data	AJ584624	AJ585396
C11	3.00	No Data	AJ584625	AJ585397
C12	1.80	AJ585062	AJ584626	AJ585398
C13	1.50	AJ585063	AJ584627	AJ585399
C14	1.90	AJ585064	AJ584628	AJ585400
C15	1.75	AJ585065	AJ584629	AJ585401
C16	3.10	AJ585066	AJ584630	AJ585402
C17	2.50	AJ585067	AJ584631	AJ585403
C18	1.40	AJ585068	AJ584632	AJ585404
C19	1.70	AJ585069	AJ584633	AJ585405
C20	1.40	AJ585070	AJ584634	AJ585406
C21	3.00	AJ585071	AJ584635	AJ585407
C22	2.40	AJ585072	AJ584636	AJ585408
B. angustum	1.75	AJ585073	AJ584637	AJ585409

there were thirty-six polymorphic nucleotides, twenty-four of which were parsimony informative (lrRNA-ND1 = 11 sites [1.14%] > CO1 = 10 sites [1.06%] > ITS-2 = 3 sites [0.84%]). Details of nucleotide polymorphism within and between populations of *B. vagans* are given in Tables 4 and 5. In both populations, the number of segregating nucleotides per sequence (S) was similar (except with lrRNA-ND1). The Pooks Hill population consistently had a greater number of average sequence differences (κ) among individuals than Las Cuevas, despite fewer haplotypes overall. Within each population, our results suggest that differences in directional selection are not detectable (neither population deviates significantly from neutrality) and that demographic conditions are relatively stable (non-significant Tajima's D statistic).

Table 2.—Mean nucleotide composition of genetic fragments of *B. vagans*.

Fragment	Length (bp)	Adenine (A)	Cytosine (C)	Guanine (G)	A + T (%)
CO1	963	0.206	0.121	0.229	65.0
lrRNA rRNA	498	0.349	0.156	0.159	68.5
$tRNA^{(LEU)}$	53	0.377	0.131	0.113	75.6
ND1	396	0.331	0.174	0.110	71.5
5.8S rRNA	119	0.197	0.267	0.309	42.4
ITS-2	193	0.187	0.294	0.296	41.0
28S rRNA	45	0.330	0.252	0.209	53.9

For both the CO1 and lrRNA-ND1 fragments, the largest proportion of sequence diversity was attributable to differences between populations. Estimates of gene flow and population structure were difficult to determine with ITS-2. This was surprising, as this region is often large and variable in arthropods, and hence widely used for phylogeographic studies. Estimates of between population gene flow between using F_{ST} values were much lower from nuclear ITS-2 than from either mitochondrial fragment, reflecting its short length and relative invariance. Overall, results

Table 3.—Nucleotide composition of CO1 across exemplar Araneae. * = Complete.

[Infraorder] Family	Genus	Accession	% A	% C	% G	Length	A + T
[Aran.] Agelenidae	*Tegenaria*	AY138836	22.7	15.3	19.8	450	64.9
[Aran.] Araneidae	*Argiope*	AY731171	27.5	12.1	18.8	1536*	69.1
[Aran.] Desidae	*Badumna*	AF218280	25.4	11.2	20.7	552	68.1
[Aran.] Eresidae	*Stegodyphus*	AY611805	26.6	11.1	19.7	1000	69.2
[Aran.] Linyphiidae	*Frontinella*	DQ029220	23.8	13.0	19.5	954	67.5
[Aran.] Lycosidae	*Rabidosa*	DQ029232	23.9	11.6	20.4	942	68.0
[Aran.] Oecobiidae	*Uroctea*	DQ973166	22.8	13.2	21.2	964	65.7
[Aran.] Salticidae	*Habronattus*	NC005942	26.8	11.4	18.7	1542*	69.9
[Aran.] Thomisidae	*Xysticus*	AY297423	24.8	12.7	17.9	1047	69.4
[Aran.] Tetragnathidae	*Nephila*	NC008063	27.1	11.7	18.7	1536	69.7
[Aran.] Dysderidae	*Dysdera*	AF244321	21.2	14.0	22.9	471	63.1
[Aran.] Hypochilidae	*Hypochilus*	AF303527	23.3	14.0	22.4	1536	63.6
[Mygal.] Antrodiaetidae	*Antrodiaetus*	AY297423	23.7	11.4	21.0	1008	67.6
[Mygal.] Atypidae	*Sphodros*	AF303528	21.3	11.9	21.2	1047	66.9
[Mygal.] Cyrtaucheniidae	*Apomastus*	DQ389886	25.7	11.6	19.0	810	69.4
[Mygal] Hexathelidae	*Atrax*	AAL11676	25.4	12.9	19.6	658	67.5
[Mygal.] Theraphosidae	*Ornithoctonus*	NC_005925	24.7	12.2	21.4	1536*	66.4
[Mygal.] Theraphosidae	*Brachypelma*	AJ584636	20.7	12.2	22.9	963	64.9
[Suborder Mesothelae] Heptathelidae	*Heptathela*	NC005924	28.2	17.4	15.1	1533*	67.5
Average (all Araneae)			24.5	12.7	20.0	1057	67.3
Average (only Araneomorphae)			24.6	12.5	20.1	1090	67.4
Average (only Mygalomorphae)			24.2	12.8	20.0	1079	67.1

indicated that the Pooks Hill population is more structured than at Las Cuevas, even though fewer individuals were sampled at Pooks Hill. An equally plausible explanation is that the Pooks Hill population is older (under neutrality). Estimates of the effective numbers of migrating individuals per generation (Nm) were surprisingly low from both mitochondrial fragments, almost to a level where it is difficult to distinguish between low and non-existent levels of gene flow among populations, suggesting a high degree of mitochondrial sequence isolation.

DISCUSSION

Non-lethal DNA sampling by autospasy.—The evaluation of non-lethal DNA sampling techniques is important in any genetic studies where the goal is conservation. Here, we induced limb autospasy from fossorial tarantulas for genetic material. We refer to this induced response as autospasy rather than autotomy, which has been incorrectly used elsewhere and strictly applies to a reflex action alone (after Piéron 1907; Wood 1926; Roth & Roth 1984). In general, the ability to cast limbs is found in a wide variety of arthropods. In most spiders, limb separation involves rupture at the coxa-trochanter boundary, achieved by snapping the coxa upwards while the femur is kept static (Bauer 1972). This is followed by muscle contraction around the internal margin of the coxa to close the wound and minimize hemolymph loss (after Wood 1926; Bauer 1972). In some cases, autospasy can occur at the patella-tibial joints in certain long-legged Linyphiidae and perhaps Filistatidae (Roth & Roth 1984). A third type of autospasy has been described between the femur and patella, at the patellar cleavage plane, but only been in two genera of Agelenidae (Roth 1981). Autospasy can be easily induced in other arachnids, especially Opiliones, but not in Scorpiones (Wood 1926) or some primitive spiders (Roth 1981). Therefore, while limb removal is probably a useful method to obtain non-lethal DNA samples from most spiders, it is not universally applicable for all arachnids.

To reduce trauma to *B. vagans* during tissue sampling, we considered CO_2 anesthetization, as used to attach radio-transmitters (Janowski-Bell & Horner 1999) or insert transponders (Reichling & Tabaka 2001). However, because autospasy is partly voluntary, we suggest that

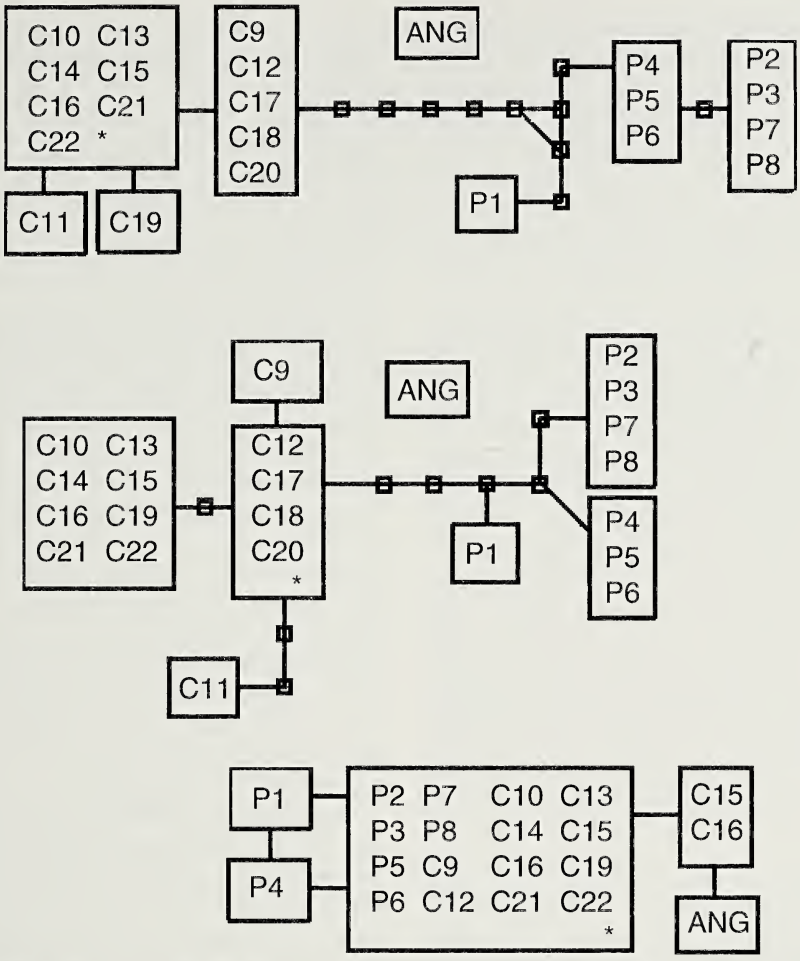


Figure 3.—Haplotype networks for the two *B. vagans* populations (top = from lrRNA-ND1; middle = CO1; bottom = ITS-2). In all cases gaps = missing data. Asterisked boxes notify ancestral sequences. Black lines represent single mutational steps, and small squares lost/un-sampled haplotypes. P = Pooks Hill population of *B. vagans*, C = Las Cuevas population of *B. vagans*, and ANG = *B. angustum*.

the method would be damaging to anesthetized specimens and result in excessive fluid loss (as shown by Bonnet 1930). Without anesthetization, one captive tarantula did show more hemolymph loss than other similar sized spiders after autospasy, either in captive or field populations. Even after the application of artificial coagulants (corn starch and nail

Table 4.—DNA polymorphism within populations.

Gene	Population	Haplo-types	(S)	Nucleotide differences (κ) -gene	θ (S) -gene	Tajimas' D	Significance
COI	Las Cuevas	4	6	1.626	1.887	−0.495	> 0.1 = N/s
	Pooks Hill	3	6	2.428	2.314	0.231	> 0.1 = N/s
lrRNA-ND1	Las Cuevas	4	3	0.780	0.943	−0.529	> 0.1 = N/s
	Pooks Hill	3	7	2.393	2.699	−0.541	> 0.1 = N/s
ITS-2	Las Cuevas	2	1	0.303	0.331	−0.195	> 0.1 = N/s
	Pooks Hill	2	1	0.250	0.385	−1.055	> 0.1 = N/s

Table 5.—DNA polymorphism between populations.

Gene	Fixed nucleotide differences	Avg. nucleotide differences (K) between populations	F_{ST}	Nm
COI	3	7.679	0.736	0.18
lrRNA-ND1	6	9.786	0.838	0.10
ITS-2	0	0.519	0.520	4.56

hardener) the wound re-opened when this spider moved, though fluid-loss finally ceased when it was artificially restrained. Closer examination showed abdominal darkening characteristic of the pre-molt phase, and the spider successfully molted two weeks later. The molt occurred without any regeneration of the lost leg, which was not observed until a subsequent molt (8 mo later). As tarantulas are unwilling to accept prey items during their molting period, linking forced autospasy with prey-lure collection techniques may lower the probability of capturing pre-molt individuals in field studies, and prevent this potential problem. However, if needed, tarantulas can willingly cast limbs while in pre-molt, though this may be the most vulnerable time for limb removal.

Alternative strategies for genetic studies of *Brachypelma*.—DNA can be easily extracted from exuviae (Peterson et al. 2007), which provides an alternative non-lethal tissue source than limb removal for arthropods. While DNA yields are low, it is possible to amplify high-copy fragments (like lrRNA). However, current attempts to use tarantula exuviae have either failed to sequence large fragments or been confounded by fungal contaminants (Longhorn 2001; Peterson et al. 2007). With more taxon specific primers, exuviae will doubtless provide a useful, albeit challenging tissue source for DNA samples, especially for captive spiders. That said, exuviae are little used in genetic studies of natural populations, mainly due to DNA degradation and contamination. Furthermore, most adult tarantulas molt yearly (or less), which is unlikely to coincide with a short research period and these spiders often keep old exuviae deep inside their burrows, making it difficult for the investigator to access without undue damage. The most valuable use for exuviae may be for genetic investigations of tarantulas seized during wildlife enforcement (Peterson et al. 2007), although investigators must again wait for a molt, probably an intolerable delay. Regardless of approach, it is critical to promote non-lethal DNA sources in conservation-focused studies, and build a resource of characterized genetic data. For the genus *Brachypelma*, a DNA reference collection may be extremely useful to wildlife-trade enforcement and conservation efforts. At best, such a genetic resource would be based on samples with clear provenance and a supporting morphological determination. Our genetic samples of *B. vagans* fulfil these criteria for this species and provide a useful reference for comparison with similar spiders of uncertain history or collection location.

Population structure in *Brachypelma*.—Due to the small number of genetic fragments sampled, it is unknown whether the discrepancies in F_{ST} between the three genetic regions can be attributed to differences between nuclear and mitochondrial gene flow in general. However, the two-mitochondrial fragments did not suggest differential selection pressures. Reasonable levels of polymorphism diversity in these two fragments allowed gene flow estimates (Nm) to be calculated with more confidence than from the less variable nuclear ITS-2. However, the validity of gene flow estimates based on indirect measures such as F_{ST} is unclear. Slatkin (1989) suggested that when samples are large (around 10 or more individuals, as here), values for Nm are robust estimators of gene flow. More recently, it has been proposed that measures of genetic variability based on F_{ST} may be valuable, but their transformation into quantitative estimates of gene flow may be unnecessary at best, and misleading at worst (Whitlock & McCauley 1999). Theoretical concerns aside, estimates of gene flow are important to understand species integrity. In general, gene flow acts to counter the effects of isolation. Broadly, gene flow influences the persistence

of local populations and facilitates the spread of adaptive traits among complex landscapes (Hanski & Gilpin 1997). Direct measures of migration may be the most preferable approach to estimate gene flow, but such measures have only been applied cursorily in tarantulas with implanted tags (Reichling & Tabaka 2001) or radio-transmitters (Janowski-Bell & Horner 1999). As well as the time-constraints and technological difficulties, direct measures of dispersal may not reflect the actual movement of genetic material. For gene flow to have occurred, the migrant must also reproduce effectively in the new location. This is probably not the case for the majority of male tarantulas, which can fall prey to predators, harsh environmental factors, or even unreceptive females.

Indirect estimation of gene flow in tarantulas.—The estimation of gene flow in fossorial tarantulas is complicated by the influence of sex-biased dispersal. In all species of *Brachypelma*, mature females display strong site fidelity while mature males show long-range dispersal. Due to these sex-specific aspects, estimates of population subdivision from maternally inherited mitochondrial DNA can be over-estimates compared to bi-parentally inherited nuclear DNA (Gómez-Zurita & Vogler 2003). In fossorial tarantulas, direct measures of gene flow from male migration may provide the best estimates of long-range gene flow, but these overlook finer scale effects of juvenile dispersal and colony formation. The indirect estimation of gene flow from maternally inherited mitochondrial fragments may present the clearest picture of fine sub-structure and gene flow. We concede that inferences from mitochondrial markers are probably not suitable to track genetic admixture from long-range male dispersal, but suggest that finer scale genetic differences within populations are equally interesting.

For our samples of *B. vagans*, results are consistent with the Pooks Hill population having more connectivity with neighboring populations than those at Las Cuevas. Field observations agreed as the Pooks Hill population was from a semi-open grassland habitat indistinguishable from nearby regions where it was likely that *B. vagans* also occurred in high densities. In contrast, the Las Cuevas population was restricted to an isolated grass clearing enclosed by moist broadleaf forest where intensive searches failed to reveal further *B. vagans* burrows (but these have since been reported to exist at low densities).

Overall, the geographic distance between the two *B. vagans* populations (about 50 km) reflects the entire range of other, more threatened species in the genus *Brachypelma* (Locht et al. 1999; West 2005). As a result, inferences from our localized geographic sampling of *B. vagans* may provide a useful baseline to compare against the overall genetic divergences from other species of *Brachypelma*. However, ecologies of *B. vagans* slightly differ from other species, and comparisons of divergences across taxa should be treated with caution since different *Brachypelma* species probably differ in their abilities to colonize new areas.

Alternative sources of nuclear genes.—The nuclear ITS-2 was not suitable to resolve genetic sub-structure in *B. vagans* due to its short size and relative invariance. At the time of this study, the only known spider ITS-2 were from the araneomorph families Theridiidae and Nesticidae at ~150–250 bp (Hedin 1997b), reflecting the paucity of genetic regions amenable to study at that time. Aside from ITS-2 and neighboring rRNA, we initially had few other choices of regions to select, given a lack of nuclear data for any spiders, especially Theraphosidae. There has since been a gradual accumulation of PCR targeted nuclear sequences of spiders in public domain, plus several thousand expressed sequence tags (ESTs) from the tarantula *Acanthoscurria gomesiana* Mello-Leitão 1923 (Lorenzini et al. 2006). Together, these new data can provide a foundation for genetic studies of theraphosid spiders. Several studies have suggested that nuclear gene introns are often the most suitable region for population level genetic studies. However, with EST data, the location of introns is often unknown, as most are derived from mature mRNA. Before additional nuclear markers can be derived from spider ESTs, gene-specific primers need to be designed and the location, size and variability of introns identified at the genomic region of interest. Overall, it would be desirable to explore estimates of population sub-structure in fossorial tarantulas using several nuclear fragments. However, this study confirms that selected segments of the mitochondrial genome can alone provide valuable genetic

data for phylogeographic studies of these spiders.

Of the three genetic regions, the mitochondrial lrRNA-ND1 was best suited for the characterization of population subdivision and genetic polymorphism in *B. vagans*. Similar conclusions arose from both lrRNA-ND1 and CO1, which confirmed the suitability of both these mitochondrial markers for inferring population sub-structure. The knowledge of appropriate molecular makers is vital to facilitate future genetic studies with tarantulas. Both mitochondrial fragments were able to distinguish individuals of *B. vagans* from different collection sites, and both regions revealed a greater degree of population sub-structure than anticipated. Such information is critical for conservation, which is often focused around saving as much of the discrete genetic diversity as possible. Taken to the extreme, the spiders from different collection locations could be considered as different phylogenetic species, or at least, discrete geographical lineages, equally worth conservation efforts reflecting their distinct identities.

For the genus *Brachypelma* as a whole, habitat fragmentation continues to threaten the cohesion of natural populations, particularly for the species endemic to the Pacific coast of Mexico. Thankfully, the collection of spiders in the genus *Brachypelma* is now restricted by both national and international laws, including CITES. The next critical step to the conservation of these spiders will be to enhance understanding of the genetic affinities and population sub-structure of each threatened species. At best, future genetic studies of *Brachypelma* will ensure the future survival of viable populations, define species limits, and be used to conserve the maximum genetic diversity of each discrete lineage in this high-profile genus.

ACKNOWLEDGMENTS

We thank the Forestry Department of Belize, and staff at the LCRS, particularly Chris Minty and Chapal Bol. We thank Vicki and Ray Snaddon for their hospitality at Pook's Hill Lodge. This work was funded by a LCRS research grant, the American Tarantula Society, and British Airways "Conservation Scheme." We are indebted to Steve Reichling for field advice, and to Andrew Smith for his faith that genetics may someday be useful in theraphosid systematics. We thank Susan Masta and Jesus Gómez-Zurita for valuable comments, Pierre Paquin for French translation, and Paula Cushing and Gail Stratton for assistance with the manuscript. This work formed part of a Masters thesis (2001) of the first author from Imperial College, London, UK.

LITERATURE CITED

Anonymous. 1998. Checklist of CITES Species: A Reference to the Appendices to the Convention on International Trade in Endangered Species of Wild Fauna and Flora 1998. WCMC, Cambridge, UK.

Arnedo, M.A. & R.G. Gillespie. 2006. Species diversification patterns in the Polynesian jumping spider genus *Havaika* Proszynski, 2001 (Araneae, Salticidae). Molecular Phylogenetics and Evolution 41:472–495.

Ayoub, N.A. & S.E. Riechert. 2004. Molecular evidence for Pleistocene glacial cycles driving diversification of a North American desert spider, *Agelenopsis aperta*. Molecular Ecology 13: 3453–3465.

Baerg, W.J. 1958. The Tarantula. University of Kansas Press, Lawrence, Kansas. 88 pp.

Bauer, K.H. 1972. Funktionsmechanisms der autotomie bei Spinnen (Araneae) und morphologischen Voraussetzungen. Zeitschrift für Morphologie der Teire. 72:173–202.

Bond, J.E., M.C. Hedin, M.G. Ramirez & B.D. Opell. 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian costal dune endemic trapdoor spider *Aptostichus simus*. Molecular Ecology 10: 899–910.

Bond, J.E. 2004. Systematics of the Californian euctenizine spider genus *Apomastus* (Araneae: Mygalomorphae: Cyrtaucheniidae): the relationship between molecular and morphological taxonomy. Invertebrate Systematics 18:361–376.

Bond, J.E., D.A. Beamer, T. Lamb & M. Hedin. 2006. Combining genetic and geospatial analyses to infer population extinction in mygalomorph spiders endemic to the Los Angeles region. Animal Conservation 9:145–157.

Bonnet, P. 1930. La mue, l'autotomie et la regeneration chez les Araignées, avec une etude des *Dolomèdes* d'Europe. Bulletin de la Société d'Histoire Naturelle de Toulouse 59:237–700.

Brautigam, S.E. & M.H. Persons. 2003. The effect of limb loss on the courtship and mating behavior of the wolf spider *Pardosa milvina* (Araneae: Lycosidae). Journal of Insect Behavior 16:571–587.

Breene, R.G., III. 1998. The ATS Arthropod Medical Manual: Diagnosis and Treatment. The

American Tarantula Society, New Mexico. 38 pp.

Clement, M., D. Posada & K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

Cleva, S. 1998. Investigation probes a web of smuggling. Fish & Wildlife News Sept/Oct:12–13.

Gómez-Zurita, J. & A.P. Vogler. 2003. Incongruent nuclear and mitochondrial phylogeographic patterns in the *Timarcha goettingensis* species complex (Coleoptera, Chrysomelidae). Journal of Evolutionary Biology 16:833–843.

Hanski, I.A. & M.E. Gilpin. 1997. Metapopulation Biology: Ecology, Genetics and Evolution. Academic Press, New York. 512 pp.

Hedin, M.C. 1997a. Molecular phylogenetics at the population/species interface in cave spiders of the Southern Appalachians (Araneae: Nesticidae: *Nesticus*). Molecular Biology & Evolution 14: 309–324.

Hedin, M.C. 1997b. Speciational history in a diverse clade of habitat-specialised spiders (Araneae: Nesticidae: *Nesticus*): inferences from geographic based sampling. Evolution 51:1929–1945.

Hedin, M.C. 2001. Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae; Hypochilidae). Molecular Phylogenetics & Evolution 18:238–251.

Hedin, M.C. & W.P. Maddison. 2001. A combined molecular approach to phylogeny of the jumping spider subfamily Dendryphantinae (Araneae: Salticidae). Molecular Phylogenetics & Evolution 18:386–403.

Hedin, M.C. & W.P. Maddison. 2003. Phylogeny of *Habronattus* jumping spiders (Araneae: Salticidae), with consideration of genital and courtship evolution. Systematic Entomology 28:1–22.

Hendrixson, B.E. & J.E. Bond. 2005. Testing species boundaries in the *Antrodiaetus unicolor* complex (Araneae: Mygalomorphae: Antrodiaetidae): 'Paraphyly' and cryptic diversity. Molecular Phylogenetics & Evolution 36:405–416.

Hormiga, G., M. Arnedo & R.G. Gillespie. 2003. Speciation on a conveyor belt: sequential colonization of the Hawaiian Islands by *Orsonwelles* spiders (Araneae, Linyphiidae). Systematic Biology 52:70–88.

Hudson, R.R., M. Slatkin & W.P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132:583–589.

Janowski-Bell, M.E. & N.V. Horner. 1999. Movement of the male brown tarantula, *Aphonopelma hentzi* (Araneae, Theraphosidae) using radio telemetry. Journal of Arachnology 27:503–512.

Johnson, S.A. & E.M. Jakob. 1999. Leg autotomy in a spider has minimal costs in competitive ability and development. Animal Behaviour 57:957–965.

Joyce, D.A. & A.S. Pullin. 2003. Conservation implications of the distribution of genetic diversity at different scales: a case study using the marsh fritillary butterfly (*Euphydryas aurinia*). Biological Conservation 114:453–461.

Locht, A., M. Yáñez & I. Vázquez. 1999. Distribution and natural history of Mexican species of *Brachypelma* and *Brachypelmides* (Theraphosidae, Theraphosinae) with morphological evidence for their synonymy. Journal of Arachnology 27:196–200.

Longhorn, S.J. 2001. Genetic evaluation of CITES II protected spiders of the genus *Brachypelma* (Theraphosidae); including an outline of non-lethal tissue sampling. M.Sc. Thesis. Imperial College, University of London, London, UK.

Longhorn, S.J. 2002. Non-lethal DNA sampling from CITES II protected 'Tarantula' spiders of Belize. Las Cuevas Newsletter 9:8–9.

Lorenzini, D.M., P.I. Silva, M.B. Soares, P. Arruda, J. Setubal & S. Daffre. 2006. Discovery of immune-related genes expressed in hemocytes of the tarantula spider *Acanthoscurria gomesiana*. Developmental & Comparative Immunology 30: 545–556.

Lushai, G., W. Fjellsted, O. Marcovitch, K. Aagaard, T. Sherratt, J.A. Allen & N. Maclean. 2000. Application of molecular techniques to non-lethal tissue samples of endangered butterfly populations (*Parnassius apollo* L.) in Norway for conservation management. Biological Conservation 94:43–50.

Mallatt, J.M., J.R. Garey & J.W. Schultz. 2004. Ecdysozoan phylogeny and Baysian inference: first use of nearly complete 28S and 18S rRNA gene sequences to classify arthropods and their kin. Molecular Phylogenetics & Evolution 31:178–191.

Masta, S.E. 2000a. Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations? Evolution 54:1699–1711.

Masta, S.E. 2000b. Mitochondrial sequence evolution in spiders: intraspecific variation in tRNAs lacking the TψC arm. Molecular Biology & Evolution 17:1091–1100.

Masta, S.E. & J.L. Boore. 2004. The complete mitochondrial genome sequence of the spider *Habronattus oregonensis* reveals rearranged and extremely truncated tRNAs. Molecular Phylogenetics & Evolution 21:893–902.

M'Rabet, S.M., Y. Hémaut, R. Rojo & S. Calmé. 2005. A not so natural history of the tarantula *Brachypelma vagans*: interaction with human activity. Journal of Natural History 39:2515–2523.

Murphy, N.P., V.W. Framenau, S.C. Donnellan, M.S. Harvey, Y.-C. Park & A.D. Austin. 2006.

Phylogenetic reconstruction of the wolf spiders (Araneae: Lycosidae) using sequences from the 12S rRNA, 28S rRNA, and NADH1 genes: implications for classification, biogeography and the evolution of web building behavior. Molecular Phylogenetics and Evolution 38:583–602.

Nicholas, M. 2002. Hunting the Mexican red rump tarantula in Belize. Journal of the British Tarantula Society 18:9–20.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. 513 pp.

Paquin, P. & M. Hedin. 2004. The power and perils of 'molecular taxonomy': a case study of eyeless and endangered *Cicurina* (Araneae: Dictynidae) from Texas caves. Molecular Ecology 13:3239–3255.

Petersen, S.D., T. Mason, S. Akber, R. West, B. White & P. Mason. 2007. Species identification of tarantulas using exuviae for international wildlife law enforcement. Conservation Genetics 8: 497–502.

Piéron, H. 1907. Autotomie et "Autospasie" Comte Rendu hebdomadaire Séances. Memoires Société Biologie, Paris 63:425–427.

Qiu, Y., D. Song, K. Zhou & H. Sun. 2005. The mitochondrial sequences of *Heptathela hangzhouensis* and *Ornithoctonus huwena* reveal unique gene arrangements and atypical tRNAs. Journal of Molecular Evolution 60:57–71.

Reichling, S.B. 1999. Nearest neighbour relationships among theraphosid spiders in Belize. The Southwestern Naturalist 44:518–521.

Reichling, S.B. 2000. Group dispersal in juvenile *Brachypelma vagans* (Araneae, Theraphosidae). Journal of Arachnology 28:248–250.

Reichling, S.B. 2003. Tarantulas of Belize. Kreiger Publishing, Malabar, Florida. 127 pp.

Reichling, S.B. & C. Tabaka. 2001. A technique for individually identifying tarantulas using passive integrated transponders. Journal of Arachnology 29:117–118.

Roth, V.D. 1981. A new genus of spider (Angelenidae) from California exhibiting a third type of leg autospasy. *In* Contributions to arachnid systematics in honor of Willis J. Gertsch, on the occasion of his seventy-fifth birthday. Bulletin of the American Museum of Natural History 170: 101–105.

Roth, V.D. & B.M. Roth. 1984. A review of appendotomy in spiders and other arachnids. Bulletin of the British Arachnological Society 6:137–146.

Rozas, J. & R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.

Shillington, C. & B. McEwen. 2006. Activity of juvenile tarantulas in and around the maternal burrow. Journal of Arachnology 34:26–265.

Shillington, C. & P. Verrell. 1997. Sexual strategies of a North American 'tarantula' (Araneae: Theraphosidae). Ethology 103:588–598.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu & P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87:651–701.

Slatkin, M. 1989. Detecting small amounts of gene flow from phylogenies of alleles. Genetics 121: 609–612.

Slatkin, M. & W.P. Maddison. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics 123:603–613.

Smith, A.M. 1994. Pp. 174–176. *In* Theraphosid Spiders of the New World, Volume 2. Tarantulas of the USA and Mexico. Fitzgerald Publishing, London, UK.

Striffler, B. & A. Graminske. 2003. *Brachypelma*—die bunten Vogelspinnen Mexikos. DRACO 4: 52–61.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105: 437–460.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Templeton, A.R., E. Routman & C. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambrystoma tigrinum*. Genetics 140:767–782.

Watterson, G.A. 1975. On the number of segregating sites in genetical models without recombination. Theoretical Population Biology 7:256–276.

West, R.C. 2005. The *Brachypelma* of Mexico. Journal of the British Tarantula Society 20:108–119.

Whitlock, M.C. & D.E. McCauley. 1999. Indirect measures of gene flow and migration: Fst ≠ 1/(4Nm+1). Heredity 82:117–125.

Wood, F.D. 1926. Autotomy in Arachnida. Journal of Morphology 42:143–192.

Woodman, J.D., J.E. Ash & D.M. Rowell. 2006. Population structure in a saproxylic funnelweb spider (Hexathelidae: *Hadronyche*) along a forested rainfall gradient. Journal of Zoology 268: 325–333.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:323–354.

Yáñez, M. & G. Floater 2000. Spatial distribution and habitat preference of the endangered tarantula, *Brachypelma klaasi* (Araneae: Theraphosidae) in Mexico. Biodiversity and Conservation 9:795–810.

Manuscript received 10 June 2005, revised 25 April 2007.

2007. The Journal of Arachnology 35:293–306

CHROMOSOMES OF *CROSSOPRIZA LYONI* (BLACKWALL 1867), INTRAINDIVIDUAL NUMERICAL CHROMOSOME VARIATION IN *PHYSOCYCLUS GLOBOSUS* (TACZANOWSKI 1874), AND THE DISTRIBUTION PATTERN OF NORs (ARANEOMORPHAE, HAPLOGYNAE, PHOLCIDAE)

Rosângela Martins Oliveira,[1] **Aline Carolina de Jesus,**[1] **Antonio Domingues Brescovit**[2] and **Doralice Maria Cella**[1]: [1]Universidade Estadual Paulista-UNESP, Instituto de Biociências, Departamento de Biologia, Caixa Postal 199, CEP 13506-900, Rio Claro, State of São Paulo, Brazil. E-mail: characid@yahoo.com.br [2]Instituto Butantan, Laboratório de Artrópodes Peçonhentos, Av. Vital Brasil, n. 1500; CEP 05503-900, São Paulo, State of São Paulo, Brazil

ABSTRACT. Pholcidae (Haplogynae) encompasses 967 described species, of which only 14 have been cytogenetic analyzed. Several chromosomal features have already been described including presence of meta- and sub-metacentric chromosomes and sex determination chromosome system (SDCS) of the X, X_1X_2Y, and X_1X_2 types, which contrast with the telo- and acrocentric chromosomes and SDCS of the X_1X_2 type typical of entelegyne spiders. To obtain further cytogenetic information for the family, we examined two pholcid species, *Crossopriza lyoni* (Blackwall 1867) and *Physocyclus globosus* (Taczanowski 1874) using both conventional staining and silver staining techniques. *Crossopriza lyoni* exhibited 2n = 23 = 22 + X in males and 2n = 24 = 22 + XX in females, while *P. globosus* showed 2n = 15 = 14 + X and 4n = 30 = 28 + 2X, both in male adults, 2n = 16 = 14 + XX in female adults and embryos, and 2n = 15 = 14 + X in male embryos. Both species revealed predominately metacentric and submetacentric chromosomes and a SDCS of the X/XX type. The cytogenetic data obtained in this work and those already recorded for *C. lyoni* indicate interpopulational and intraspecific numerical chromosome variation, suggesting the presence of chromosomal races or cytotypes in this species. The intraindividual numerical chromosome variation observed in male adult specimens of *P. globosus* may be explained by the presence of cytoplasmatic bridges between germ cells. The use of the silver staining technique to reveal the nucleolar organizer region (NOR) showed that chromosome pairs 4 and 6 and the X chromosome in *C. lyoni* are telomeric NOR-bearers, and that the chromosome pair 2 in *P. globosus* possesses a proximal NOR in the long arm.

Keywords: Chiasma, chromosome rearrangements, meiosis, syncytial, tetraploidy

The family Pholcidae currently has 81 genera and 967 described species (Platnick 2007) and is included in the Haplogynae (Coddington & Levi 1991; Ramírez 2000), which is considered less morphologically derived than Entelegynae. Chromosome analyses within Pholcidae were initiated by Painter (1914) in *Spermophora senoculata* (Dugès 1836) (as *Spermophora meridionalis* Hentz 1837). Due to the inefficient cytogenetic techniques of the time, Painter noted only that the chromosome complement consisted of small metacentric chromosomes and that the sex determination chromosome system (SDCS) was of the X_1X_2 type. Since then, considering the number of named species, little cytogenetic information on Pholcidae has been added to the literature. Cytogenetic data currently exist for 14 pholcid species (Table 1), which represent less than 2% of the total known. The Indian *Crossopriza lyoni* (Blackwall 1867) has provided the greatest amount of chromosomal data that encompassed several populations. Bole-Gowda (1958) described the presence of 2n = 27 = 13II + X with metacentric autosomes and sex chromosomes; Sharma et al. (1959) recorded 2n = 24 = 11II + X_1X_2 with metacentric autosomes and acrocentric sex chromosomes; Srivastava & Shukla (1986) observed 2n = 25 = 12II + X but did not provide any information regarding chromosome morphology, and finally Parida & Sharma (1987) and Shar-

Table 1.—Cytogenetic characterized Pholcidae species with their respective diploid numbers (2n), chromosome morphology (CM) and biogeographical region of origin (BR). A = acrocentric; M = metacentric; SM = submetacentric; T = telocentric. * as *Artema atlanta*; ** as *Pholcus affinis* (Schenkel 1913); *** as *Spermophora meridionalis* Hentz 1837.

Species	2n/male	CM	BR	Authors
*Artema atlanta** Walckenaer 1837	32 = 15II + X_1X_2	——	Oriental	Parida & Sharma (1987); Sharma & Parida (1987)
Crossopriza lyoni (Blackwall 1867)	27 = 13II + X	26M + XM	Oriental	Bole-Gowda (1958)
C. lyoni	24 = 11II + X_1X_2	22M + X_1X_2A	Oriental	Sharma et al. (1959)
C. lyoni	25 = 12II + X	——	Oriental	Srivastava & Shukla (1986)
C. lyoni	23 = 11II + X	——	Oriental	Parida & Sharma (1987); Sharma & Parida (1987)
C. lyoni	23 = 11II + X	22M + XM	Neotropical	This work
Holocnemus caudatus (Dufour 1820)	23 = 11II + X	16M + 6SM + XM	Palearctic	Král et al. (2006)
Mesabolivar luteus (Keyserling 1891)	15 = 7II + X	14M + XM	Neotropical	Araujo et al. (2005b)
Micropholcus fauroti (Simon 1887)	17 = 8II + X	17M or SM	Neotropical	Araujo et al. (2005b)
*Pholcus manueli*** Gertsch 1937	25 = 12II + X	A-T + XSM	Palearctic	Xiuzhen et al. (1997)
Pholcus crypticolens Bösenberg & Strand 1906	24 = 11II + X_1X_2	22M + X_1X_2A	Palearctic	Suzuki (1954)
Pholcus phalangioides (Fuesslin 1775)	24 = 11II + X_1X_2	——	Neotropical	Rodríguez-Gil et al. (2002)
P. phalangioides	25 = 11II + X_1X_2Y	18M + 4SM + X_1M + X_2SM + YM	Palearctic	Král et al. (2006)
Pholcus sp.	26 = 12II + X_1X_2	——	Oriental	Sharma & Parida (1987)
Physocyclus californicus Chamberlin & Gertsch 1929	15 = 7II + X	14M + XM	Nearctic	Cokendolpher (1989)
Physocyclus enaulus Crosby 1926	15 = 7II + X	14M + XM	Nearctic	Cokendolpher (1989)
Physocyclus globosus (Taczanowski 1874)	15 = 7II + X	6M + 8SM + XM	Neotropical	This work
Physocyclus sp.	15 = 7II + X	14M + XM	Nearctic	Cokendolpher (1989)
*Spermophora senoculata**** (Dugès 1836)	X_1X_2	X_1X_2M	Nearctic	Painter (1914)
S. senoculata	25 = 11II + X_1X_2Y	22M + X_1X_2YM	Palearctic	Král et al. (2006)

ma & Parida (1987) reported the presence of 2n = 23 = 11II + X, also with no description of chromosome morphology. In *Physocyclus* Simon 1893, all cytogenetic described species (*Physocyclus californicus* Chamberlin & Gertsch 1929, *Physocyclus enaulus* Crosby 1926, and *Physocyclus* sp.) occur in the Nearctic region (Cokendolpher 1989) and show a great karyotypic uniformity in relation to diploid number (2n = 15), metacentric chromosome morphology, and X/XX sex determination chromosome system type.

Recently, cytogenetic analyses were carried out in three pholcid species. *Pholcus phalangioides* (Fuesslin 1775) revealed 2n = 24 = 11II + X_1X_2 in males with metacentric autosomes and acrocentric sex chromosomes (Rodríguez-Gil et al. 2002); *Mesabolivar luteus* (Keyserling 1891) showed 2n = 15 = 7II + X in males and 2n = 16 = 7II + XX in females with a metacentric chromosome morphology; in the male specimens of *Micropholcus fauroti* (Simon 1887), the diploid number was 2n = 17 = 8II + X, with the chromosomes being described as biarmed (Araujo et al. 2005b).

Existing karyotypic descriptions for pholcid species (Table 1) show that the diploid number varies from 2n = 15 to 2n = 32, that the predominant chromosome morphology is metacentric and that the most frequent SDCS is of the X/XX type. In addition to this type of SDCS, some species of this family presented $X_1X_2/X_1X_1X_2X_2$ and $X_1X_2Y/X_1X_1X_2X_2$ types in a decreasing succession of occurrence.

In the other 11 haplogyne families, specifically Diguetidae, Drymusidae, Dysderidae, Filistatidae, Leptonetidae, Ochyroceratidae, Plectreuridae, Scytodidae, Segestriidae, Sicariidae, and Tetrablemmidae, from which 28 species have been cytogenetically characterized (Hackman 1948; Suzuki 1954; Beçak & Beçak 1960; Diaz & Sáez 1966a, 1966b; Benavente & Wettstein 1980; Silva 1988; Tugmon et al. 1990; Silva et al. 2002; Král et al. 2006), the diploid number varies from 2n = 7 to 2n = 37, the predominant chromosome morphology is also metacentric, and the SDCS may be of the X (43%), X_1X_2 (26%), X_1X_2Y (24%) or XY (7%) types.

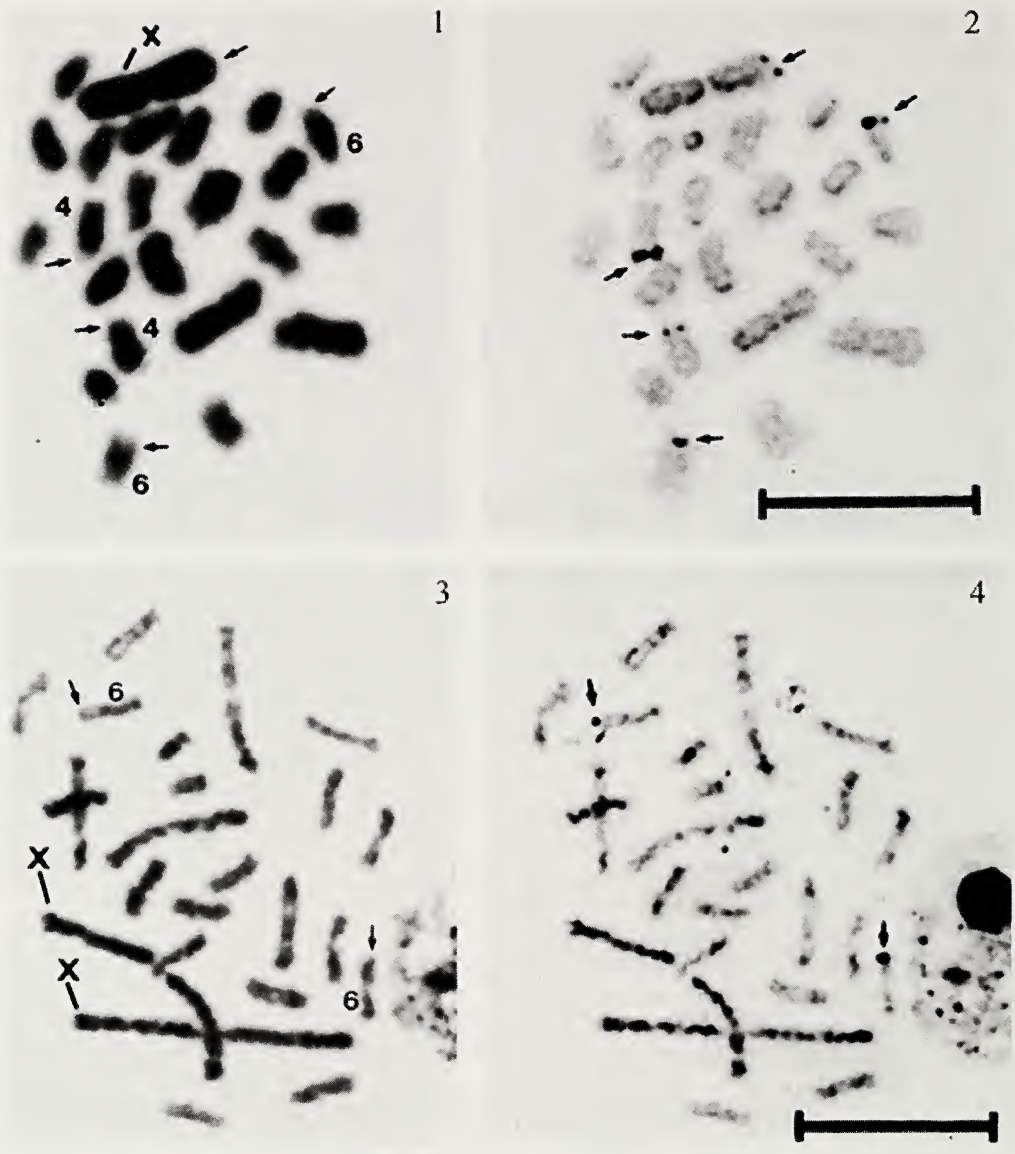
The majority of cytogenetically analyzed araneomorph species belong to Entelegynae. Approximately 500 species of entelegyne spiders, representing nearly 30 families, have a diploid number varying between 2n = 14 and 2n = 52, a predominantly acrocentric chromosome morphology, and a SDCS of the X_1X_2 type in most species. The few cytogenetically studied species of Pholcidae and other haplogyne families exhibit karyotypic peculiarities, such as predominantly metacentric chromosome morphology and X/XX sex determination chromosome system, that contrasts with those of Entelegynae. The cytogenetic analysis of other haplogyne species will probably provide additional information that can be useful in establishing some strategies of karyotype differentiation among the species of this group and also between Haplogynae and Entelegynae.

Considering the karyotypic peculiarities of Pholcidae, the present work aims to characterize the cytogenetics of two species of this family, *Crossopriza lyoni* and *Physocyclus globosus* (Taczanowski 1874). The diploid number, chromosome morphology, SDCS type, and behavior of chromosomes during meiosis were determined with conventional staining, and the distribution pattern of the active nucleolar organizer regions (NORs) in the chromosomes was established using silver staining.

METHODS

The chromosomal characterization in *C. lyoni* was performed through the analysis of 27 adult specimens (24 males and 3 females); in *P. globosus*, 10 adult specimens (7 males and 3 females) and 12 embryos were used. The individuals of both species were collected from natural populations in the city of Rio Claro (22°05′S, 47°30′W), State of São Paulo, Brazil. The adult specimens were deposited in the collection of the Butantan Institute, city of São Paulo, State of São Paulo, Brazil. All analyzed adult specimens were collected in August 2003 and *P. globosus* embryos were collected in January 2004.

The gonadal and embryonic chromosome preparations were obtained using the technique described by Webb et al. (1978), although a few were prepared from testicles not submerged in colchicine solution. Conventional staining was performed using a 3% Giemsa solution for 12 to 15 minutes. The NOR silver staining was carried out according to the



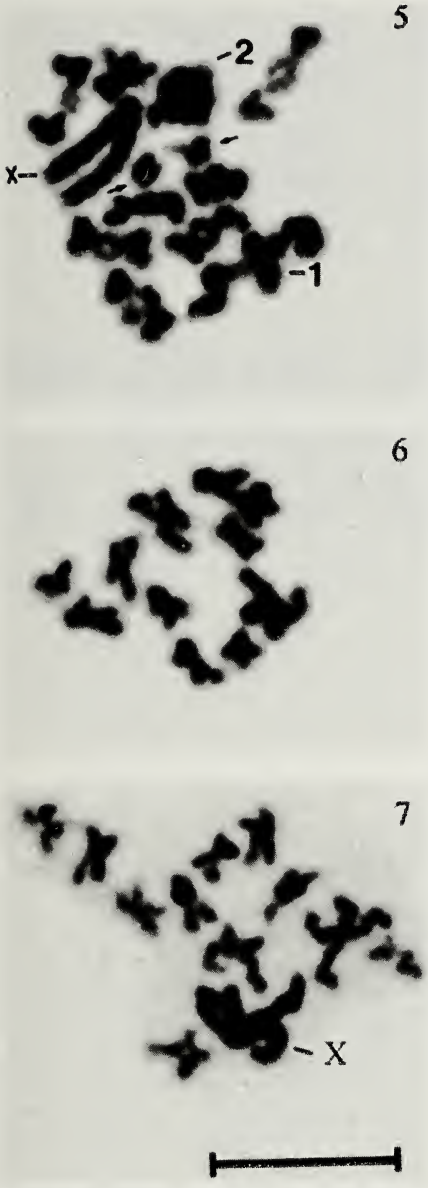
Figures 1–4.—Gonadal mitotic metaphases from adult *Crossopriza lyoni* individuals. 1, 3. Conventionally stained, male with 2n = 23 and female with 2n = 24. 2, 4. Same cells seen in 1 and 3, respectively, submitted to silver staining. Arrows indicate the NOR-bearing chromosomes. Scale bar = 10 μm.

methodology described by Howell & Black (1980).

RESULTS

Cytogenetics of *Crossopriza lyoni*.—Analysis of 356 gonadal cells of *C. lyoni* using conventional staining revealed 2n = 23 chromosomes in the spermatogonial metaphases (Fig. 1) and 2n = 24 in the oogonial metaphases (Fig. 3), a meiotic formula of 2n = 11II + X in the spermatocytes I (Fig. 5), and the occurrence of n = 11 or n = 12 = 11 + X with metacentric and submetacentric chromosomes in the metaphases II of males (Figs. 6, 7). These data showed the occurrence of the X/XX sex determining system in *C. lyoni*.

The conventionally stained mitotic metaphases of *C. lyoni* showed chromosomes with little morphological definition (Figs. 1, 3). In these cells, the chromosome elements of pair 1 and the X sex chromosome were always easily identified as being the largest elements



Figures 5–7.—Spermatocytes of adult *Crossopriza lyoni* specimens in conventional staining. 5. Meiocyte in prophase I, with 2n = 11II + X; note the cross or ring configuration of bivalents 1 and 2, evidencing the occurrence of one and two chiasmata, respectively; the arrows indicate the chromosomal elements of a bivalent with precocious separation. 6, 7. Metaphases II, with n = 11 and n = 12 = 11 + X, respectively, showing the presence of metacentric and submetacentric chromosomes. Scale bar = 10 μm.

of the complement; the other chromosomes represented a series of gradually decreasing size. Additionally, the X chromosome was positively heteropycnotic in most gonadal metaphases and spermatogonial anaphases.

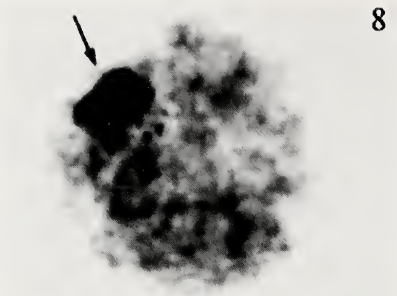
The silver-stained spermatogonial metaphases exhibited 5 telomeric NORs occupying the short arm of the chromosome elements of pair 4, the long arm of the chromosome elements of pair 6, and one of the arms of the metacentric X chromosome (Fig. 2). The oogonial metaphases showed only two telomeric NORs in the long arm of the pair 6 chromosomes (Fig. 4), reflecting an intersexual heterogeneity in the activity of these regions.

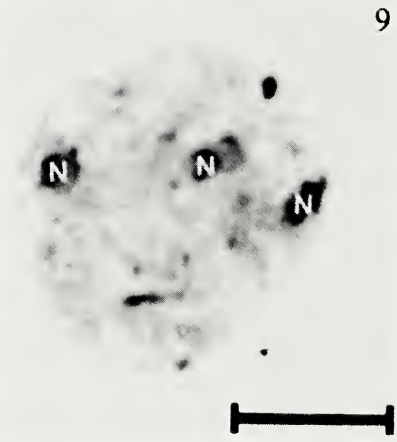
In the diplotene and diakinesis of the male *C. lyoni* specimens, the autosomal bivalents showed a regular meiotic behavior of pairing and staining, and the X chromosome appeared as an extremely large and isopycnotic univalent (Fig. 5). In these cells, most of the bivalents possessed an interstitial chiasma with a cross configuration, with the exception of bivalents 1 and 2 that showed two chiasmata, assuming a ring configuration. In some of these cells, the smallest bivalent showed a precocious separation, but exhibited a regular segregation in the subsequent meiotic phases (Fig. 5).

The metaphases II of male *C. lyoni*, with n = 11 or n = 12 = 11 + X, confirmed the regular reductional segregation of all the chromosomes in the preceding anaphase I and the meta- and submetacentric morphology of the chromosomes (Figs. 6, 7). In the metaphases II with n = 12, the X chromosome was identified through its large size and positive heteropycnosis.

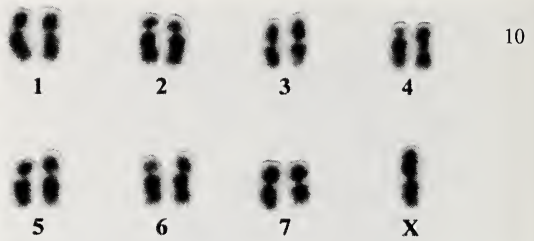
Silver-stained spermatocytes I and II did not show NOR markings on the chromosomes. However, early prophasic nuclei I from male specimens exhibited a strongly stained nucleolus.

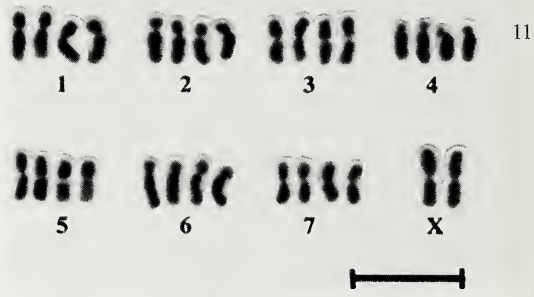
Conventionally stained interphasic nuclei of males and females showed one and two positive heteropycnotic chromatinic blocks, respectively, which probably corresponded to a sexual chromatin (Fig. 8). The silver staining of the interphasic nuclei of males resulted in the marking of three nucleoli (Fig. 9), corroborating the results obtained regarding the number of Ag-NOR-bearing chromosomes in the spermatogonial metaphases, i.e., pairs 4 and 6 and the X chromosome. In the inter-





Figures 8, 9.—Interphasic nuclei of male *Crossopriza lyoni*. 8. Conventionally stained, showing a conspicuous heteropycnotic-positive chromatinic block (arrow). 9. Silver-stained, evidencing three nucleoli (N). Scale bar = 10 μm.





Figures 10, 11.—Male *Physocyclus globosus* karyotypes submitted to conventional staining. 10. 2n = 15 chromosomes. 11. 4n = 30 chromosomes. Scale bar = 10 μm.

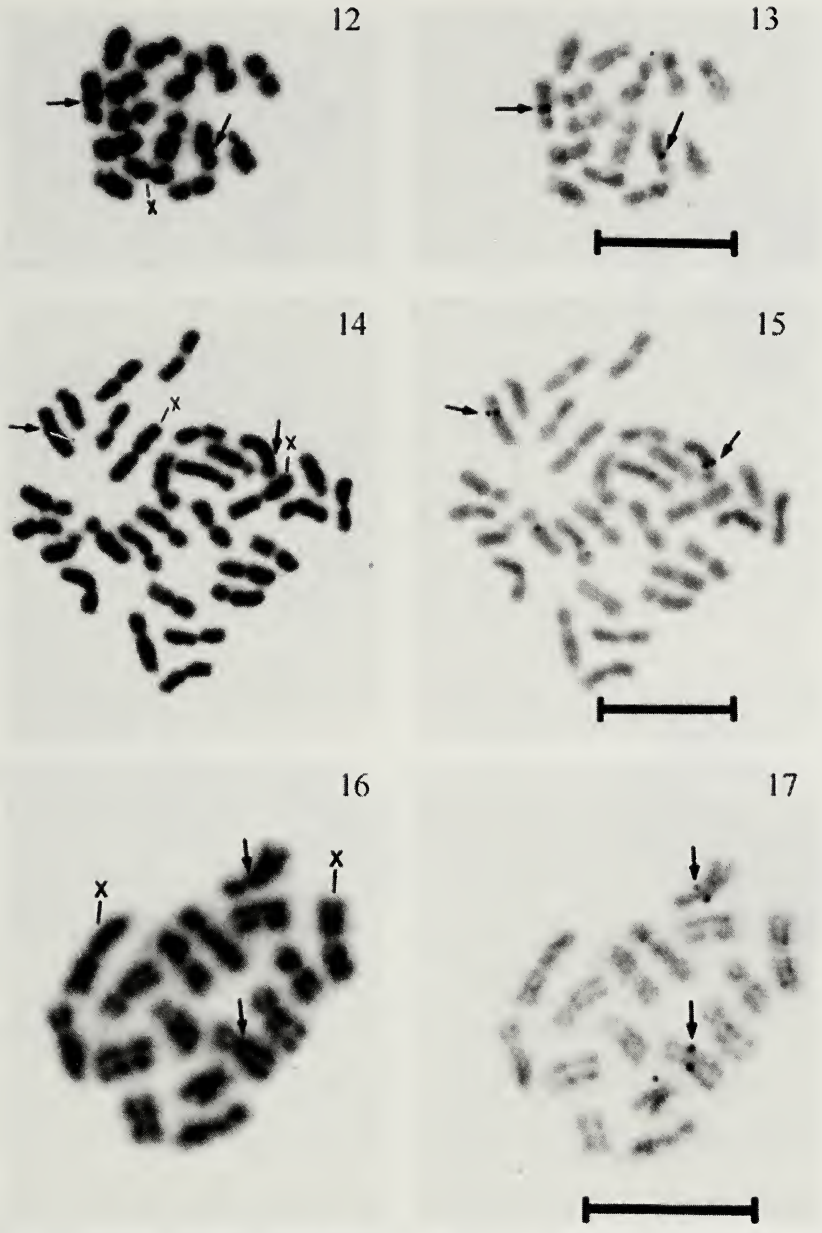
phasic nuclei of female specimens, only one strongly stained nucleolus was observed, confirming the number of active NORs verified in the oogonial metaphases.

Cytogenetics of *Physocyclus globosus*.— The testicular cells of the 7 analyzed adult specimens of *P. globosus* showed intraindividual variation in the number of chromosomes, i.e., of the 208 metaphases obtained from these individuals, 125 exhibited 15 = 14 + X chromosomes (Figs. 10, 12), and 83 showed 30 = 28 + 2X chromosomes (Figs. 11, 14). Of approximately 30 spermatogonial metaphases obtained from each individual, about 60% of cells showed 15 chromosomes and about 40% possessed 30 chromosomes. The analysis of 136 oogonial and embryonic metaphases showed the occurrence of 16 = 14 + XX chromosomes (Fig. 16) in 9 females (3 adults and 6 embryos) and 15 = 14 + X chromosomes in 6 male embryos.

The spermatocytes of the adult specimens of *P. globosus* also exhibited intraindividual variation in the number of chromosomes. Spermatocytes I showed the meiotic formula 7II + X (Fig. 18) or 14II + 2X (Fig. 19) in prophase I and metaphase I; spermatocytes II exhibited 7 chromosomes (Fig. 20) or 8 = 7 + X chromosomes (Fig. 21) in the metaphases II, indicating that these came from the spermatocytes I with 7II + X. Or they possessed 15 = 14 + X chromosomes (Fig. 22), indicating that they originated from spermatocytes I with 14II + 2X.

Considering the chromosome numbers obtained in the testicular, ovarian and embryonic cells of *P. globosus*, the diploid number and the chromosomal sex determination system were established: 2n = 15 = 14 + X = 7II + X in males and 2n = 16 = 14 + XX = 7II + XX in females. The testicular cells with 30 = 28 + 2X = 14II + 2X were interpreted as tetraploids, indicating an intraindividual numerical chromosome variation in the male specimens of adult *P. globosus*.

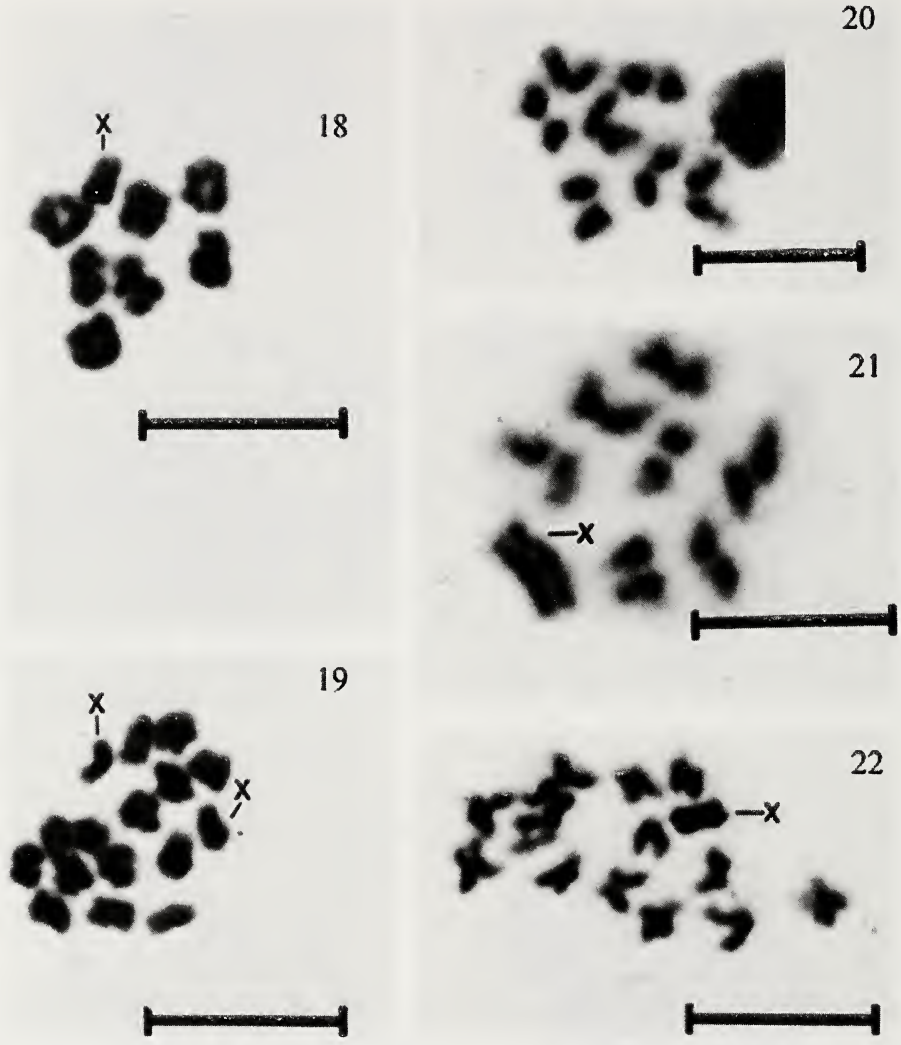
The gonadal and embryonic somatic metaphases revealed that pairs 1, 2, 4 and 6 of the *P. globosus* karyotype are submetacentric, and



Figures 12–17.—Gonadal mitotic metaphases of *Physocyclus globosus*. 12, 14, 16. Conventionally stained with 2n = 15 (male), 4n = 30 (male tetraploid cell), and 2n = 16 (female), respectively. 13, 15, 17. Same cells seen in 12, 14 and 16, respectively, stained with silver nitrate, showing the NORs in the chromosomes of pair 2 (arrows). Scale bar = 10 μm.

that pairs 3, 5 and 7 and the X chromosome are metacentric (Fig. 10). The autosomal pairs could be arranged in a series of gradually decreasing size and the X chromosome was a size intermediate between chromosome pairs 1 and 2.

Only mitotic metaphases of males and females were subjected to silver staining, which revealed a NOR in the proximal region of the long arm of the second pair of chromosomes (Figs. 13, 15, 17). The metaphases with 2n = 15 and those with 2n = 16 exhibited a maximum number of two NOR-bearing chromosomes, while the metaphases with 30 chromosomes presented a maximum number of four NOR-bearing chromosomes; in these



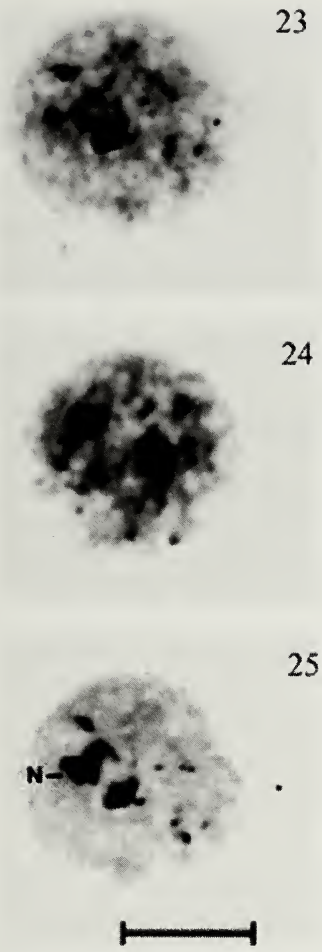
Figures 18–22.—Conventionally stained meiotic cells of male *Physocyclus globosus*. 18, 19. Diplotenes exhibiting 2n = 7II + X and 4n = 14II + 2X, respectively. 20, 21, 22. Metaphases II with 7 chromosomes, 8 = 7 + X chromosomes and 15 = 14 + X chromosomes, respectively. Scale bar = 10 μm.

metaphases, the NORs were always marked in the chromosomes of pair 2.

In the *P. globosus* testicular chromosome preparations, meiocytes with 15 or 30 chromosomes were found in all the phases of meiosis I. In the subphases prophase I and metaphases I, autosomal bivalents and the X univalent always occurred, even in the cells with 30 = 14II + 2X (Figs. 18, 19). In the diplotene cells, the occurrence of an interstitial chiasma was observed in three bivalents, in the cells with 15 = 7II + X, and in six bivalents, in the cells with 30 = 14II + 2X. Metaphases II showed chromosome numbers that confirmed the reductional segregation of the chromosomes during the preceding anaphase I, including the asynaptic X chromosomes present in the cells with 30 = 14II + 2X.

The silver-stained testicular meiocytes provided no information on NORs or nucleolar material, with the exception of pachytene cells that exhibited a single strongly stained block of nucleolar material.

Conventionally stained testicular and ovarian interphasic nuclei revealed the presence of one or two large heteropycnotic-positive blocks, respectively, which are probably related to the sex chromatin (Figs. 23, 24). The occurrence of two chromatinic blocks in a few



Figures 23–25.—Testicular interphasic nuclei of *Physocyclus globosus*. 23, 24. Conventionally stained, emphasizing one and two conspicuous heteropycnotic-positive chromatinic blocks, respectively. 25. Same nucleus seen in 23 submitted to silver staining, evidencing one nucleolus (N). Scale bar = 10 μm.

testicular interphasic nuclei (Fig. 24) suggested the presence of cells with two X chromosomes in males. Silver-stained interphasic nuclei exhibited a single marked nucleolus (Fig. 25).

DISCUSSION

The cytogenetical data obtained from *C. lyoni* and *P. globosus* in relation to the metacentric and submetacentric morphology of all the chromosomes of the complement and the presence of an X/XX sex determination system are similar to those described for related species belonging to the Nearctic, Neotropical, Oriental and Palearctic regions (Bole-Gowda 1958; Srivastava & Shukla 1986; Parida & Sharma 1987; Sharma & Parida 1987; Cokendolpher 1989; Araujo et al. 2005b; Král et al. 2006). However, the studied species showed some particularities regarding chromosome number when compared with related species described in the literature.

The *C. lyoni* specimens analyzed in this work showed a diploid number (2n = 23 = 22 + X) similar to the one found by Parida & Sharma (1987) and Sharma & Parida (1987) in specimens from the same species from two different Indian populations. Nevertheless, this diploid number differs from those described by Bole-Gowda (1958) - 2n = 27 = 26 + X, Sharma et al. (1959) - 2n = 24 = 22 + X_1X_2, and Srivastava & Shukla (1986) - 2n = 25 = 24 + X, for individuals belonging to other, more geographically distant Indian populations.

Chromosome rearrangements of the centric fission, followed or not by pericentric inversion, and/or centric fusion types are suggested in order to explain the interpopulational and intraspecific numerical variation found in *C. lyoni*. The presence of predominantly metacentric or submetacentric autosomes and of a SDCS of the X_1X_2 type, with acrocentric X chromosomes, or of the X type, with a metacentric X chromosome, corroborate such mechanisms of karyotypic differentiation.

Chromosomal variations of the diploid number have already been described for some species of entelegyne spiders, such as *Agelena limbata* Thorell 1897 (Agelenidae), *Delena cancerides* Walckenaer 1837 (Sparassidae) and *Evarcha hoyi* (Peckham & Peckham1883) [as *Pellenes hoyi* (Peckham & Peckham 1909)] (Salticidae). In each one of these species, karyotypes belonging to different populations were characterized as chromosomal races that appeared to have originated mainly by centric or tandem fusion, involving only autosomes or autosomes and sex chromosomes (Maddison 1982; Rowell 1985, 1990, 1991; Tsurusaki et al. 1993; Hancock & Rowell 1995). Likewise, the different karyotypes present in *C. lyoni* could represent chromosomal races or cytotypes.

There are three works that focus on the phylogeny of pholcid spiders (Huber 2000; Bruvo-Madaric et al. 2005; Astrin et al. 2006) and could be used to hypothesize the origin

of the *C. lyoni* cytotypes. However, the study of Astrin et al. (2006) did not include *Spermophora senoculata*, which Bruvo-Madaric et al. (2005) considered basal to all pholcines and part of Holocneminae. Due to its type of SDCS, this species was also considered to be the most basal of all pholcid species already analyzed from the cytogenetic point of view (Král et al. 2006). The phylogeny proposed by Huber (2000) was based on morphological characters and that of Bruvo-Madaric et al. (2005) was made using both morphological and molecular data.

Considering the phylogeny described by Bruvo-Mararic et al. (2005), *S. senoculata*, with 2n = 25 = 11II + X_1X_2Y (Král et al. 2006), is basal in relation to *C. lyoni*. The X_1X_2Y system of basal pholcid species could give rise to an X_1X_2 system, such as that registered for one *C. lyoni* Oriental population with 2n = 24 = 11II + X_1X_2 (Sharma et al. 1959), by gradual heterochromatinization and erosion of the Y sex chromosome. Taking into account this process of SDCS differentiation, the 2n = 24 = 11II + X_1X_2 could represent the basic karyotype of *C. lyoni*. The other diploid numbers obtained for this species could be derived from this basic number. The process of Y sex chromosome heterochromatinization and erosion have been detected in many groups of arthropods and considered a usual event involved in SDCS evolution (White 1973; Smith & Virkki 1978; Steinemanm & Steinemanm 1998). On the other hand, the possibility of conversion of an X_1X_2Y system into an X system, as postulated by Král et al. (2006) for some Haplogynae species, can not be excluded, especially if we consider the 2n = 23 = 11II + X of *Holocnemus caudatus* (Král et al. 2006), which represents a genus closely related to *Crossopriza* (Huber 2000; Bruvo-Madaric et al. 2005). If so, the *C. lyoni* populations with 2n = 23 = 11II + X could be ancestral.

The proposal that 2n = 24 originated the chromosomal races in *C. lyoni* suggests karyotypic evolution by raising or lowering the diploid number of chromosomes and an origin of the X_1X_2 sex determination chromosome system from the X_1X_2Y system. The proposal that 2n = 23 is the ancestral condition requires an increase in the chromosome number and origin of an X system from an X_1X_2Ysystem. These propositions differ from those elaborated by other researchers, such as Suzuki (1951, 1954), Postiglioni & Brum-Zorrila (1981), Maddison (1982), Rowell (1985, 1990) and Datta & Chatterjee (1988), in order to explain the karyotypic differentiation of most spider species.

Considering that the highest chromosome numbers occur in some less morphologically derived spider species (Mesothelae and Mygalomorphae) and that the acrocentric chromosome morphology and X_1X_2 sex determination chromosome system are the most frequent among Araneae, these researchers have suggested that the above-mentioned karyotypic characteristics would be ancestral to Araneae. Lower chromosome numbers and other types of chromosome morphology and sex determination systems, particularly of the X and $X_1X_2X_3Y$ types, would be derived mainly from centric fusions followed or not by pericentric inversions, or by tandem fusions. Alternatively, some of these researchers postulated that the existence of an X sex determination chromosome system as an ancestral condition can not be ruled out and X chromosome centric fission from species with a metacentric X chromosome could give rise to an X_1X_2 system.

Nevertheless, cladistic analyses have indicated that Mesothelae, Mygalomorphae and Araneomorphae (Haplogynae and Entelegynae) have undergone independent processes of morphological differentiation. Independent processes also seem to have promoted the diversification between haplogyne and entelegyne spiders (Platnick et al. 1991; Griswold et al. 1999; Ramírez 2000). These data raise the possibility of an independent karyotypic differentiation in the Mesothelae, Mygalomorphae, Haplogynae and Entelegynae spiders, i.e., the karyotypic differentiation of extant spiders may occur by a raise or lowering of the basic chromosome number.

Unfortunately, we do not have enough evidence to determine the process of karyotypic differentiation among the *C. lyoni* cytotypes. Cytogenetical analysis of other *Crossopriza* species will certainly provide additional information that will allow a more secure establishment of the characteristics of the basic karyotype of the species of this genus, as well as the mechanisms involved in the origin of the chromosomal races or the cytotypes of *C. lyoni*.

Considering that *C. lyoni* is a species with a wide geographic distribution, the karyotype diversity recorded for this species is not surprising and the occurrence of other cytotypes can not be excluded. Therefore, it is not possible to disregard the hypothesis that *C. lyoni* represents a species complex. Cytogenetic analyses may be useful in understanding the taxonomy of this species, that is, if distinct cytotypes are sympatric and there are not hybrid karyotypes. In a few animal groups whose species are very morphologically similar, cytogenetic studies coupled with morphological analyses have promoted the discovery of new species (Silva & Yonenaga-Yassuda 1998; Bertollo et al. 2000).

In the *P. globosus* sample analyzed, the number of chromosomes found in females, 2n = 16 = 14 + XX, and male embryos, 2n = 15 = 14 + X, is coincident with those described by Cokendolpher (1989) for other *Physocyclus* species, namely *P. californicus*, *P. enaulus* and *Physocyclus* sp. However, an intraindividual variation in the number of chromosomes, i.e., 2n = 15 = 14 + X and 4n = 30 = 28 + 2X, was observed in the testicular cells of the adult specimens of *P. globosus*.

The presence of tetraploid cells in the male germ line of *P. globosus* is probably related to the occurrence of cytoplasmatic bridges between cells of the same cyst. These bridges form a syncytium and promote synchronization in cell division and cell differentiation (Alberti & Weinmann 1985; Alberts et al. 2002; Michalik et al. 2003). Due to some peculiarities of these cytoplasmatic bridges, cell couples from a single cyst remained connected during chromosome preparation, leading to the formation of cells that were apparently tetraploid, and of interphasic nuclei with two sexual chromatinic blocks. In fact, the chromosomes of the resulting tetraploid cells exhibited the same degree of condensation and meiotic behavior. In the pholcid *Mesabolivar luteus*, some diplotene cells appeared in pairs, and the authors also suggested that the organization of the testicular cells was responsible for this apparent tetraploidy (Araujo et al. 2005b).

Cokendolpher & Brown (1985) also verified the presence of a few polyploid cells in *Physocyclus* sp., which was attributed to the cell treatment with a colchicine solution. In *P. globosus*, the numerical chromosome variation was certainly not due to the cell treatment with the colchicine solution, because this variation was also observed in preparations where the cells were not subjected to this solution.

The meiotic testicular cells of *C. lyoni* and *P. globosus* showed that the autosomal bivalents and the univalent X chromosome exhibited a regular behavior similar to those described by Suzuki (1954), Bole-Gowda (1958), Cokendolpher (1989), Tugmon et al. (1990), Gorlov et al. (1995), Gorlova et al. (1997), Shyh-Hwang (1999) and Rodríguez-Gil et al. (2002) for most Araneae in terms of condensation, synapsis, chiasma number and chromosome segregation.

The occurrence of the nucleolus or NORs associated with specific chromosomes has been described in some spiders based on ultrastructural analysis of bisected testicular cells using transmission electron microscopy (Benavente & Wettstein 1980; Wise 1983) or on analysis of silver impregnated gonadal and embryonic metaphases using light microscopy (Araujo et al. 2005a; Král et al. 2006). In these analyses, the nucleolar material was associated with the X chromosome in some haplogyne spiders, such as *Dysdera crocata* Koch 1838 (Dysderidae), with 2n = 11 = 10 + X (Benavente & Wettstein 1980), *Ochyrocera* sp. Simon 1891 (Ochyroceratidae), with 2n = 13 = 12 + X (Král et al. 2006), *Scytodes thoracica* (Latreille 1802) (Scytodidae), with 2n = 19 = 18 + X (Král et al. 2006), and *Monoblemma muchmorei* Shear 1978 (Tetrablemmidae), with 2n = 23 = 22 + X (Král et al. 2006), and with autosomes of some entelegyne species, such as two autosomal bivalents of *Allocosa georgicola* (Walckenaer 1837) (as *Lycosa georgicola*) (Lycosidae, Entelegynae), with 2n = 28 = 26 + X_1X_2 (Wise 1983), and three autosomal pairs of *Nephilengys cruentata* (Fabricius 1775) (Nephilidae), with 2n = 22 + $X_1X_1X_2X_2$ (Araujo et al. 2005a).

In *C. lyoni*, five NORs were found occupying the telomeric regions of the pair 4, pair 6 and X chromosomes, while in *P. globosus*, two NORs were found in the interstitial region of the pair 2 chromosomes. The presence of a NOR in the X chromosome of *C. lyoni*, *D. crocata*, *Ochyrocera* sp., *Scytodes thoracica*, and *Monoblemma muchmorei*, all bearing an X/XX sex determination system type, sug-

gests that the X chromosome can represent one of the elements that constitutes the basic NOR pattern in "Higher Haplogynes"(*sensu* Coddington & Levi 1991). The absence of a NOR marking in the X chromosome of *P. globosus* is possibly due to chromosome rearrangements or differential activation of this region. Considering the low number of species whose chromosomes have been subjected to silver staining, it is not yet possible to determine a quantitative pattern of active NORs in spiders.

The interpopulational and intraindividual numerical variations respectively found in *C. lyoni* and *P. globosus* have different origins and meanings in the two species. In *C. lyoni*, the interpopulational variation shows that structural chromosome rearrangements are acting upon the karyotypic evolution of this species. On the other hand, the apparent tetraploidy of the spermatogonial metaphases in *P. globosus* is a result of the tissue organization of the spermatogonia. Thus, variation in *P. globosus* seems to have no relationship to chromosomal evolution of this species, but reflects a mechanisms which guarantees that great quantities of spermatozoids are simultaneously differentiated at the moment of reproduction.

ACKNOWLEDGMENTS

We thank D. Araujo and M.C. Schneider from Universidade Estadual Paulista (UNESP), Departamento de Biologia, Rio Claro, State of São Paulo, Brazil, and two anonymous reviewers for comments, suggestions, and critical review of the manuscript.

LITERATURE CITED

Alberti, G. & C. Weinmann. 1985. Fine structure of spermatozoa of some labidognath spiders (Filistatidae, Segestriidae, Dysderidae, Oonopidae, Scytodidae, Pholcidae; Araneae; Arachnida) with remarks of spermiogenesis. Journal of Morphology 185:1–35.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts & P. Walter. 2002. Molecular Biology of the Cell. Garland Science, New York. 1616 pp.

Araujo, D., D.M. Cella & A.D. Brescovit. 2005a. Cytogenetic analysis of the neotropical spider *Nephilengys cruentata* (Araneomorphae, Tetragnathidae): standard staining, NORs, C-bands and base-specific fluorochromes. Brazilian Journal of Biology 65:193–202.

Araujo, D., A.D. Brescovit, C.A. Rheims & D.M. Cella. 2005b. Chromosomal data of two pholcids (Araneae, Haplogynae): a new diploid number and the first cytogenetical record for the new word clade. Journal of Arachnology 33:591–596.

Astrin, J.J., B.A. Huber, M. Bernhard & C.F.C. Klütsch. 2006. Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): evaluation of species identification methods using CO1 and 16S rRNA. Zoologica Scripta 35:441–457.

Bertollo, L.A.C., G.G. Born, J.A. Dergam, A.S. Fenocchio & O. Moreira-Filho. 2000. A biodiversity in the neotropical Erythrinidae fish, *Hoplias malabaricus*. Karyotypic survey, geographic distribution of cytotypes and cytotaxonomic considerations. Chromosome Research 8:603–613.

Beçak, W. & M.L. Beçak. 1960. Constituição cromossômica de duas espécies de aranhas do gênero *Loxosceles*. Revista Brasileira de Biologia 20:425–427.

Benavente, R. & R. Wettstein. 1980. Ultrastructural characterization of the sex chromosomes during spermatogenesis of spiders having holocentric chromosomes and a long diffuse stage. Chromosoma 77:69–81.

Bole-Gowda, B.N. 1958. A study of the chromosomes during meiosis in twenty-two species of Indian spiders. Proceedings the Zoological Society of Bengal 11:69–108.

Bruvo-Madaric, B., B.A. Huber, A. Steinacher & G. Pass. 2005. Phylogeny of pholcid spiders (Araneae: Pholcidae): combined analysis using morphology and molecules. Molecular Phylogenetics and Evolution 37:661–673.

Coddington, J.A. & H.W. Levi. 1991. Systematics and evolution of spiders (Araneae). Annual Review of Ecology and Systematics 22:565–592.

Cokendolpher, J.C. 1989. Karyotypes of three spider species (Araneae: Pholcidae: *Physocyclus*). Journal of the New York Entomological Society 97:475–478.

Cokendolpher, J.C. & J.D. Brown. 1985. Air-dry method for studying chromosomes of insects and arachnids. Entomological News 96:114–118.

Datta, S.N. & K. Chatterjee. 1988. Chromosomes and sex determination in 13 araneid spiders of North-Eastern India. Genetica 76:91–99.

Diaz, M.O. & F.A. Sáez. 1966a. Investigaciones citogeneticas sobre algunas species de araneidos Uruguayos. Congresso Latino Americano de Zoologia, Anais 2:3–9.

Diaz, M.O. & F.A. Sáez. 1966b. Karyotypes of South-American Araneida. Memórias do Instituto Commemorativo 33:153–154.

Gorlov, I.P., O.Y. Gorlova & D.V. Logunov. 1995. Cytogenetic studies in Siberian spiders. Hereditas 122:211–220.

Gorlova, O.Y., I.P. Gorlov, E. Nevo & D.V. Logunov. 1997. Cytogenetic studies on seventeen spi-

der species from Israel. Bulletin of the British Arachnological Society 10:249–252.

Griswold, C.E., J.A. Coddington & N.I. Platnick. 1999. Towards a phylogeny of Entelegyne spiders (Araneae, Araneomorphae, Entelegynae). Journal of Arachnology 27:53–63.

Hackman, W. 1948. Chromosomenstudien an Araneen mit besonderer Berucksichtigung der Geschlechtschromosomen. Acta Zoologica Fennica 54:1–101.

Hancock, A.J. & D.M. Rowell. 1995. A chromosomal hybrid zone in Australian huntsman spider, *Delena cancerides* (Araneae: Sparassidae). Evidence for a hybrid zone near Canberra, Australia. Australia Journal of Zoology 43:173–180.

Howell, W.M. & D.A. Black. 1980. Controlled silver staining of nucleolus organizer regions with protective colloidal developer: a 1-step method. Experientia 36:1014–1015.

Huber, B.A. 2000. New World pholcid species (Araneae: Pholcidae): a revision at generic level. Bulletin of the American Museum of Natural History 254:1–347.

Král, J., J. Musilová, F. Št'áhlavský, M. Řezáč, Z. Akan, R.L. Edwards, F.A. Coyle & C.R. Almerje. 2006. Evolution of the karyotype and sex chromosome systems in basal clades of araneomorph spiders (Araneae: Araneomorphae). Chromosome Research 14:859–880.

Maddison, W.P. 1982. XXXY sex chromosomes in males of the jumping spiders genus *Pellenes* (Araneae: Salticidae). Chromosoma 85:23–37.

Michalik, P., M.R. Gray & G. Alberti. 2003. Ultrastructural observations of spermatozoa and spermiogenesis in *Wandella orana* Gray, 1994 (Araneae: Filistatidae) with notes on their phylogenetic implications. Tissue & Cell 35: 325–337.

Painter, T.S. 1914. Espermatogenesis in spiders. Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere 38:509–576.

Parida, B.B. & N.N. Sharma. 1987. Chromosome number, sex mechanism and genome size in 27 species of Indian spiders. Chromosome Information Service 43:11–13.

Platnick, N.I., J.A. Coddington, R.R. Forster & C.E. Griswold. 1991. Spinneret morphology and the phylogeny of haplogyne spiders (Araneae, Araneomorphae). American Museum Novitates 3016:1–73.

Platnick, N.I. 2007. The World Spider Catalog, Version 7.5. American Museum of Natural History, New York. Online at: http://research.amnh.org/entomology/spiders/catalog/index.html.

Postiglioni, A. & N. Brum-Zorrilla. 1981. Karyological studies on Uruguayan spiders. II. Sex chromosomes in spiders of the genus *Lycosa* (Araneae: Lycosidae). Genetica 56:47–53.

Ramírez, M.J. 2000. Respiratory system morphology and the phylogeny of haplogyne spiders (Araneae, Araneomorphae). Journal of Arachnology 28:149–157.

Rodríguez-Gil, S.G., L.M. Mola, A.G. Papeschi & C.L. Scioscia. 2002. Cytogenetic heterogeneity in common haplogyne spiders from Argentina (Arachnida, Araneae). Journal of Arachnology 30:47–56.

Rowell, D.M. 1985. Complex sex-linked fusion heterozygosity in the Australian huntsman spider *Delena cancerides* (Araneae: Sparassidae). Chromosoma 93:169–176.

Rowell, D.M. 1990. Fixed fusion heterozygosity in *Delena cancerides* Walck. (Araneae: Sparassidae): an alternative to speciation by monobrachial fusion. Genetica 80:139–157.

Rowell, D.M. 1991. Chromosomal fusion and meiotic behaviour in *Delena cancerides* (Araneae: Sparassidae). I. Chromosome pairing and X-chromosome segregation. Genome 34:561–566.

Sharma, G.P., B.L. Gupta & R. Parshad. 1959. Cytological studies on the Indian spiders. III. An analysis of the chromosomes in the male germ cells of the spider *Crossopriza lyoni* (Blackwall), Fam. Pholcidae. Research Bulletin of the Panjab University 10:49–53.

Sharma, G.P. & B.B. Parida. 1987. Study of chromosome in spiders from Orissa. Pranikee 8:71–76.

Shyh-Hwang, C. 1999. Cytological studies on six species of spiders from Taiwan (Araneae: Theridiidae, Psechridae, Uloboridae, Oxyopidae, and Ctenidae). Zoological Studies 38:423–434.

Silva, D. 1988. Estudio cariotípico de *Loxosceles laeta* (Araneae: Loxoscelidae). Revista Peruana de Entomologia 31:9–12.

Silva, M.J. & Y. Yonenaga-Yassuda. 1998. Karyotype and chromosomal polymorphism of an undescribed *Akodon* from Central Brazil, a species with the lowest known diploid chromosome number in rodents. Cytogenetics and Cell Genetics 81:46–50.

Silva, R.W., D.R. Klisiowicz, D.M. Cella, O.C. Mangili & I.J. Sbalqueiro. 2002. Differential distribution of constitutive heterochromatin in two species of brown spider: *Loxosceles intermedia* and *Loxosceles laeta* (Araneae, Sicariidae), from the metropolitan region of Curitiba, PR (Brazil). Acta Biológica Paranaense 31:123–136.

Smith, S.G. & N. Virkki. 1978. Animal Cytogenetics. 3. Insecta. 5. Coleoptera. Gebrüder Borntraeger, Berlin.

Srivastava, M.D.L. & S. Shukla. 1986. Chromosome number and sex determing mechanism in forty seven species of Indian spiders. Chromosome Information Service 41:23–26.

Steinemanm, M. & S. Steinemanm. 1998. Enigma of Y chromosome degeneration: Neo-Y and Neo-X chromosomes of *Drosophila miranda* a model

for sex chromosome evolution. Genetica 102/103:409–420.

Suzuki, S. 1951. Cytological studies in spiders. I. A comparative study of the chromosomes in the family Argiopidae. Journal of Science of the Hiroshima University (B-1) 12:67–98.

Suzuki, S. 1954. Cytological studies in spiders. III. Studies on the chromosomes of fifty-seven species of spiders belonging to seventeen families, with general considerations on chromosomal evolution. Journal of Science of the Hiroshima University (B-1) 15:23–136.

Tsurusaki N., Y. Ihara & T. Arita. 1993. Chromosomes of the funnel-web spider *Agelena limbata* (Araneae: Agelenidae). Acta Arachnologica 42: 43–46.

Tugmon, C.R., J.D. Brown & N.V. Horner. 1990. Karyotypes of seventeen USA spiders species (Araneae, Araneidae, Gnaphosidae, Loxoscelidae, Lycosidae, Oxyopidae, Philodromidae, Salticidae and Theridiidae). Journal of Arachnology 18:41–48.

Wang, X.-Z., S.-J. Cui, Z.-L. Yang, J.-P. Wang & Y.-J. Wang. 1997. On karyotype of the *Pholcus affinis* (Araneae: Pholcidae). Acta Arachnologica Sinica 6:19–22.

Webb, G.C., M.J.D. White, N. Contreras & J. Cheney. 1978. Cytogenetics of the parthenogenetic grasshopper *Warramaba* (formerly *Moraba*) *virgo* and its bisexual relatives. IV. Chromosome banding studies. Chromosoma 67:309–339.

White, M.J.D. 1973. Animal Cytology and Evolution. 3rd Edition. Willian Clowes & Sons, London. 961 pp.

Wise, D. 1983. An electron microscope study of the karyotypes of two wolf spiders. Canadian Journal of Genetics and Cytology 25:161–168.

Manuscript received 13 March 2006, revised 5 April 2007.

2007. The Journal of Arachnology 35:307–312

A NOVEL TRAP TO CAPTURE BALLOONING SPIDERS

Chris Woolley, C. F. George Thomas and **Linda Hutchings**: School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, Devon, PL4 8AA, UK. E-mail: c.woolley@plymouth.ac.uk

Sara Goodacre and **Godfrey M. Hewitt**: School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

Steve P. Brooks: Statistical Laboratory, Centre for Mathematical Sciences, Wilberforce Road, Cambridge, CB3 0WB, UK

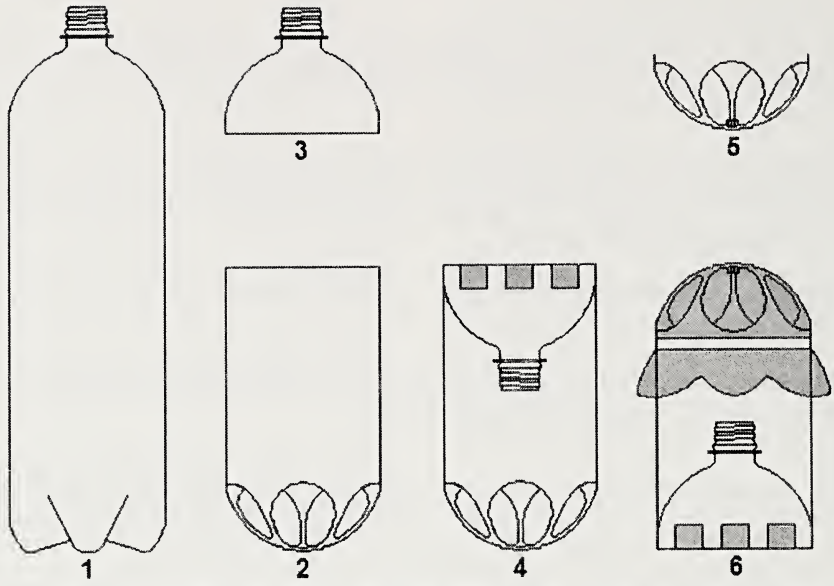
ABSTRACT. An unattended trap was designed to sample and retain spiders dispersing from agricultural grassland and crops. Traps comprised a removable bottle-trap fixed to the top of a vertical metal rod or "climbing-stick" that spiders climbed during normal pre-ballooning behavior. Bottle-traps caught over eight times more spiders than sticks treated with insect trapping adhesive. Draping sticks with nets increased the effective area of the traps and increased the catch size threefold. On average, 9.1% of spiders were lost from traps during the daytime sampling period. No difference in average rate of loss of spiders from the bottle-traps was observed between night and daylight hours. The bottle-trap design is economical and simple to construct, erect and operate. Continuous sampling also allows multiple traps to be used simultaneously in various locations.

Keywords: Aerial dispersal, sampling, bottle-trap, climbing-stick

Aerial dispersal by ballooning is a key strategy in the life histories of many spiders, especially pioneers of disturbed, patchy habitats exemplified by linyphiids in agricultural landscapes (Thomas et al. 2003a). Quantifying the dispersal power of these species is a necessary prerequisite for accurately modeling spatial population dynamics and developing successful sustainable management strategies. Various techniques that actively or passively intercept airborne spiders have been used to measure aspects of aerial dispersal. For example: the use of nets and sticky traps to measure aerial density at one or more altitudes (Greenstone et al. 1987; Greenstone 1991; Thomas et al. 2003b); manual collection from fences, wire, or string to quantify numbers passing a point or line per unit time (Vugts & Van Wingerden 1976; Thomas et al. 2003b); or water traps to quantify deposition rates per unit area (Weyman et al. 1995; Thomas & Jepson 1999). These methods are either labor intensive, require operator attendance, cannot easily sample several locations at the same time, or may be cumbersome or expensive.

An alternative sampling method exploits the climbing behavior normally exhibited by spiders as a precursor to ballooning (Blackwall 1827): spiders climb to a high point where a silk line can be produced above the surrounding vegetation and where suitable atmospheric conditions for successful ballooning are likely to occur (Suter 1999). Sticks, canes or similar objects inserted into the ground, provide artificial platforms that stand higher than the surrounding vegetation. Spiders climbing and attempting to balloon from these can be observed, or caught and counted, to give a relative indication of ballooning activity over a given period. Thorbek et al. (2002), in a validation of this technique, found that numbers of spiders observed climbing a 30 cm stick correlated well with numbers obtained from an aerial suction trap. Using a similar technique to sample several habitats over time, Duffey (1956) applied a tacky adhesive to the tops of canes to trap climbing spiders. However, the adhesive was adversely affected by hot, cold or wet weather and became clogged with winged insects during summer months.

This paper describes and evaluates a novel



Figures 1–6.—Trap construction. 1. Two liter soft-drinks bottle. 2. Bottle bottom with the five reinforcements removed. 3. Top removed and section below discarded. 4. Inverted top inserted into the remaining section and secured with adhesive tape. 5. Screw cap glued underneath the central hub. 6. Finished trap with fine gauze fastened in place with a rubber band.

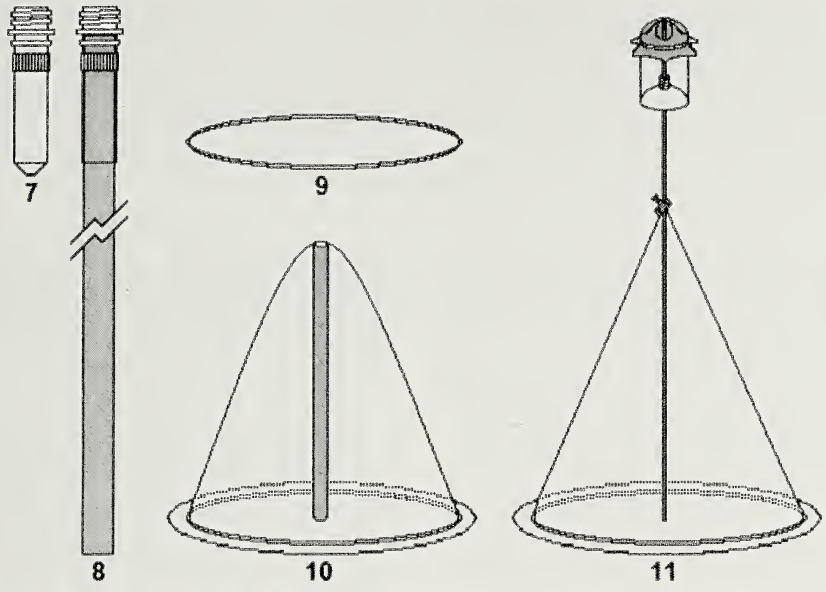
design that develops the climbing-stick into a trap to allow continuous unattended sampling without the use of adhesive. Attached to the top of a climbing-stick is a "bottle-trap" operating on the lobster-pot principle. Climbing spiders are retained within the bottle-trap until it is removed or replaced. In the present paper we compare the trapping efficiencies of climbing sticks either with bottle-traps or with adhesive.

The trap collects spiders climbing from the underlying vegetation before they first become airborne, and spiders already airborne arriving at the trap from sources upwind. In the present paper we do not differentiate between these two potential sources. However, we evaluate the effect of suspending a net skirt from the climbing-stick to increase the effective vertical and horizontal cross-sectional area of the trap. This increases both the source area of spiders emerging from the ground and the interception of airborne spiders.

METHODS

Trap construction.—The "lobster-pot" part comprising the bottle-trap was constructed from a standard straight-sided, clear plastic, 2-liter soft-drinks bottle (Fig. 1). The body of the trap was made by first removing, with a heated scalpel blade, the material between the five reinforcing moldings in the base (Fig. 2). The top section of the bottle was then removed, just below the shoulder, approximately 9 cm from the top of the bottle opening (Fig. 3). A band approximately 7 cm deep was cut away from the main body and discarded. The removed top section was then inverted and fixed into the remaining base section of the bottle using adhesive tape (Fig. 4), ensuring no gaps remained between the two sections. A 2 ml micro-tube screw-cap (Sarstedt™, A.G. Sarstedt & Co, Nümbrecht, Germany) was glued with super-glue (Loctite™, Henkel, Düsseldorf, Germany) centrally beneath the now inverted base section and above the original bottle top opening forming the new base (Fig. 5). A 20 × 20 cm square of white voile gauze fabric was then fastened tightly over the five cut-away openings with a rubber band (Fig. 6). The cut-away openings covered in fine gauze voile material allowed vertical air flow, general ventilation and, when removed, the extraction of spiders from the trap.

The climbing-stick was made from a 1.5 m length of 7.9 mm diameter aluminum rod. The surface was roughened with sandpaper to assist climbing spiders.



Figures 7–11.—Trap construction. 7. Micro-tube. 8. Micro-tube with bottom removed, pushed over the end of the climbing-stick and glued in position. 9. Circular wire frame. 10. Netting pulled over pole with circular wire frame placed over netting. 11. Finished trap with bottle-trap screwed on and net clipped to stick.

An attachment for the bottle-trap was made using the body of the 2 ml micro-tube from which came the cap that had been glued to the bottle-trap. The bottom section of the main body was removed just above the taper (Fig. 7). A small amount of rapid drying epoxy resin (Araldite™, Huntsman Advanced Materials, Everberg, Belgium) was applied to the inside of the tube, which was then placed over the end of the climbing-stick with the thread end uppermost and extending approximately 5 mm above the end (Fig. 8).

The net was constructed from 2 cm mesh bird netting made from a natural-fibre twine. Sufficient material to form a small tent was draped over a 1.2 m wooden pole. A 3.14 m length of 2 mm fencing wire, formed into a 1 m diameter circle (Fig. 9) was placed over the netting and pole to weigh down the base of the net and keep it splayed out. The netting was pulled taut over the pole, arranged evenly around the frame, and its hem secured to the circular base with wire ties before cutting away excess material. (Fig. 10).

Setting and operating the trap.—To set the trap, the climbing-stick was pushed vertically into the ground, and a bottle-trap placed over and screwed to the top of the stick. If a net was also used, this was first pulled up to form a cone and the climbing-stick placed through the apex before the stick was pushed into the ground. The net was then clipped to the stick using a small bulldog clip set at an angle to ensure the spiders continued climbing. The circular wire base was held down with wire pegs or stones. The bottle-trap was then screwed to the top of the stick (Fig. 11).

For continual sampling, bottle-traps were unscrewed and replaced with empty ones. For daily samples reported here, traps were typically changed each evening after ballooning behavior had finished. Removed traps were placed in plastic bags in the field before returning to the lab. Spiders were extracted from traps by removing the voile gauze and shaking vigorously over a tray from which spiders were collected with an aspirator. Any spiders remaining in the trap were removed either with an aspirator or, if there was a lot of silk in the trap, with a small paint brush.

Trap evaluation.—Experiments were performed with traps set along a transect in an 8 ha grass field on the estate farm at the Seale-Hayne Faculty, Newton Abbot, Devon, in the southwest of the UK. The temporary grass ley was approximately 150 mm tall at the time of sampling. The transect, orientated north-south, traversed the brow of a hill, the mid-section

Table 1.—Total number of spiders caught per trap over an 11 day period from climbing-sticks with bottle-traps and climbing-sticks with adhesive.

Trap number	1	2	3	4	5	6	7	8	9	10
Bottle-trap	18	17	14	46	78	107	131	75	53	25
Adhesive	1	6	8	9	6	5	16	7	8	0

being elevated relative to the extremities. An electric fence was used to protect the transect from disturbance by sheep and cattle that periodically grazed the field.

Three aspects of the trap were evaluated: catch size from climbing-sticks with bottle-traps compared with climbing-sticks with a polybutene-based insect trapping adhesive (Oecotak A5™, Oecos Ltd, Kimpton, Hertfordshire, England) applied to the uppermost 15 cm of the stick; catch size from climbing-sticks and bottle-traps with and without nets; retention of spiders left in bottle-traps during the day and overnight.

To compare catch size from climbing-sticks with either bottle-traps or adhesive, 10 traps of each design were set alternately at 10 m intervals. Bottle-traps were emptied on each of 11 successive days in March 2003; climbing-sticks with adhesive accumulated spiders over the same period. Climbing sticks with adhesive were checked periodically to ensure that the accumulation of trapped spiders or insects was not excessive and that there was ample exposed adhesive to maintain capture efficiency. Total numbers caught per trap were recorded at the end of the sampling period. For catch size evaluations comparing climbing-sticks and bottle-traps with and without nets, 10 traps of each design were set alternately at 10 m intervals. Samples were taken and recorded daily over a 13 day period in March 2004. For the retention study, 10 climbing-sticks with bottle-traps were placed in the field as above. Numbers of spiders in each bottle-trap were recorded after 24 h at 17:00. Traps were then relocated to a tarmac substrate away from ground vegetation to minimize further ingress of spiders. Numbers of spiders remaining in the traps were again recorded at 09:00 and at 17:00 the following day.

RESULTS

Comparison between climbing-sticks with bottle-traps and climbing-sticks with adhesive.—For all traps, catch sizes were higher for climbing-sticks with bottle-traps than for climbing-sticks with adhesive (Table 1). Total catch size over the period for climbing-sticks with bottle-traps was 564 spiders and for climbing-sticks with adhesive, 66 spiders.

Comparison between bottle-traps with and without nets.—Climbing-sticks with nets caught greater numbers of spiders than those without nets for 7 days out of the 13 day period (Table 2). Spiders were not recorded in any trap on 22, 23, 24, 28, and 29 March when high wind speeds suppressed ballooning activity. No differences were recorded on 26 March though catch size was very low with only 2 spiders recorded in all traps together. The total numbers of spiders caught by climbing-sticks with and without nets were 641 and 218 respectively.

Retention of spiders in bottle-traps.—Of a total of 413 spiders in 10 bottle-traps recorded at 17:00, 69 (15.3% ± 11.8%) had escaped by 09:00 the following morning. A further 35 (9.1% ± 7.7%) escaped between 09:00 and 17:00. The average loss over 24 h was 24.4% ± 16.6%. A significant linear regression (adjusted R^2 = 63.6%, P = 0.004) between initial numbers caught and numbers lost after 24 h indicated losses to be largely den-

Table 2.—Daily totals of spiders caught for all traps with and without nets.

Date	18/3	19/3	20/3	21/3	22/3	23/3	24/3	25/3	26/3	27/3	28/3	29/3	30/3
Nets	14	324	41	46	0	0	0	147	4	1	0	0	64
No nets	2	137	8	7	0	0	0	57	1	1	0	0	5

sity independent. Mean rate of loss (± SE) from traps between 17:00 and 09:00 was 0.431 ± 0.141 spiders per hour and from 09:00 to 17:00, 0.438 ± 0.148 spiders per hour. No significant difference in rate of loss was observed between night and day hours ($F_{(1,18)} = 0.01$, $P = 0.976$).

DISCUSSION

Climbing-sticks with bottle-traps are extremely effective, cheap and easy to make and use. We estimate the cost of construction materials to be less than $9 US per trap at current prices. Apart from the greater catch size, which, in total, was over eight times that of climbing sticks with adhesive, the bottle-traps also retain the advantage of easy replication and the ability to simultaneously sample different habitats at large spatial and/or short temporal scales. The retention of live spiders means trapping agents such as adhesive or water and detergent are not required. Furthermore, additional behavioral, ecological or genetic studies can be carried out on the trapped spiders if required.

The addition of nets to climbing sticks with bottle traps increased catch size almost three fold. The trials reported here were conducted in short grass. However, in other trials conducted in taller crops, such as wheat, it was necessary to use 2.5 m climbing-sticks to raise the nets and bottle-traps above the crop in order to intercept airborne spiders. For comparative work sampling airborne spiders above crops of differing height, traps should be set at a constant height above the roughness length of the vegetation.

Although losses from traps left operating for several consecutive days can be estimated, it is recommended that the traps are emptied daily, unless spiders are being collected only for laboratory studies. This avoids large amounts of silk accumulating inside the bottle-traps which makes separation of the spiders from the silk difficult and extraction much more time-consuming. Similarly, when large numbers of spiders were caught within a single day, we found traps were best emptied immediately after collection because of the quantity of silk produced if left overnight. We found traps were best removed in the evening after ballooning had finished. If traps cannot be changed until the morning, it should be carried out very early during summer months in order to prevent cross contamination with the previous day's sample. If longer duration sampling is required and live spiders are not, a preserving fluid could be introduced into the bottom section of the bottle-trap. Spiders would fall into this, thereby reducing losses and minimizing any build-up of silk.

A large variation in catch size was observed along the transect, particularly for the bottle-traps. This was possibly due to the greater trapping efficiency of the bottle-traps coupled with the undulating nature of the field, the greatest catch size being recorded at the highest elevation.

Linyphiids were by far the commonest spiders caught by the traps, being highest both in numbers and in occurrence throughout the year. Other spiders caught in lesser numbers belonged to the families Thomisidae and Araneidae. Though immature thomisids were observed ballooning, adults of these families may have been present in traps as an accident of other behaviours such as rigging, locating shelter/feeding sites or web building. Care must therefore be taken before attributing dispersal by ballooning to all spiders caught.

The bottle-traps sometimes caught other insects including bush crickets, cantharid beetles, ephemeropterans, plecopterans, tipulids and various other dipterans. Some of this bycatch might prey on spiders but we did not see any evidence for this. Other potential losses are likely from predation among spiders but this was not quantified and is likely only if traps are left operating unchanged for longer periods.

ACKNOWLEDGMENTS

This work was funded through BBSRC grants D14032, D20476 and D14036. We would like to thank all the technical and farm staff at Seale-Hayne for their assistance in this work.

LITERATURE CITED

Blackwall, J. 1827. Observations and experiments, made with a view to ascertain the means by which the spiders that produce gossamer effect their aërial excursions. Transactions of the Linnean Society of London 15:449–459.

Duffey, E. 1956. Aerial dispersal in a known spider population. Journal of Animal Ecology 25:85–111.

Greenstone, M.H. 1991. Aerial dispersal of arthropod natural enemies: altitudinal differences in

taxonomic distributions of dispersers. Pp. 104–106. *In* Proceedings of the 10th Conference on Biometeorology and Aerobiology and the Special Session on Hydrometeorology. American Meteorological Society, Boston, Massachusetts.

Greenstone, M.H., C.E. Morgan, A.-L. Hultsch, R.A. Farrow & J.E. Dowse. 1987. Ballooning spiders in Missouri, USA, and New South Wales, Australia: family and mass distributions. Journal of Arachnology 15:163–170.

Suter, R.B. 1999. An aerial lottery: the physics of ballooning in a chaotic atmosphere. Journal of Arachnology 27:281–293.

Thomas, C.F.G., R.P. Blackshaw, L. Hutchings, C. Woolley, S. Goodacre, G.M. Hewitt, K. Ibrahim, S. Brooks & R. Harrington. 2003a. Modelling life-history/dispersal-strategy interactions to predict and manage linyphiid spider diversity in agricultural landscapes. Pp. 167–172. *In* International Organization for Biological Control WPRS Bulletin, Volume 26. Landscape Management for Functional Biodiversity. (W.A.H. Rossing, H-M. Poehling & G. Burgio, eds.). University of Bologna, Italy.

Thomas, C.F.G., P. Brain & P.C. Jepson. 2003b. Aerial activity of linyphiid spiders: modeling dispersal distances from meteorology and behaviour. Journal of Applied Ecology 40:912–927.

Thomas, C.F.G. & P.C. Jepson. 1999. Differential aerial dispersal of linyphiid spiders from a grass and a cereal field. Journal of Arachnology 27: 294–300.

Thorbek, P., C.J. Topping & K.D. Sunderland. 2002. Validation of a simple method for monitoring aerial activity of spiders. Journal of Arachnology 30:57–64.

Vugts, H.F. & W.K.R.E. Van Wingerden. 1976. Meteorological aspects of aeronautic behaviour of spiders. Oikos 27:433–444.

Weyman, G.S., P.C. Jepson & K.D. Sunderland. 1995. Do seasonal-changes in numbers of aerially dispersing spiders reflect population density on the ground or variation in ballooning motivation? Oecologia 101:487–493.

Manuscript received 5 June 2006, revised 27 January 2007.

2007. The Journal of Arachnology 35:313–317

A REVIEW OF THE WOLF SPIDER GENUS *HIPPASELLA* (ARANEAE, LYCOSIDAE, SOSIPPINAE)

Éder S. S. Álvares[1,2] and **Antonio D. Brescovit**[1]: [1]Laboratório de Artrópodes, Instituto Butantan, São Paulo, São Paulo, Brazil [2]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil. E-mail: essalvares@yahoo.com.br

ABSTRACT. The monotypic genus *Hippasella* Mello-Leitão 1944 is revised, and its type-species *H. nitida* Mello-Leitão 1944 is considered a junior synonym of *Tarentula guaquiensis* Strand 1908, from Bolivia. *Hippasella guaquiensis* (Strand) comb. nov. is redescribed and the female genitalia are illustrated for the first time. This species now is recorded from Peru, Bolivia and Argentina. It appears to prefer vegetation near water.

RESUMO. O gênero monotípico *Hippasella* Mello-Leitão 1944 é revisado e sua espécie-tipo *H. nitida* Mello-Leitão 1944 é considerada um sinônimo júnior de *Tarentula guaquiensis* Strand 1908, da Bolívia. *Hippasella guaquiensis* (Strand) comb. nov. é redescrita e a genitália da fêmea é ilustrada pela primeira vez. Esta espécie é agora conhecida do Peru, Bolívia e da Argentina, onde parece preferir a vegetação próxima à água.

Keywords: Neotropical, taxonomy, redescription

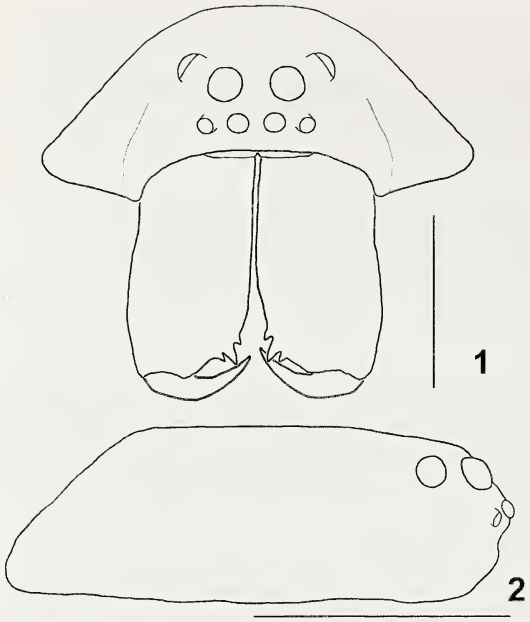
The genus *Hippasella* was proposed by Mello-Leitão (1944) based on *Hippasella nitida* Mello-Leitão 1944, a species known only from a male specimen collected in La Plata, Argentina. The type-specimen of *H. nitida* is an adult male, but it is fragmented and in bad condition. Capocasale (1990) studied the type specimen of *H. nitida* and synonymized *Hippasella* with *Sosippus* Simon 1888, based on the eye arrangement observed on the carapace fragments of the type specimen and on the absence of a palea and of a terminal apophysis in the male pedipalp. However, Sierwald (2000), refering to the figures of Capocasale (1990, figs. 12, 13), pointed out that the male pedipalp of *H. nitida* does not have a long finger-shaped apophysis (apophysis *a* in Sierwald 2000: 136, fig. 7) shared by all *Sosippus* species. Moreover, the original size ratio of the eyes described by Mello-Leitão (1944: 343) for *H. nitida* does not match the ratio observed in the remaining *Sosippus* species. Based on these observations, Sierwald (2000) revalidated the genus *Hippasella*.

For a long time the only known specimen of *H. nitida* was the fragmented type specimen. Recently, after examining Lycosidae material housed in the Museu de Ciências Naturais, Porto Alegre, and in the Museo de Historia Natural San Marcos, Lima, we found some additional specimens of this species, including females. Moreover, after examining the types of *Tarentula guaquiensis* Strand 1908, housed in the Museum Wiesbaden, Wiesbaden, and kindly loaned by Dr. Michael Apel, we detected a new synonym for *H. nitida*. In this paper, we present a more detailed redescription of this genus and the first illustrations of the female genitalia.

METHODS

Descriptions and terminology follow Santos & Brescovit (2001). All measurements are in millimeters. The abbreviations used in the text are the following: ALE, anterior lateral eyes; AME, anterior median eyes; PLE, posterior lateral eyes; PME, posterior median eyes.

The material examined are deposited in the following collections: IBSP, Instituto Butantan, São Paulo, Brazil; MHNSM, Museo de Historia Natural San Marcos, Lima, Peru; MCN, Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil; MWNH, Museum Wiesbaden, Wiesbaden, Germany; SMF, Naturmuseum Senckenberg, Frankfurt, Germany.



Figures 1–2.—*Hippasella guaquiensis* (Strand 1908), female from Huatajata, Bolivia: 1. Carapace, frontal view; 2. Carapace, lateral view. Scale bars = 2.00 mm.

TAXONOMY

Family Lycosidae Sundevall 1833
Subfamily Sosippinae Dondale 1986
Hippasella Mello-Leitão 1944

Hippasella Mello-Leitão 1944:342; Roewer 1955: 313; Roewer 1960:1002.

Sosippus Simon: Capocasale 1990:140 (synonymy rejected by Sierwald 2000:138).

Type species.—*Hippasella nitida* Mello-Leitão 1944, by original designation and monotypy.

Diagnosis.—Males of *Hippasella* can be distinguished from males of other genera of Sosippinae by the tegular lobe in the pedipalp with a small and pointed lateral apophysis (Figs. 4, 5); a small and membranous median apophysis (Figs. 4, 5); and by a small lobe on the apical edge of the tegulum seen in the ventral view (Fig. 4). Females can be distinguished by the large and flattened median septum, and by spermathecae with a long and sigmoid curved stalk and with a small and not bilobate base (Fig. 8).

Description.—Small lycosids (length 5.81–8.40 mm). Carapace piriform, flattened dorsally (Fig. 1). Eyes: anterior ocular row slightly procurve (Fig. 2); ocular quadrangle trapezoidal (Figs. 2, 3). Chelicerae strong; promargin with three teeth, the median bigger than lateral ones; retromargin with three big, equal and equidistant teeth. Sternum longer than wide, brownish, covered with grayish setae. Spinnerets: anterior lateral spinnerets conical, posterior median small, posterior lateral with distal segment not elongated. Male pedipalp: tibia cylindrical, 1.67 times longer than wide; cymbium piriform, without distal spines and with basal retrolateral edge dilated; tegulum large, with spermatic ducts visible ventrally, sigmoid; in ventral view (Fig. 4), the apical border bearing a small and transversally elongated projection at median region; retrolateral margin of tegulum with a developed and ventrally pointed tegular lobe (Fig. 5). Median apophysis small, membranous and elongated, with distal end curved ventrally. Embolus with broad base, and distal area filiform and located below the base of median apophysis (Fig. 6). Terminal apophysis absent.

Epigynum: median septum wider than long, flattened, with copulatory openings located anteriorly at its lateral borders (Fig. 7); epigynal plate and posterior half of median septum covered with small setae; atrium reduced, forming a narrow depression at lateral side of median septum. Internally (Fig. 8, 9) spermathecae hardly sclerotized, with small and not bilobate base, located dorsally at the stalk (Fig. 8); stalk elongated, curved in an "S" like shape. Head small, globular. Copulatory ducts large, flattened, and located medially to the base. Fertilization ducts small, membranous, located at posterior margin and curved dorsally (Fig. 9).

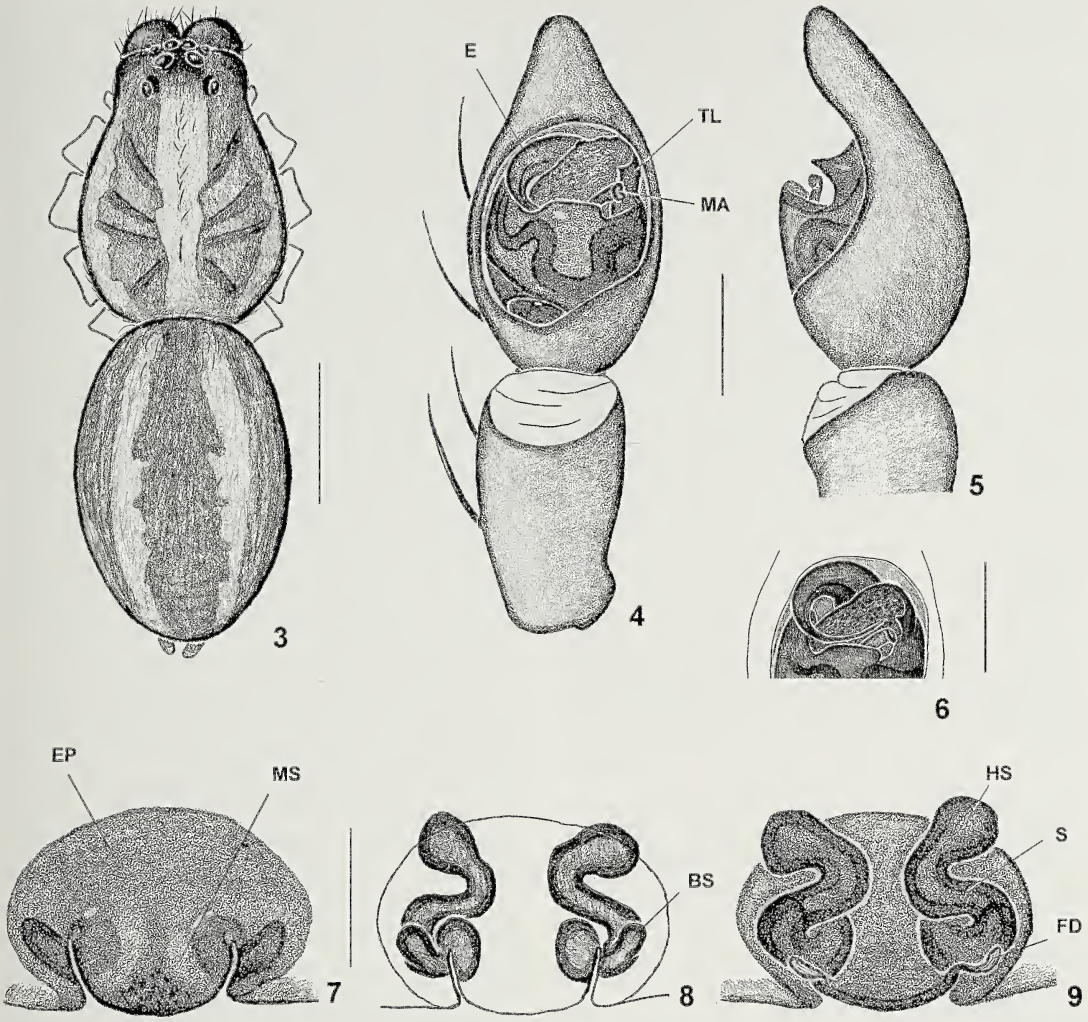
Remarks.—The placement of *Hippasella* in Sosippinae is based on the absence of a terminal apophysis and of a developed palea in the male pedipalp. Moreover, retrolaterally the base of the male pedipalpal cymbium of *Hippasella* is enlarged, as seen in species of *Sosippus*, *Aglaoctenus* Tullgren 1905 and *Diapontia* Keyserling 1876, and this character can be added to the diagnosis of Sosippinae.

***Hippasella guaquiensis* (Strand 1908)**
comb. nov.
Figs. 1–10

Tarentula guaquiensis Strand 1908:252.

Lycosa guaquiensis (Strand): Petrunkevitch 1911: 559.

Hippasella nitida Mello-Leitão 1944:343, fig. 32;



Figures 3–9.—*Hippasella guaquiensis* (Strand 1908), from Huatajata, Bolivia: 3. Female body, dorsal view; 4. Male pedipalp, ventral view; 5. Male pedipalp, retrolateral view; 6. Male pedipalp, antero-ventral view; 7. Female epigynum, ventral view; 8. Cleared female epigynum, ventral view; 9. Female epigynum, dorsal view. Abbreviations: BS = base of spermatheca, E = embolus, EP = epigynal plate, FD = fertilization duct, HS = head of spermatheca, MA = median apophysis, MS = median septum, S = stalk of spermatheca, TL = tegular lobe. Scale bars: Figure 3 = 2.00 mm; Figures 4–9 = 0.25 mm.

Roewer 1955:313; Sierwald 2000:138. **New synonymy.**

Trochosa guaquiensis (Strand): Roewer 1955:301.

Sosippus nitidus (Mello-Leitão): Capocasale 1990: 139, figs. 12, 13; Platnick 1993:508.

Type specimens.—*Tarentula guaquiensis*: BOLIVIA: 1 male (without pedipalps), 1 female syntype, Guaqui (not Peru) (16.5°S, 68.8°W), 1907, K. Seyd (MWNH #448); 1 pedipalp of the male syntype mounted on a microscope slide (SMF #13521-138).

Hippasella nitida: ARGENTINA: male holotype, La Plata, Buenos Aires (34.9°S, 57.9°W), M. Birabén (MLP #16035).

Other material examined.—PERU: *Departamento de Pasco:* 2 ♀, Pucayacu (10°39.2′S, 76°14.0′W), 8 May 2005, W. Paredes, D. Causso (MHNSM); *Departamento de Cusco:* 1 ♀, Cusco (13°30.4′S, 71°59.0′W), June–July 1983, M. del Castillo (MHNSM); 2 ♀, Quebrada Jalunmoco Huayco (13°35.7′ S, 71°58.5′W), 23 March 2005, W. Paredes (MHNSM); *Departamento de Puno:* 1 ♀, Arapa, border of Lake Titicaca (15°07.4′S,

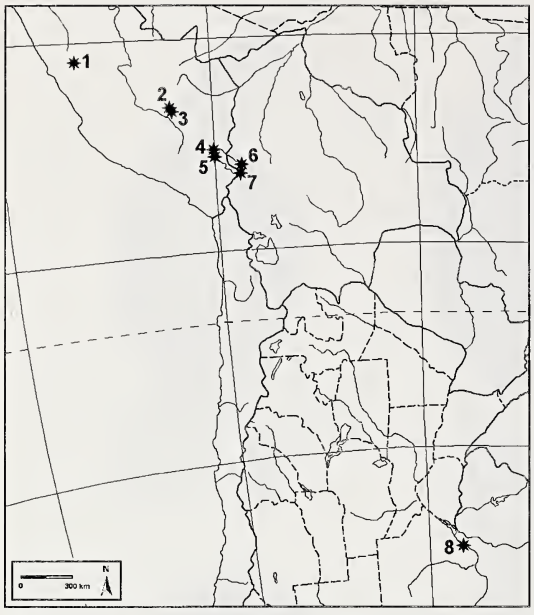


Figure 10.—*Hippasella guaquiensis* (Strand 1908), known records. Peru: 1. Pucayacu; 2. Cusco; 3. Quebrada Jalunmoco Huayco; 4. Arapa; 5. Puno. Bolivia: 6. Huatajata; 7. Guaqui. Argentina: 8. La Plata.

70°06.3′W), November 1948, F. Blancas (MHNSM); 1 ♂ Puno (15°49.6′S, 70°01.3′W), November 1948, F. Blancas (MHNSM); BOLIVIA: *Departamento de La Paz:* 1 ♂, 1 ♀, Huatajata, border of Lake Titicaca (16°06.0′S, 68°42.0′W), 8 August 1993, A.D. Brescovit & H. Höfer (MCN #23788); 1 ♀, same data (IBSP #66977).

Diagnosis.—The same for the genus.

Description.—*Male (Huatajata, Bolivia MCN #23788):* Carapace brownish with gray setae and with 1 dorsal and 1 submarginal pale band, with dark radial bands; eyes surrounded by black areas. Chelicerae, sternum and labium brown; coxae and endites yellowish. Legs: dorsum of femora, patellae, and tibiae yellowish with dark spots; metatarsi and tarsi light brown; venter of femora and tibiae yellowish with brown spots; patellae, metatarsi, and tarsi brown. Abdomen: dorsum with a dorsal longitudinal brown band delineated by black lines and bordered by a pale longitudinal band on each side; sides brown with 4–6 yellowish parallel longitudinal lines; venter yellowish with two median parallel black lines, extending from the epigastric furrow to the middle of abdomen. Spinnerets yellowish. Total length: 5.81; carapace length: 2.98; carapace width: 2.13. Eye diameters: AME 0.14; ALE 0.12; PME 0.22; PLE 0.17. Eye interdistances: AME–AME 0.08; AME–ALE 0.04; PME–PME 0.14; PME–PLE 0.16; PLE–PLE 0.59. Clypeus: 0.07 height. Leg I: femur 1.80/patella 1.05/tibia 1.48/metatarsus 1.48/tarsus 0.92/total 6.73; leg II: 1.79/0.99/1.30/1.39/0.92/6.39; leg III: 1.76/0.82/1.22/1.61/0.89/6.30; leg IV: 2.13/1.07/1.74/2.40/1.17/8.51. Legs, spination: femur I: p0-0-2, d1-1-0, r0, II: p0-1-1, d1-1-0, r0; III: p0-1-1, d1-1-1, r0-0-1; IV: p0 (or p1-0-0, or p0-0-1), d1-1-1, r1-0-0; patellae I-II: p0, r0; III: p1, r1; IV: p1, r1; tibia I: p1-1, d0, r0-1 v2-0-2; II: p1-1, d0-0, r0-1 v2-2-2 or v1r-2-2; III-IV: p1-1, d0-1, r1-1, v2-2-2; metatarsus I: p0-1-1, r0, v2-2-3; II: p0-1-1, r0-1-1, v2-2-3; III-IV: p1-1-1, r1-1-1, v2-2-3 (v3-2-3 in metatarsus IV). Tarsus and distal end of metatarsus of legs I and II weakly scopulate. Pedipalp (Figs. 4–6): see description in the genus.

Female (Huatajata, Bolivia MCN #23788): Coloration as in males except carapace with lateral pale bands almost marginal (Fig. 3), dorsum of abdomen with lateral pale bands darker, and venter of abdomen with a median longitudinal dark band bordered by a lateral pale band on each side. Total length: 8.18; carapace length: 3.96; carapace width: 3.13. Eye diameters: AME 0.20; ALE 0.16; PME 0.29; PLE 0.25. Eye interdistances: AME–AME 0.10; AME–ALE 0.08; PME–PME 0.21; PME–PLE 0.26; PLE–PLE 0.89. Leg I: femur 2.55/patella 1.48/tibia 1.79/metatarsus 1.84/tarsus 1.17/total 8.83; leg II: 2.37/1.45/1.66/1.79/1.12/8.39; leg III: 2.24/1.33/1.48/1.96/1.10/8.11; leg IV: 2.98/1.52/2.22/2.93/1.38/11.03. Leg spination as male, except: femur I: p0-0-1; IV: p0, r0; tibia I: p0, d0, r0, v0-1p-2; II: p0-0-1, d0, r0, v 0-1r-2; III-IV: d0; metatarsus I: p0; II: p0-1-0 or p0, v0. Epigynum (Figs. 7–9): see generic description.

Variation.—Nine females. Total length: 6.96–8.92; carapace length: 3.67–4.07; length of femur I: 2.16–2.55. Three males. Total length: 5.29–7.60; carapace length: 2.98–3.85; length of femur I: 1.80–2.55.

Remarks.—The type locality of *Tarentula guaquiensis* was previously considered to be located in Peru. However, there is no locality called Guaqui in Peru, but there is a locality of the same name in Bolivia, near the edge of

Lake Titicaca, situated close to the border of Peru and Bolivia.

Natural history.—Very little is known about the habits of this species. As the specimens from Huatajata, Bolivia, and Arapa, Peru, were collected near the border of Lake Titicaca, we believe that this species lives in vegetation near water as some other South American Sosippinae.

Distribution.—The only known records are from Peru, Bolivia and Argentina (Fig. 10).

ACKNOWLEDGMENTS

We would like to thank Adriana Davanzzo for revising the English version of the manuscript, Volker Framenau, Petra Sierwald, Mark Harvey, and the editors of the Journal of Arachnology for comments and suggestions. We are grateful to the following curators supplying material for study, D. Silva (MHNSM), E.H. Buckup (MCN), M. Apel (MWNH), and P. Jäger (SMF); we are particularly grateful to M. Apel who kindly located and sent us the Strand types. Financial support was provided by FAPESP (n. 99/05446-8 and 02/11275-6). This study is part of the BIOTA/FAPESP–the Virtual Institute Program (www.biotasp.org.br) and of the Doctoral thesis in Zoology by the first author, developed in the Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

LITERATURE CITED

Capocasale, R.M. 1990. Las especies de la subfamilia Hippasinae de America del Sur (Araneae, Lycosidae). Journal of Arachnology 18:131–141.

Mello-Leitão, C.F. 1944. Arañas de la provincia de Buenos Aires. Revista del Museo La Plata (Nova Serie, Zoología) 3:311–393.

Petrunkevitch, A. 1911. A synonymic index-catalogue of spiders of North, Central and South America with all adjacent islands, Greenland, Bermuda, West Indies, Terra del Fuego, Galapagos, etc. Bulletin of the American Museum of Natural History 29:1–791.

Platnick, N.I. 1993. Advances in Spider Taxonomy 1988–1991, with Synonymies and Transfer 1940–1988. New York Entomological Society, in association with the American Museum of Natural History, New York. 846 pp.

Roewer, C.F. 1955. Katalog der Araneae von 1758 bis 1940, bzw. 1954. Bruxelles, 2:1–1751.

Roewer, C.F. 1960. Araneae Lycosaeformia II (Lycosidae) (Fortsetzung und Schluss). Exploration du Parc National de l'Upemba Mission G.F. De Witte 55:519–1040.

Santos, A.J. & A.D Brescovit. 2001. A revision of the South American spider genus *Aglaoctenus* Tullgren, 1905 (Araneae, Lycosidae, Sosippinae). Andrias 15:75–90.

Sierwald, P. 2000. Description of the male of *Sosippus placidus*, with notes on the subfamily Sosippinae (Araneae, Lycosidae). Journal of Arachnology 28:133–140.

Strand, E. 1908. Exotisch araneologisches.—I. Amerikanische hauptsächlich in Peru, Bolivien und Josemitetal in Californien gesammelte Spinnen. —II. Spinnen aus Kamerun. —III. Übersicht der bekanten Hysterocrates-Arten. —IV. Zur Kenntnis der *Araneae rufipalpis* (Luc). Jahrbücher des Nassauischen Verreins für Naturkunde 61:223–295.

Manuscript received 2 October 2006, revised 2 May 2007.

2007. The Journal of Arachnology 35:318–324

A NEW SPECIES OF *EUKOENENIA* (PALPIGRADI, EUKOENENIIDAE) FROM MOROCCO

Pablo Barranco and **Jaime G. Mayoral**: Departamento de Biología Aplicada, Cite II-B, Universidad de Almería, 04120 Almería, Spain. E-mail: pbvega@ual.es

ABSTRACT. The new species *Eukoenenia maroccana* is described from six specimens (two males, two females and two immatures) collected in Kef Aziza Cave, Morocco, and is distinguished from all other *Eukoenenia* species by the presence of thickened opisthosomal glandular setae in males on sternites IV–VI. The genitalia and chaetotaxy of both adult sexes show differences from other species of *Eukoenenia* and are discussed in this paper.

RESUMEN. Se describe *Eukoenenia maroccana* a partir de seis ejemplares (dos machos, dos hembras y dos inmaduros) capturados en la gruta de Kef Aziza, Marruecos. Lo más destacable y del todo singular de esta nueva especie es la particular presencia de setas glandulares esternales engrosadas del macho, la genitalia y resto de quetotaxia de ambos sexos.

Keywords: *Eukoenenia maroccana*, taxonomy, North Africa, morphology

Two species of Palpigradi have been previously reported from Morocco (Harvey 2003). The endogenous species *Eukoenenia mirabilis* (Grassi & Calandruccio 1885) has been collected from a range of locations (Rémy 1952a, 1956b, 1957), which were summarized by Harvey et al. (2006, fig. 2). A second endogenous species, *Eukoenenia hanseni* (Silvestri 1913), was recorded by Condé (1951) [see also Rémy (1952a, 1957)]. Canals & Viñas (1960) captured a specimen within Kez Aziza (also named Kef Aziza) cave, which was identified as *Koenenia* sp., but this material has not been restudied and is now lost (Condé 1984, 1996). The study of a recent collection of several palpigrade specimens from Kef Aziza cave has revealed the presence of a previously undescribed species.

The specimens examined in this study are deposited in the National Museum of Natural Sciences, Madrid, Spain (MNCN) and the University of Almería, Almería, Spain (UAL). All measurements are expressed in micrometers and were taken using an ocular micrometer with a compound microscope. The following abbreviations were utilized: L, total length of body (without flagellum which is lost in all specimens); B, length dorsal shield; P, pedipalpus; I and IV, legs I and IV; ti, tibia; bta1, basitarsus 1; bta2, basitarsus 2; bta3, basitarsus 3; bta4, basitarsus 4; ta1, tarsus 1; ta2, tarsus 2; ta3, tarsus 3; a, width of basitarsus IV at level of seta *r*; *er*, distance between base of basitarsus IV and insertion of seta *r*; *grt*, length of tergal seta; *gla*, length of lateral seta; *r*, length of stiff seta; *t/r*, ratio between length of basitarsus IV and stiff seta length; *t/er*, ratio between length of basitarsus IV and distance to insertion of stiff seta; *gla/grt*, ratio between lengths of lateral and tergal setae; B/bta, relation between lengths of prosomal shield and basitarsus IV; bta/ti, ratio between lengths of basitarsus IV and tibia IV. Setal nomenclature follows Condé (1974, 1971, 1984, 1988, 1989, 1992, 1993, 1994).

TAXONOMY

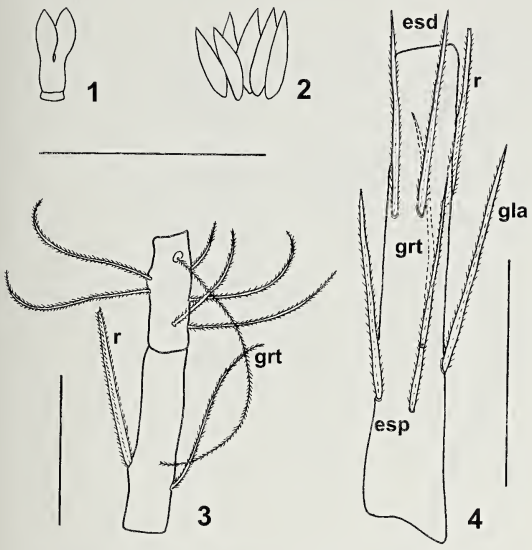
Family Eukoeneniidae Petrunkevitch 1955
Genus *Eukoenenia* Börner 1901

Koenenia Grassi & Calandruccio 1885:165 [junior primary homonym of *Koenenia* Beushausen 1884 (Mollusca: Bivalvia)].

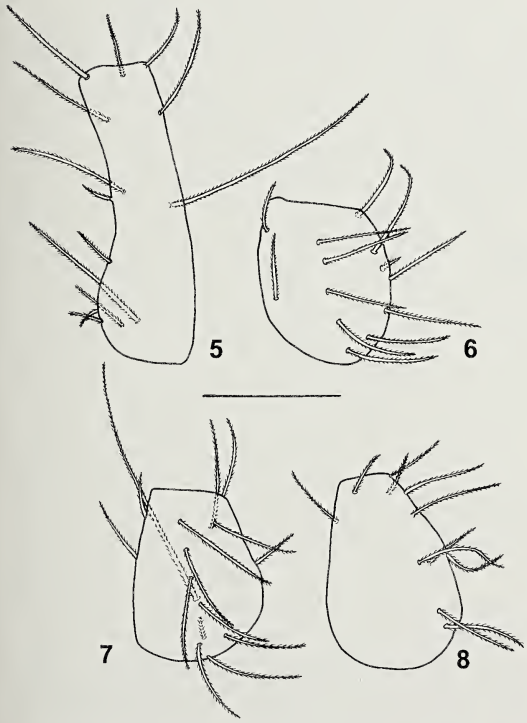
Koenenia (*Eukoenenia*) Börner 1901:551.

Type species.—*Koenenia mirabilis* Grassi & Calandruccio 1885, by monotypy.

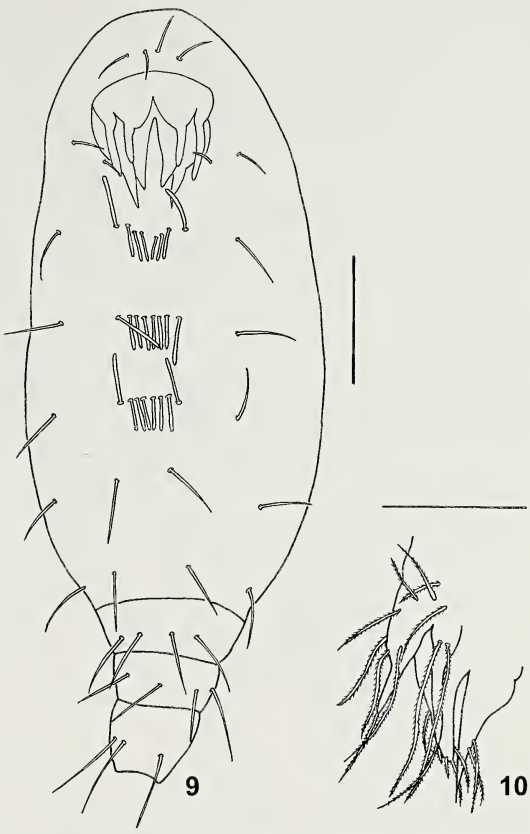
Remarks.—*Eukoenenia* includes 60 species and is the most diverse genus of Palpigradi (Harvey 2002, 2003; Mayoral & Barranco 2002a). It is cosmopolitan with 25 species in Europe, 21 in Africa, 14 in Asia, 9 in America and 2 in Australia; some of them appear on different continents. Most species



Figures 1–4.—*Eukoenenia maroccana* new species: 1. Frontal organ, dorsal view; 2. Lateral organ, dorsal view; 3. Basitarsus 3–4 of leg I; 4. Basitarsus IV. Scale bars 100 μm.



Figures 5–8.—*Eukoenenia maroccana* new species: 5. Coxa I; 6. Coxa II; 7. Coxa III; 8. Coxa IV. Scale bar 100 μm.



Figures 9–10.—*Eukoenenia maroccana* new species: 9. Opisthosoma of male, ventral view; 10. Male genitalia, lateral view. Scale bars 100 μm.

are found in soil, but 27 are from caves. New species have been described recently (Mayoral & Barranco 2002b; Montaño & Francke 2006). The distribution of some endogean species suggests human intervention (Savory 1974; Condé 1986; Harvey et al. 2006).

The genus *Eukoenenia* is characterized by the absence of ventral sacs in opisthosomal sternites IV–VI, and segment IX is narrower than VIII, but larger than XI (Monniot 1966).

Eukoenenia maroccana new species
Figs. 1–13

Material examined.—MOROCCO: *Errachidia:* Holotype adult male, Kef Aziza cave, Tazougerte, Bouclenib [32°01′46″N, 03°47′17″W, 1040 m], July 1997, C. Hernando (MNCN 20.02/14845). Paratypes: MOROCCO: *Errachidia:* 1 adult male, same locality and collector (UAL-Pp-022), 2 adult females, same locality and collector (UAL-Pp-023, MNCN 20.02/14846); 2 immature females,

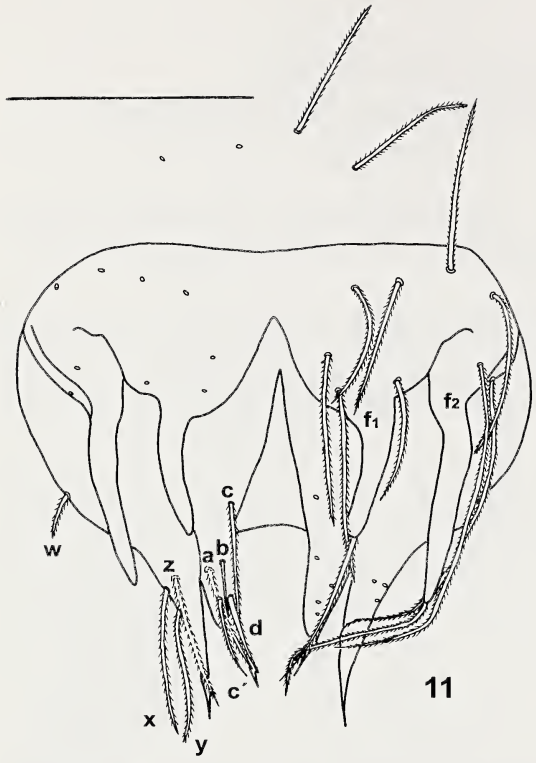


Figure 11.—*Eukoenenia maroccana* new species: male genitalia. Scale bar 100 μm.

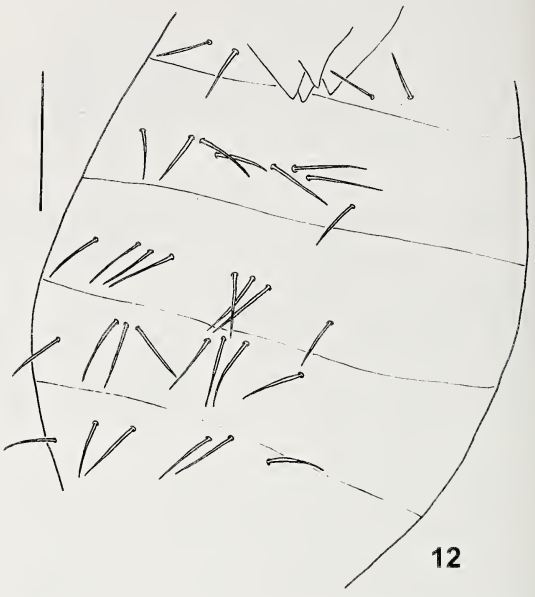


Figure 12.—*Eukoenenia maroccana* new species: opisthosoma of female, ventral view. Scale bar 100 μm.

(type A), same locality and collector (UAL-Pp-024, MNCN 20.02/14847).

Diagnosis.—This species differs from all other species of the genus by the combination of the presence of six lateral organs and the characteristic chaetotaxy and genitalia: males with 4 + 4 thickened secretory setae (a) and only one seta (s) on sternites IV–VI; different ventral opisthosomal chaetotaxy in both sexes; the presence of strongly developed fusules on very long dilated digitiform processes in male genitalia; and the shape of genitalia in females.

Description.—*Male: Prosoma:* frontal organ with 2 expanded granulate branches, blunt apically and each over 2.8 times longer than wide (Fig. 1). Lateral organ with 6 pointed blades, each 6 times longer than wide (Fig. 2). Dorsal shield with 10 + 10 short setae. Free segment of opisthosoma with 3 + 3 setae (t_1, t_2, t_3), all of similar length. Chelicerae with 9 teeth on each side of chelicera and with 6 dorsal and 1 ventral setae. Four deuto-tritosternal seta in linear arrangement. Chaetotaxy of coxae I–IV: 13, 13, 15 and 11 (Figs. 5–8). Basitarsus 3 of leg I slender, 4 times longer than broad, with 2 setae: stiff (*r*) and (*grt*) (Fig. 3), *r* shorter than the segment (120/95, *t/r* = 1.26), inserted in proximal half and surpassing hind edge (32.5/112.5, *s/er* = 0.29). Basitarsus IV with 7 setae (2 *esd*, 2 *esp, gla, grt*, and *r*) (Fig. 4), *bta/ti* 0.89. Stiff seta *r* 2.60 times shorter than tergal edge of article (*t/r* = 2.60) and inserted in its distal third (*t/er* = 1.66) very close to both *grt*. Both *esd* proximally inserted, followed by *gla* and *grt*, more or less at same level, all of them in proximal third.

Opisthosoma: tergites II–VI with 3 + 3 dorsal setae, 2 pair of setae (t_1, t_3) between both slender seta (*s*), 2 + 2 on VII and VIII, only *t* seta present, and without *s*. Sternites II–III with 2 + 2 setae. Sternites IV–VI with 4 + 4 thickened setae in middle of opisthosoma (a_1, a_2, a_3, a_4), outer ones (a_4, 71) longer than others (a_1-a_3, 52.5). In addition, only one normal seta (*s*) present on each side, as long as a_4. Sternites with VII–VIII 2 + 2 setae (Fig. 9). Chaetotaxy of segments IX–XI each with 8 setae.

Genitalia: 3 lobes present, with 44 setae; first lobe with deep medial indentation that separates two sides with a subtrapezoidal aspect, 13 + 13 setae (including 2 + 2 fusules). Fusules inserted on a dilated digitiform base, external fusules longer, reaching past distal

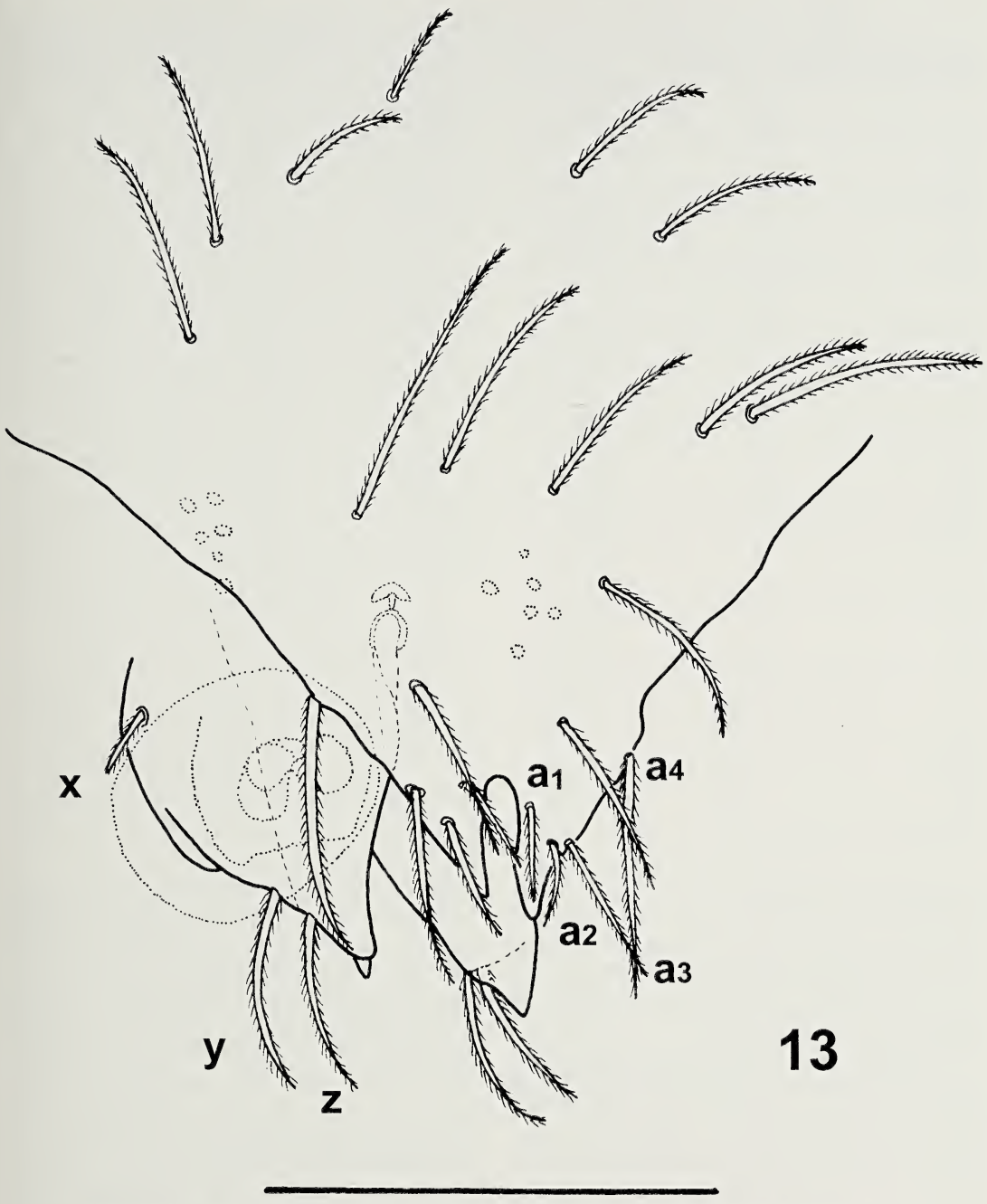


Figure 13.—*Eukoenenia maroccana* new species: female genitalia. Scale bar 100 μm.

margin of second lobe. Outer 4 subapical setae extremely long, reaching past apex of third lobe. Second lobe with a large, single and pointed apical part, with 5 + 5 setae (*a, b, c, c', d*). Third lobe of similar shape, but with 4 + 4 setae (*x, y, z, w*) (Figs. 10, 11).

Female: generally similar to male but differing as follows:

Opisthosoma: 4 deuto-tritosternal seta in linear arrangement. Ventral setal formula: sternite III with 2 + 2 setae, sternites IV–VI with 3 + 3 setae (a_1, a_2, a_3) and a single *s* on each side; sternite VII with 2 + 2 setae (a_1, a_2) and one *s* on each side, sternite VIII with only 2 + 2, seta *s* absent (Fig. 12).

Genitalia: first lobe with 11 + 11 setae in

Table 1.—Measurements (μm) of selected body parts of *Eukoenenia maroccana*.

Body part	Male 1, holotype	Male 2	Female 1	Female 2	Immature
L	1725	1604	1866	1115	936
B	584	605	697	605	—
Pti	205	207	220	210	158
Pbta1	68	70	70	73	53
Pbta2	80	90	88	93	73
Pta1	43	50	45	43	38
Pta2	55	53	68	65	53
Pta3	80	90	98	95	55
Iti	227	—	247	238	190
Ibta1 + 2	168	165	173	165	128
Ibta3	110	123	120	110	110
Ibta4	70	70	80	85	55
Ita1	50	50	53	43	38
Ita2	53	55	58	58	48
Ita3	188	190	200	190	163
IVbta	208	212	212	215	168
IVti	233	225	242	235	185
IVta1	85	70	75	70	70
IVta2	125	120	125	133	105
a	23	—	25	33	23
er	125	130	145	143	103
grt	93	100	103	—	80
gla	90	88	103	95	78
r	80	85	80	83	65
t/r	2.60	2.49	2.65	2.59	2.58
t/er	1.66	1.63	1.46	1.50	1.63
gla/grt	0.97	0.88	1.00	—	0.98
B/bta	2.81	2.85	3.10	2.81	—
bta/ti	0.89	0.94	0.88	0.91	0.91

5 transverse rows, 4 sternal 2 + 2, 2 + 2, 2 + 2, 1 + 1 and distal 4 + 4, of which a_1 and a_2 of same length (25) which are shorter than a_3 (42.5–48) and a_4 (55–58). Second lobe with 3 + 3 setae (Fig. 7); 5 glandular orifices. Spermathecae elliptical (Fig. 13).

Immature (type A, immature female): Generally similar to adults, but lateral organs with 4 blades; 3 deuto-tritosternal setae; and 8 cheliceral teeth. Basitarsus IV with 6 setae: 1 *esp* absent. Ventral and dorsal chaetotaxy as for female. One of the immatures was too damaged to be measured.

Dimensions (μm).—See Table 1.

Etymology.—The specific name *maroccana* refers to the country, Morocco, where the species was found.

Remarks.—The prosoma of *Eukoenenia maroccana* bears six lateral organs, similar to that of *E. depilata* Remy 1960 (6), *E. remyi* Condé 1974 (4–6), *E. spelaea* (Peyerimhoff 1902) (5–6), *E. hanseni* (Silvestri 1913) (3–6) and *E. cf. lyrifer* Condé 1974 (6). *Eukoenenia maroccana* has 9 + 9 cheliceral teeth, while *E. depilata*, *E. remyi*, and *E. spelaea* have 8 + 8, *E. hanseni* has 7 + 7 and *E. cf. lyrifer* also has 9 + 9. *Eukoenenia depilata*, *E. remyi* and *E. cf. lyrifer* are only known from females. The ventral chaetotaxy of *E. depilata* and *E. remyi* has only one secretory seta (*a*) on sternites IV–VI, three *s* setae, which are slightly thickened, on each side in *E. depilata* (Remy 1960), only two *s* setae in *E. remyi* (Condé 1974). Although Condé (1974) did not describe the chaetotaxy of *E. cf. lyrifer*, it is supposed that it is the same as that of *E. lyrifer*, which has two *a* and only one *s* setae. All of these species differ from the female of *E. maroccana* (with 1 + 3 + 3 + 1 and no thickened setae) and also the first lobe of the female genitalia is more rounded in these three species than in *E. maroccana*.

The ventral chaetotaxy in males of *E. maroccana* have 4 + 4 *a* setae, which is similar

to that of *E. spelaea*, *E. hanseni*, and *E. florenciae* (which have 3 lateral organs), *E. strinatii* (4 lateral organs), *E. patrizzi* (8–10 lateral organs), *E. maros* (4–5 lateral organs) and partially *E. bouilloni* (5 lateral organs), which has 5 + 5 secretory setae on sternite IV. All of these species have two *s* setae while *E. maroccana* has only one and none of them have the thickened secretory setae, which appears to be exclusively found in males of *E. maroccana*. These thickened setae are similar to those on the sternites V–VI in females of *E. paulinae* Condé 1994, which are present only on sternites IV and V (Condé 1994), and in *E. angolensis* (Remy 1956) and *E. hesperia* (Remy 1953) (Remy 1953, 1956a; Condé 1992, 1994). Other species with 5 lateral organs are *E. pyrenaica* and *E. naxos*, both with different sternal chaetotaxies, with two pair of *a* setae between a pair of *s* for the first one and 5 + 5 secretory setae in sternites V–VI between two *s* setae in *E. naxos*.

The presence of fusules on dilated processes is frequent in males of the genera *Eukoenenia* and *Koeneniodes* (Condé 1994). These processes can be only slightly developed, as in *E. fossati* Remy 1960, *E. brignolii* Condé 1979 and *E. gasparoi* Condé 1988 (Remy 1960; Condé 1979, 1988), moderately developed, as in *E. pauli* Condé 1979, *E. lawrencei* Remy 1987, *E. grassii* (Hansen 1901) and *E. janetscheki* Condé 1993 (Condé 1979, 1981, 1993), or strongly developed, as in *E. patrizii* (Condé 1956) and *E. maroccana*.

It is unusual to have different ventral opisthosomal chaetotaxy in both sexes of the same species. This situation is also seen in *E. janetscheki* Conde 1993 where the female has an additional secretory seta on sternites IV–VI (Condé 1993).

The mean value of B/bta in the four adults is 2.89, very far from the value of the cavernicolous species, which should be lower than 2, but it is similar to the values for endogenous species (3–4) (Condé 1998). This situation arises because the legs of *E. maroccana* are not elongated, although the specimens have the typical large size of cave dwelling species.

ACKNOWLEDGMENTS

We are most grateful to Carles Hernando for access to the material that he collected.

LITERATURE CITED

Börner, C. 1901. Zur äusseren Morphologie von *Koenenia mirabilis* Grassi. Zoologischer Anzeiger 24:537–556.

Canals, M. & R. Viñas. 1960. Atlas 68. Espeleóleg 8:325–332.

Condé, B. 1974. *Eukoenenia remyi* n. sp., palpigrade cavernicole d'Herzégovine. Annales de Spéléologie 29:53–56.

Condé, B. 1979. Premiers Palpigrades du Gabon. Annales des Sciences Naturelles, Zoologie 1:57–62.

Condé, B. 1981. Données nouvelles sur *Eukoenenia lawrencei* Remy (Arachnides, Palpigrades). Revue Suisse de Zoologie 88:447–454.

Condé, B. 1984. Les palpigrades: quelques aspects morpho-biologiques. Revue Arachnologique 5: 133–143.

Condé, B. 1988. Nouveaux Palpigrades de Trieste, de Slovénie, de Malte, du Paraguay, de Thaïlande et de Bornéo. Revue Suisse de Zoologie 95: 723–750.

Condé, B. 1989. Palpigradi (Arachnida) de grottes d'Europe. Revue Suisse de Zoologie 96:823–840.

Condé, B. 1992. Palpigrades cavernicoles et endogés de Thaïlande et des Célèbes (1ère note). Revue Suisse de Zoologie 99:655–672.

Condé, B. 1993. Description du mâle de deux espèces de Palpigrades. Revue Suisse de Zoologie 100:279–287.

Condé, B. 1994. Palpigrades cavernicoles et endogés de Thaïlande et de Célèbes (2e note). Revue Suisse de Zoologie 101:233–263.

Condé, B. 1996. Les Palpigrades, 1885–1995: acquisitions et lacunes. Revue Suisse de Zoologie, hors de série 1:87–106.

Condé, B. 1998. Palpigradida. Pp. 913–920. *In* Encyclopaedia Biospeologica. Volume II. (C. Juberthie & V. Decu, eds.). Société Internationale de Biospeologie, Moulis (C.N.R.S.), France and Bucarest (Academie Rumanie), Roumanei.

Grassi, B. & S. Calandruccio 1885. Intorno ad un nuovo Aracnide Artrogastro (*Koenenia mirabils* [sic]) che crediamo rappresentante d'un nuovo ordine (Microteliphonida). Naturalista Siciliano 4:127–133, 162–168.

Harvey, M.S. 2002. The neglected cousins: what do we know about the smaller arachnid orders? Journal of Arachnology 30:357–372.

Harvey, M.S. 2003. Catalogue of the Smaller Arachnid Orders of the World: Amblypygi, Uropygi, Schizomida, Palpigradi, Ricinulei and Solifugae. CSIRO Publishing, Melbourne, Australia. 400 pp.

Harvey, M.S., F. Štáhlavsky & P.D. Theron. 2006. The distribution of *Eukoenenia mirabilis* (Palpigradi: Eukoeneniidae): a widespread tramp. Rec-

ords of the Western Australian Museum 23:199–203.

Mayoral, J.G. & P. Barranco. 2002a. Palpígrados: grandes desconocidos (Arachnida, Microthelyphonida). Revista Ibérica de Aracnología 5:103–110.

Mayoral, J.G. & P. Barranco. 2002b. Descripción de una nueva *Eukoenenia* Börner, 1901 del sureste Ibérico (Arachnida, Palpigradi, Microthelyphonida). Revista Ibérica de Aracnología 6:129–134.

Monniot, F. 1966. Un palpigrade interstitiel: *Leptokoenenia scurra*, n. sp. Revue d'Écologie et de Biologie du Sol 3:41–64.

Montaño, M.H. & O.F. Francke. 2006. Descripción de una nueva especie de palpígrado (Arachnida, Palpigradi) del género *Eukoenenia*, colectado en la selva lacandona, en México. Entomología Mexicana 5:162–166.

Rémy, P. 1952a. Palpigrades du Maroc et de l'Algeria occidental. Bulletin de la Société des Sciences Naturelles de Maroc 30:159–163.

Rémy, P. 1952b. Palpigrades de l'Ile de la Réunion. Mémoires de l'Institut Scientifique de Madagascar, Série A 7:69–79.

Rémy, P. 1953. Description d'un nouveau palpigrade d'Afrique Occidentale Française. Bulletin du Muséum National d'Histoire Naturelle, Paris 25: 86–89.

Rémy, P. 1956a. Contribution à l'étude de la microfaune endogée de l'Afrique tropical: Palpigrades et Pauropodes. Revue de Zoologie et de Botanique Africaines 53:327–335.

Rémy, P. 1956b. Sur la microfaune endogée des jardins de Tanger (Symphyles, Pauropodes, Pénicillates, Protoures, Palpigrades). Bulletin de la Société des Sciences Naturelles de Maroc 35: 103–108.

Rémy, P. 1957. Nouvelles stations marocaines du Palpigrade *Eukoenenia mirabilis* Grassi. Bulletin de la Société des Sciences Naturelles de Maroc 36:335–339.

Rémy, P. 1960. Palpigrades de Madagascar. II. Mémoires de l'Institut Scientifique de Madagascar, Série A 13:33–66.

Savory, T. 1974. On the arachnid order Palpigradi. Journal of Arachnology 2:43–45.

Manuscript received 3 February 2005, revised 7 March 2007.

2007. The Journal of Arachnology 35:325–333

REDESCRIPTION OF THE TYPE SPECIES OF *CYNORTA* (ARACHNIDA, OPILIONES, COSMETIDAE)

Adriano Brilhante Kury: Departamento de Invertebrados, Museu Nacional, Quinta da Boa Vista, São Cristóvão, 20.940-040, Rio de Janeiro - RJ - Brazil. E-mail: adrianok@gmail.com

Osvaldo Villarreal Manzanilla: Museo de Historia Natural La Salle, Fundación La Salle de Ciencias Naturales, Apartado 1930, Caracas 1010-A, Venezuela.

Cristiano Sampaio: Departamento de Invertebrados, Museu Nacional, Quinta da Boa Vista, São Cristóvão, 20.940-040, Rio de Janeiro - RJ - Brazil.

ABSTRACT. *Cynorta conspersa* (Perty 1833), the type species of *Cynorta* Koch 1839, is redescribed, based on abundant material from the lower Amazon basin, Brazil. A neotype is designated for this species and the species *Cynorta mayi* Mello-Leitão 1931 is herein considered a junior subjective synonym. Genital morphology of the species is described for the first time. An effort has been made to detect diagnostic characters for the genus *Cynorta*, which was used in many different senses in the past and includes a large number of unrelated Neotropical species.

RESUMEN. Es redescrita *Cynorta conspersa* (Perty 1833), especie tipo del género, con base en abundante material proveniente de la cuenca del bajo Amazonas de Brasil. Es designado un neotipo para esta especie y la especie *Cynorta mayi* Mello-Leitão 1931 es considerada como su sinónimo junior subjetivo. La morfología genital es descrita por primera vez. Ha sido hecho un esfuerzo para detectar caracteres diagnósticos del género *Cynorta*, el cual fue usado en el pasado con muchos significados diferentes, incluyendo un gran número de especies neotropicales no relacionadas.

Keywords: Neotropics, Brazil, taxonomy, new synonymy

The family Cosmetidae Koch 1839, with more than 700 nominal species, is the second most diverse of Opiliones suborder Laniatores Thorell 1876 (Kury 2003). It is distributed in the Neotropics, with the greatest abundance in Central America and the Caribbean, stretching as far north as southern U.S.A. There are also many species in the Andean realm and the lowland Amazonian rainforest. The present state of cosmetid systematics is unsatisfactory, the genera being defined by a combination of area armature and tarsal counts. The high percentage of monotypic genera in the faulty Roewerian system (e.g., Roewer 1923) has been counteracted by the recognition of large meaningless genera (Goodnight & Goodnight 1953), an equally ineffective approach to their taxonomy.

Perty (1833) described the genus *Cosmetus* with many species of Cosmetidae from Brazil, among them *Cosmetus conspersus* Perty 1833 from "Brazil." Koch (1839) was the first to narrow down the occurrence of the species from Pará, creating the genus *Cynorta* to accommodate some of Perty's species, including *C. conspersus*, *C. marginalis* Banks 1909, *C. posticata* Banks 1909, *C. dentipes* F.O. Pickard-Cambridge 1904, *C. geayi* Roewer 1912, *C. sulphurata* Roewer 1912, *C. sigillata* Roewer 1912, *C. flavoclathrata* Simon 1879, *C. vestita* Roewer 1912, *C. v-album* Simon 1879, *C. fraterna* Banks 1909, *C. albiornata* Roewer 1912, *C. scripta* Simon 1879, *C. calcarbasalis* Roewer 1912, *C. calcarapicalis* Roewer 1912 and *C. juncta* (Gervais in Walckenaer 1844), all from localities in the Antilles, Brazil, Costa Rica, Cuba, Ecuador, French Guyana, Guatemala, Guyana, and Suriname. Much later, Pickard-Cambridge (1904) designated *Cosmetus conspersus* as the type species of *Cynorta*. Mello-Leitão (1931) described *Cynorta mayi* from "Pará," but did not compare it with *C. conspersa*. The only literature records for *Cosmetus conspersus*, all

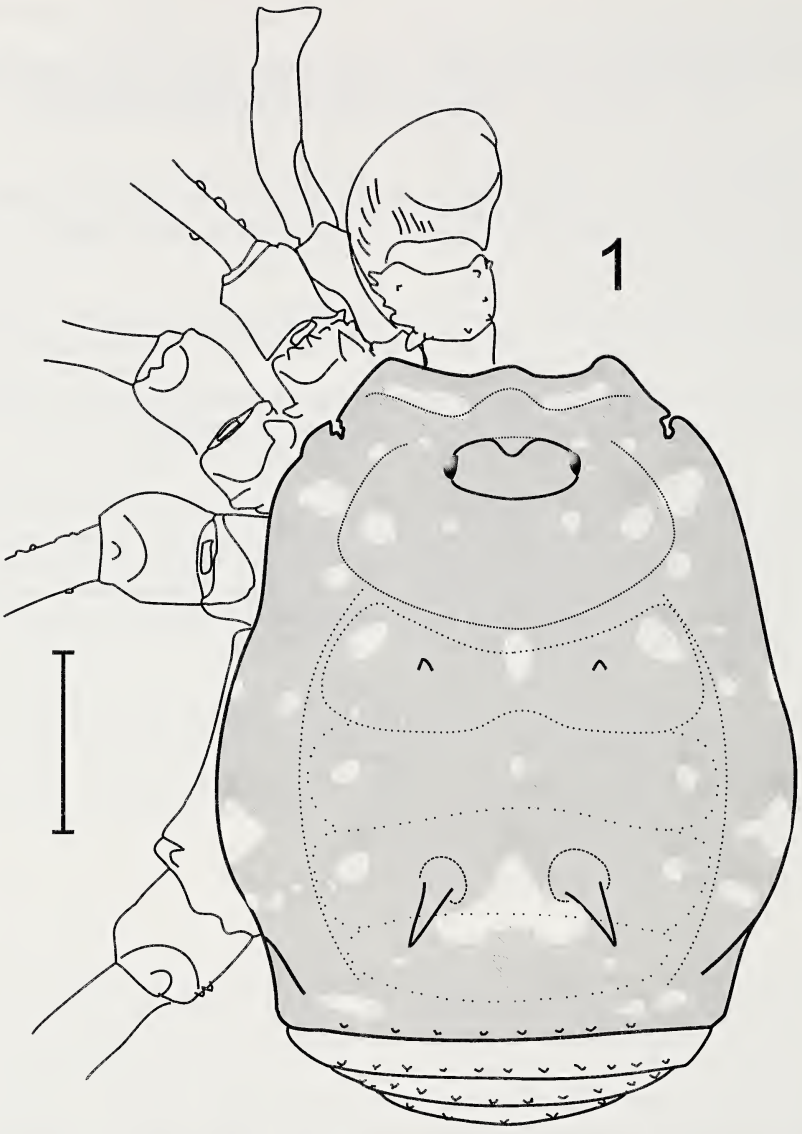


Figure 1.—*Cynorta conspersa* (Perty 1833), male neotype (MNRJ 6098) from Brazil, habitus: Dorsal view. Scale bar = 1 mm.

in the Pará state near the mouth of the Amazon River, are Cametá, at Rio Tocantins (Sørensen 1932), Belém and Tucuruí (Kury 2003).

Goodnight & Goodnight (1953), in an influential paper, using the then dominant concept of considering only tarsal segmentation to define Opiliones genera, synonymized a great number of genera of Cosmetidae into only three: *Vonones* Simon 1879, *Cynorta* Koch 1839, and *Paecilaema* Koch 1839. Most of those synonymies were disclaimed by Kury (2003); but, even so, *Cynorta* is still the largest genus of Cosmetidae, with 154 species (22% of the diversity of the family) and is the type of the subfamily Cynortinae Mello-Leitão 1933, which is currently under the synonymy of Cosmetinae.

The type material of *C. conspersa* is long lost (Roewer 1923), but we were able to examine the four syntypes of *C. mayi* in the Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil, which were compared with the descriptions and redescriptions in the literature. As a result, we here designate a lectotype from the syntypes of *C. mayi* and a neotype

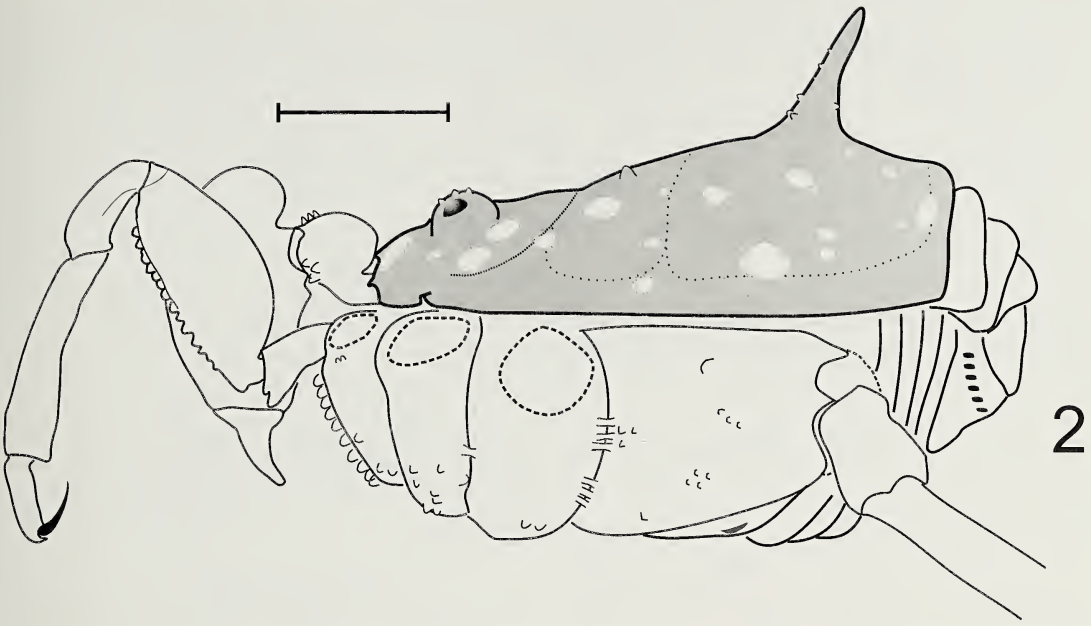


Figure 2.—*Cynorta conspersa* (Perty 1833), male neotype (MNRJ 6098) from Brazil, habitus: Lateral view. Scale bar = 1 mm.

for *C. conspersa*, to stabilize the concept of the species and consider both nominal species to be synonyms.

Abbreviations of depositories are: Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil (MNRJ); Zoologische Staatssammlung München, Germany (ZSMC). All measurements are in mm. Coordinates are in decimal degrees.

SYSTEMATICS

Family Cosmetidae Koch 1839
Genus *Cynorta* Koch 1839

Type species.—*Cosmetus conspersus* Perty 1833, by subsequent designation of Pickard-Cambridge (1904).

Diagnosis.—Outline of the dorsal scutum of the beta type; chelicerae without strong sexual dimorphism, legs I–IV long, slender and unarmed, femur IV substraight; leg I with 6 to 7 tarsomeres; basitarsomeres of leg I of male much larger than distitarsomeres; tarsal claws of legs III–IV unpectinate; penis ventral plate subrectangular, as wide basally as distally, with lateral borders parallel and distal border slightly concave and 3 + 4 lateral setae.

Cynorta conspersa (Perty 1833)
Figs. 1–10

Cosmetus conspersus Perty 1833:203.

Cynorta conspersa (Perty): Koch 1839:21; Kury 2003:43.

Poecilæma conspersum (Perty): Sørensen 1932: 336.

Cynorta mayi Mello-Leitão 1931:116, fig. 2; Mello-Leitão 1932:444, suppl. fig. 5. NEW SYNONYMY.

Type specimens.—*Cosmetus conspersus*: BRAZIL: male holotype, without further locality data (ZSMC?), lost, not examined.

BRAZIL: *Pará:* male neotype (present designation), Tucuruí (3.6903°S, 49.7213°W), April 1981, A.C. Domingos (MNRJ 6098).

BRAZIL: *Pará: Cynorta mayi*: female lectotype (present designation), 3 female paralectotypes, without further locality data, E. May (MNRJ 1368).

Other material examined.—BRAZIL: *Pará:* 5 ♂, 11 ♀, 1 juvenile, Belém (1.3904°S, 48.4490°W), 11 June 1974, W. Roth (MNRJ 6175); 12 ♂, 30 ♀, Belém, Clonal Garden (1.4300°S, 48.4564°W), insecticide blast in cacao tree, 14–15 December 1976, Hilton et al. (MNRJ 17641); 2 ♂, Belém, Utinga

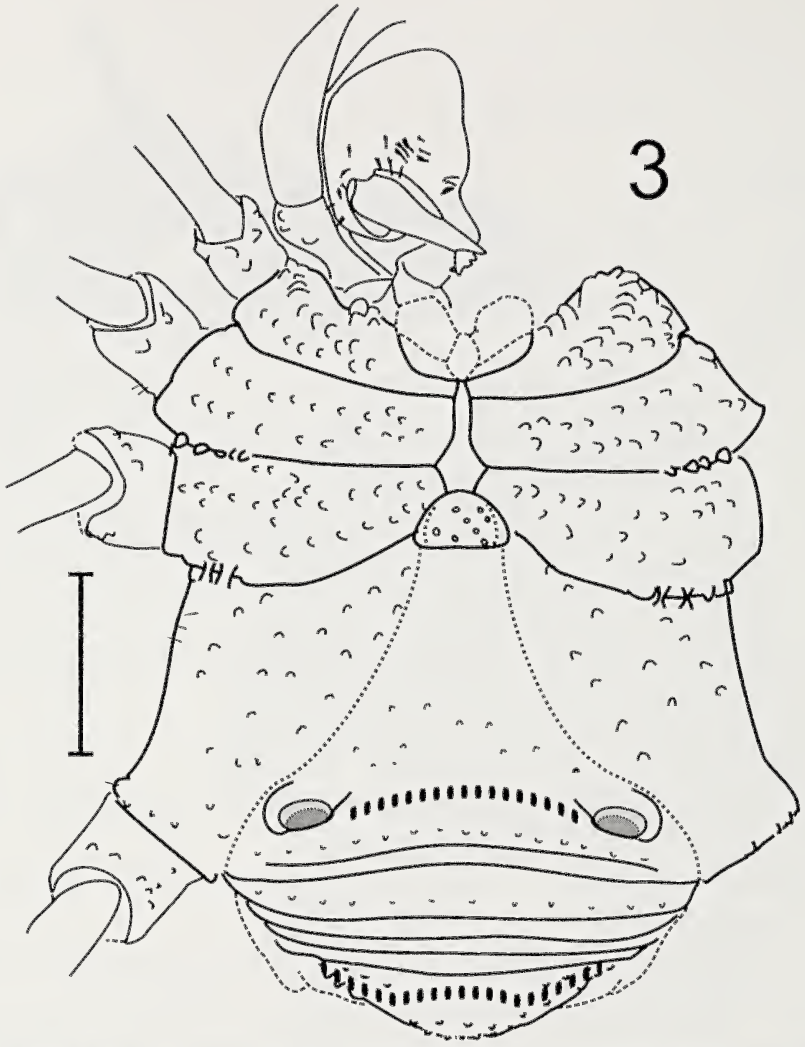
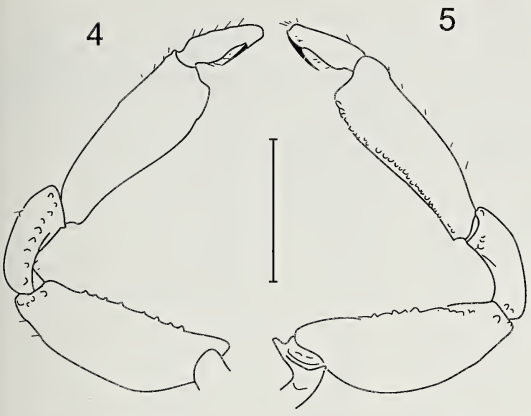


Figure 3.—Ventral view of *Cynorta conspersa* (Perty 1833), male neotype (MNRJ 6098) from Brazil. Scale bars = 1 mm.

(1.4558°S, 48.5044°W), J.C. Carvalho (MNRJ 5050); 31 ♂, 65 ♀, Tucuruí (3.6903°S, 49.7213°W), April 1981, A.C. Domingos (MNRJ 4560); 6 ♂, 13 ♀, 4 juveniles, Tucuruí (3.6903°S, 49.7213°W), 20 April 1982, W. Roth (MNRJ 6318).

Diagnosis.—Dorsal scutum pyriform with scutal areas obsolete, area I with one granule each side, III with a pair of spiniform large tubercles. Cheliceral sockets of carapace shallow, without laterofrontal projections. Cheliceral bulla marginated laterally and posteriorly by a row of tubercles, ectal most developed. Basal tarsal segments I of the male slightly swollen. Femur and tibia IV much elongate, straight and unarmed. Tarsal counts: 6–7 (3), 12–16 (3), 8–9, 9–11. Tarsal claws III–IV unpectinate. Penis: ventral plate with lateral borders straight and parallel, distal border concave, uncleft; with fourth distal curved setae cylindrical and flattened distally and three medial lateral setae; glans with a small ventro-distal projection, and dorsal process well developed; stylus with ventro-distal mat covered with very small pointed granulations.

Description of male neotype.—Measurements: dorsal scutum: carapace 1.45 long, 2.58 wide; abdominal scutum: 2.31 long, 3.26 wide; femora I–IV: 3.7, 9.3, 6.3, 10.1; tibiae I–IV: 2.6, 7.8, 3.2, 4.7. *Body dorsal (Figs. 1,*



Figures 4–5.—*Cynorta conspersa* (Perty 1833), male neotype (MNRJ 6098) from Brazil: 4. Left pedipalpus, mesal view; 5. Left pedipalpus ectal view. Scale bar = 1 mm.

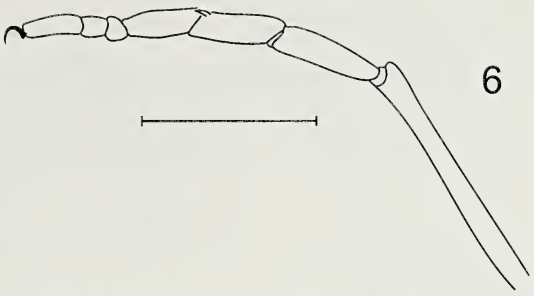
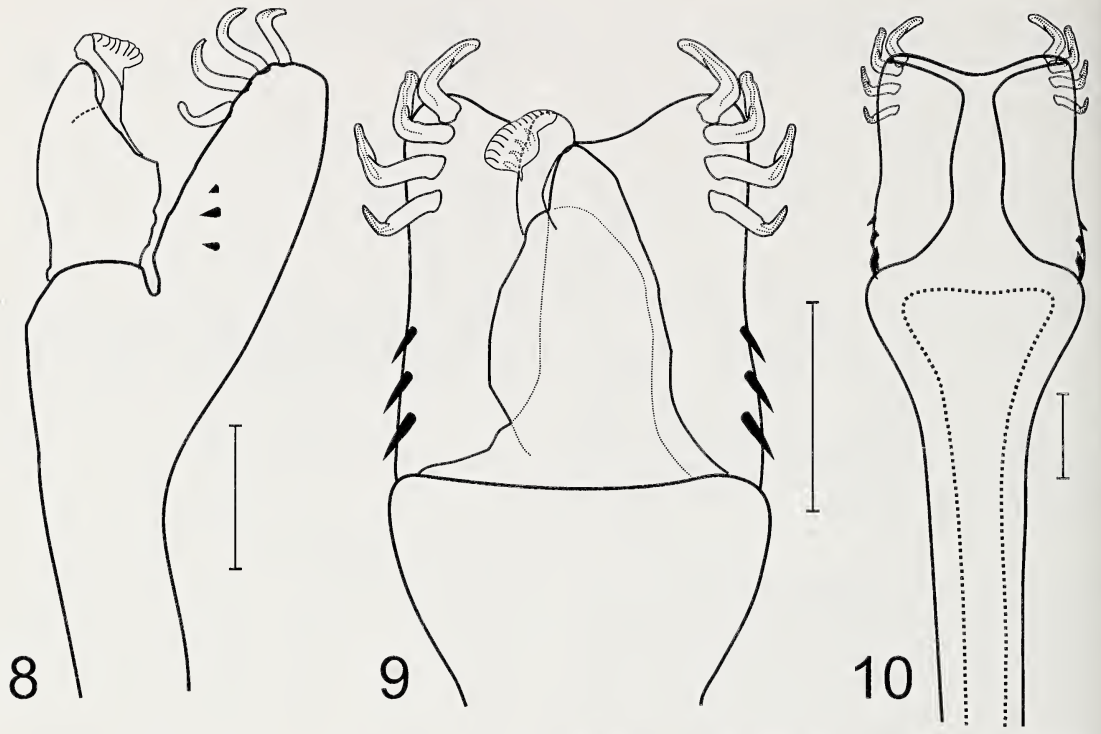


Figure 6.—*Cynorta conspersa* (Perty 1833), male neotype (MNRJ 6098) from Brazil: Tarsus of right leg I, retrolateral view. Scale bar = 1 mm.

2): dorsal scutum pyriform in dorsal view. Lateral border without granules or tubercles. Anterior margin with 2 sockets for the insertion of chelicerae, with 2 anterolateral projections. Eye mound located anteriorly on the carapace, low, wide (about 30 % of total length [TL]), with 3 dorsal granules each side. With 4 mesotergal areas with dorsal minute setae; I with 1 granule each side, II and IV unarmed; III with 2 long spiniform projections, straight with granules on its base. Posterior margin of dorsal scutum and free tergite I to III with a row of minute granules. *Body ventral (Fig. 3):* coxa I with a group of 6 anterior tubercles, 1 medial row of 8–9 tubercles, 1 posterior with 6 granules and 4 distal tubercles; II with a group of 4 anterior granules, 9 medial granules, 8–9 posterior granules and some small proximal granules between medial, posterior rows and 4 distal; III with a anterodistal row of 4 granules, a medial row of 6 granules, a posterior of 7 and 3 distal granules. Genital operculum with 2 lateroposterior small projections, and few setae circularly distributed. Stigmatic area with setae irregularly distributed. Free sternites with a row of small setiferous granules each. Anal operculum with some small granules. *Chelicera:* basichelicerite with 1 ectal row of irregularly placed tubercles and 1 mesal row of tubercles (distal larger). Bulla slightly hypertelic, movable finger with 1 basal tooth and 6–7 small distal teeth. *Pedipalpus (Figs. 4, 5):* coxa with 1 distal tubercle and 1 small ventral granule. Trochanter with 2 ventral tubercles (mesal

Figure 7.—*Cynorta conspersa* (Perty 1833), male neotype (MNRJ 6098) from Brazil: Left leg IV from trochanter to tibia, prolateral view. Scale bar = 1 mm.



Figures 8–10.—*Cynorta conspersa* (Perty 1833), male (MNRJ 4560) from Brazil, distal part of penis: 8. Lateral view; 9. Dorsal view; 10. Ventral view. Scale bars = 0.1 mm.

larger). Femur compressed, with a row of ventral tubercles all along its length. Patella foliate, depressed, with small dorsal granules and 1 mesodistal tubercle. Tibia foliate, depressed with 3 dorsal rows of small granules. Tarsus short, with some dorsal granules and setae. *Legs (Figs. 6, 7):* coxa I with 2 anterior and 1 posterior tubercles; coxa II with 2 anterior (dorsal larger) and 1 posterior fused with 1 of III; coxa III with 1 anterior fused with 1 of II; coxa IV with 3 dorsal tubercles, forming a common base. Trochanter I with 3 ventral tubercles; II with 2; III with 2 ventral and 2 retrolateral; IV with 2 retrolaterodistal granules and 1 prolaterodistal. Femora I–IV straight, with longitudinal row of very small granules and setae. Patella IV with 3 distal granules. Tibia IV slightly swollen distally. Metatarsus with 2 spiniform ventrodistal setae. Tarsi III and IV with 2 subparallel unpectinate claws, and tarsal process. Tarsal counts 7–6, ?–14, 9–9, 10–10. Distitarsi I–II with 3 articles each.

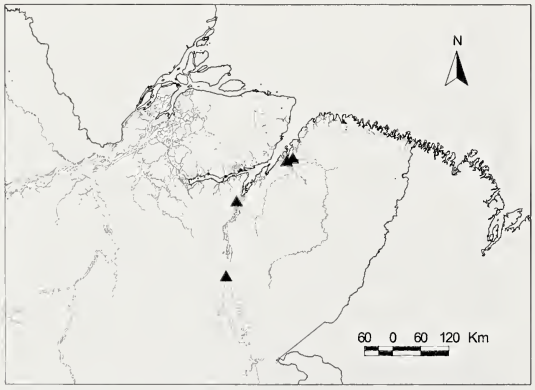


Figure 11.—Lower Amazon basin, showing distribution of *Cynorta conspersa* (black triangles) in the Brazilian State of Pará.

Female: very similar to male. Small variation in number of granules in rows of legs I–IV. Chelicerae slightly smaller.

Variation: Range of tarsal counts and length femur-tibia I–IV are given in Tables 1 and 2 respectively.

Remarks.—The type series of *C. mayi* consists of typical members of what we call *C. conspersa*, and there are no differential characters in the description by Mello-Leitão

Table 1.—Tarsal counts of males and females of *C. conspersa* (MNRJ 4560). Total number of individuals is given in parentheses.

Number of tarsomeres	Leg I (22)	Leg II (32)	Leg III (29)	Leg IV (27)
6	10 ♂, 10 ♀	—	—	—
7	2 ♀	—	—	—
8	—	—	5 ♂, 8 ♀	—
9	—	—	8 ♂, 8 ♀	3 ♂, 5 ♀
10	—	—	—	8 ♂, 8 ♀
11	—	—	—	2 ♂, 1 ♀
12	—	1 ♂, 1 ♀	—	—
13	—	3 ♂, 6 ♀	—	—
14	—	5 ♂, 5 ♀	—	—
15	—	5 ♂, 4 ♀	—	—
16	—	2 ♂	—	—

(1931) supporting his hypothesis of two different sympatric species. We are able to recognize only one "sprinkled" (Latin conspersus) species of *Cynorta* and conclude he just overlooked *C. conspersa* when he created *C. mayi*.

Distribution.—This species is known from Brazil, including Pará (Roewer 1912), Cametá, at Rio Tocantins (Sørensen 1932), Belém (Kury 2003); Tucuruí (Kury 2003), WWF Biome 01 (Tropical & Subtropical Moist Broadleaf Forests), WWF Ecoregions NT0170 (Tocantins-Araguaia-Maranhão moist forests) and NT0180 (Xingu-Tocantins-Araguaia moist forests).

DISCUSSION

The present concept of larger genera of Cosmetidae such as *Cynorta* is useless because it includes many unrelated species based only on tarsal counts and armature of tergal areas, which have been disclaimed in the recent past as superficial traits, subject to numerous independent acquisitions (e.g., Kury 1989). Past authors consistently ignored valuable morphological information to create generic diagnoses and never explored the structure of male genitalia. Furthermore, genital morphology is remarkably similar among the species of Cosmetidae, and subtle variations will have to be used as diagnostic traits. The absence of a systematic revision of the family does not allow us to offer a diagnosis for *Cynorta* supported by synapomorphic traits; however, we have made an effort to detect diagnostic characters for the genus comparable to the other genera of Cosmetidae.

The unillustrated original description by Perty (1833) of *Cosmetus conspersus* consists of five words, which are at first sight not enough for the recognition of this species. That is why a neotype is being fixed in the first place, to allow reference to a well known species. *Cynorta* is a very large genus with species from almost anywhere within the range of the family being referred to it. The unfounded extensive synonymy of Goodnight & Goodnight (1953) only exacerbated the problem. Any cosmetid with 6 tarsomeres on leg I could be referred to as *Cynorta*, as all other potential useful information was ignored. Anchoring the type species of *Cynorta* to an actual type specimen is an important

Table 2.—Appendage measurements of males and females of *C. conspersa* (MNRJ 4560), format = mean (standard deviation). Number of specimens counted = 10 for each.

Appendage	Femur ♂	Tibia ♂	Femur ♀	Tibia ♀
Leg I	4.31 (0.34)	2.51 (0.28)	4.21 (0.34)	2.53 (0.28)
Leg II	10.16 (0.84)	8.27 (0.44)	9.09 (0.49)	7.29 (0.48)
Leg III	6.21 (1.17)	3.36 (0.21)	5.86 (0.32)	3.03 (0.18)
Leg IV	9.43 (0.71)	4.89 (0.30)	8.45 (0.50)	4.43 (0.28)

step to secure the concept of this important genus.

On the bright side, the intermediate sprinkled pattern of yellowish-white (contrasting with the wide patterns or the sprayed patterns of all other species in Amazonia) on the dorsal scutum allows ready identification of this species, even without more refined morphological details in the old descriptions and redescriptions. This can be seen in Koch and Roewer's redescriptions of the species. Furthermore, locality data match well for our *C. conspersa*. Spix and von Martius collected twice (25 July to 21 August 1819 and 16 April to 13 June 1820) in Belém during the 1817–1820 expedition, which ultimately yielded the specimens described by Perty that match the known occurrence of our material.

In the comparison of *C. conspersa* with other *Cynorta*, tarsal counts are useless. Of the dozens of nominal species currently in *Cynorta*, those with heavy legs III–IV and strong cheliceral dimorphism (such as *C. refracta* Mello-Leitão, 1940) may be immediately discarded and will probably even be removed from this genus. Other *Cynorta* have wide white patterns on the scutum (or more rarely a sprayed, dust-like pattern) while *C. conspersa* has an intermediate pattern of small (but not dust-like) spots.

Out of the seven other *Cynorta* species described from Pará, six—*C. albanalis* Roewer, 1947; *C. albicurvata* Roewer, 1947; *C. albipicta* Roewer, 1947; *C. coxaepunctata* Roewer, 1947, *C. ramulata* Roewer, 1947 and *C. variegata* Roewer, 1947—are from the same locality, Santarém, and seemingly very close to each other. They show a general morphology similar to *C. conspersa*, with delicate legs and chelicerae but they possess a dorsal pattern of white markings forming elongate Ys and ribs. *Cynorta juruensis* (Mello-Leitão, 1923) has an extremely elongate body, and probably belongs to an undescribed Amazonian genus.

A most useful character that we plan to use in the comparison among genera in Cosmetidae is the outline of the dorsal scutum. Preliminary work on the family allowed us to detect four basic types of scutum outline (Fig. 12) that we here call: alpha, beta, gamma, and delta. The types can be shortly characterized as follows.

Type alpha: scutum subrectangular with laterals convex, forming two well-marked constrictions (examples *Ambatoiella*, *Erginulus*, *Flirtea*, *Rhaucus*).

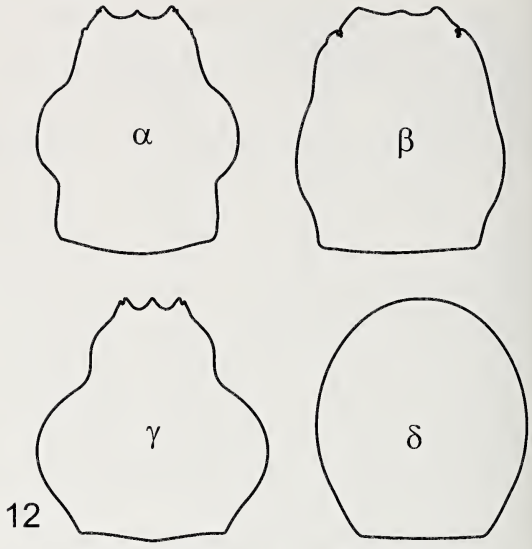


Figure 12.—Basic types of outline of dorsal scutum in Cosmetidae. Type alpha: scutum subrectangular with laterals convex, forming two well marked constrictions (examples *Ambatoiella*, *Erginulus*, *Flirtea*, *Rhaucus*). Type beta: both constrictions attenuate and posterior constriction displaced posteriorly (examples *Cosmetus*, *Cynorta*, *Metavononoides*, *Paecilaema*, *Vonones*). Type gamma: convexity of scutum much wider and displaced posteriorly, with posterior constriction almost absent and anterior constriction well marked (example *Metalibitia*). Type delta: loss of all constrictions of scutum (examples *Discosomaticus*, *Sibambea*).

Type beta: both constrictions attenuate and posterior constriction displaced posteriorly (examples *Cosmetus*, *Cynorta*, *Metavononoides*, *Paecilaema*, *Vonones*).

Type gamma: convexity of scutum much wider and displaced posteriorly, with posterior constriction almost absent and anterior constriction well marked (example *Metalibitia*).

Type delta: loss of all constrictions of scutum (examples *Discosomaticus*, *Sibambea*).

ACKNOWLEDGMENTS

This study was supported by grant #520406/98-2 from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to ABK and scholarship PIBIC to CSC. The Fundação Universitária José Bonifácio (FUJB) contributed to the equipment of the Laboratory of Arachnology of MNRJ.

REFERENCES

Goodnight, J.C. & M.L. Goodnight. 1953. The opilionid fauna of Chiapas, Mexico, and adjacent areas. American Museum Novitates 1960:1–81.

Koch, C.L. 1839. Die Arachniden getreu nach der Natur abgebildet und beschrieben. Vol. 7. C.H. Zeh'schen Buchhandlung, Nuremberg.

Kury, A.B. 1989. Notes on Mitobatinae III: a remarkable new Brazilian species of *Mitobates* Sund., 1833 (Opiliones: Laniatores: Gonyleptidae). Boletim do Museu Nacional, Rio de J. (N.S. Zool.), 328:1–12.

Kury, A.B. 2003. Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones). Revista Ibérica de Aracnología, volumen especial 1:1–337.

Mello-Leitão, C.F. de. 1931. Quatro novos opiliões. Boletim do Museu Nacional, Rio de Janeiro 7(2): 115–119.

Mello-Leitão, C.F. de. 1932. Opiliões do Brasil. Revista do Museu Paulista 17(2):1–505.

Perty, J.A.M. 1833. Delectus animalium articulatorum, quae in itinere per Brasiliam an 1817–20 peracta collegerunt J.B. de Spix et de Martius. 1830–34. Fasc. 3., published by the author, Monachii [Munich]. Pp. 125–224.

Pickard-Cambridge, F.O. 1904. Opiliones. Pp. 545–560. *In* Biologia Centrali-Americana, Arachnida, Araneidea and Opiliones. Volume 2. (F.D. Godman & O. Salvin, eds.). R.H. Porter/Dulau & Co., London.

Pickard-Cambridge, F.O. 1905. Opiliones. Pp. 561–610, pls. 53, 54. *In* Biologia Centrali-Americana, Arachnida, Araneidea and Opiliones Volume 2. (F.D. Godman & O. Salvin, eds.) Pub. for the editors by R. H. Porter/Dulau & Co., London.

Roewer, C.-F. 1912. Die Familie der Cosmetiden Opiliones-Laniatores. Archiv für Naturgeschichte, Berlin, Abteilung A, 78(10):1–122.

Roewer, C.-F. 1923. Die Webernechte der Erde. Sistematische Bearbeitung der bisher bekannten Opiliones. Gustav Fischer, Jena. 1116 pp.

Sørensen, W.E. 1932. Descriptiones Laniatorum (Arachnidorum Opilionum Subordinis). Opus posthumum recognovit et edidit Kai L. Henriksen. Det Kongelige Danske Videnskabernes Selskabs skrifter, København, Naturvidenskabelig og Mathematisk Afdeling, ser. 9, 3(4):197–422.

Manuscript received 12 June 2006, revised 5 October 2006.

2007. The Journal of Arachnology 35:334–395

MORPHOLOGY AND EVOLUTION OF COBWEB SPIDER MALE GENITALIA (ARANEAE, THERIDIIDAE)

Ingi Agnarsson,[1,2] **Jonathan A. Coddington**[1] and **Barbara Knoflach**[3]: [1]Systematic Biology-Entomology, Smithsonian Institution, NHB-105, PO Box 37012, Washington, DC 20013-7012, USA; [2]The University of British Columbia, Departments of Botany and Zoology, 3549-6270 University Blvd., Vancouver, B.C. V6T 1Z4, Canada. E-mail: ingi@zoology.ubc.ca; [3]University of Innsbruck, Institute of Ecology, Division of Terrestrial Ecology and Taxonomy, Technikerstrasse 25, A-6020 Innsbruck, Austria.

ABSTRACT. This study elucidates the homology of elements of the male palps in the spider family Theridiidae. We survey and illustrate 60 species from 29 out of the 86 currently recognized genera representing all subfamilies. The study is buttressed by a phylogenetic framework, and uses a new method to evaluate critically competing homology hypotheses based on various criteria. Among the classic criteria for homology, topology performed better than special similarity, and much better than function. Guided by those results, we propose names for and correspondences among the broad diversity of theridiid palpal tegular sclerites. We discuss the phylogenetic utility and distribution of key palpal characteristics, and evaluate existing evolutionary hypotheses of the theridiid palp and its components.

Keywords: Character homology, congruence, phylogeny, tests of homology, primary homology

Systematists in recent years broadly agree on the distinction between primary and secondary homology (e.g., de Pinna 1991). Primary homologies are almost Baconian observations—*a, b*, and *c* correspond or are similar in some way, and therefore may be the same structure modified during descent from a common ancestor. Such conjectures are what systematists call "characters," and they constitute the columns in a standard phylogenetic data matrix; features of characters, *x, y*, and *z* then being "character states." Phylogenetic analysis uses the fit of the distribution of states within characters and across characters as independent observations to choose a phylogenetic tree. Character states that provide support for nodes of the tree are termed secondary homologies (i.e., synapomorphies) because they have withstood the test of congruence under parsimony, or maximum likelihood, or whatever criterion guided tree choice. Secondary homologies, then, are primary homologies that have been tested phylogenetically (Farris 1983). Primary homologies, whether characters or character states, are background knowledge, untested postulates, assumed prior to analysis. However, congruence only tests character states—the possibility that the characters themselves may be erroneous, or that a more parsimonious sorting of states into characters may be possible, is never formally tested (e.g., Patterson 1982; Rieppel & Kearney 2002). This represents a serious problem when taxa present several similar, but independent, features. For a well known example, birds have only three digits but which of the five present is in most other vertebrates is still controversial (Wagner 2005). Spiders present their own problems in character homology, particularly the sclerites of the complex entelegyne male palpus (e.g., Griswold et al. 1998). Male spider genitalia evolve fast enough to denote species (Eberhard 1985; Huber 2003, and a wealth of revisionary work), yet are a main source of data used to define major clades (Griswold et al., 2005). Unsurprisingly then, the nomenclature—which is to say the homology hypotheses—of the parts of the male bulb are contentious (Coddington 1990).

Although Comstock (1910) was not the first to study palps, his excellent, carefully labeled illustrations of major spider clades is the seminal work in comparative studies of the parts

of the male bulb. His proposed homologies and names are still used today to describe palpal diversity. Other early work includes Menge (1866, 1868, 1869), Osterloh (1922), and Gerhardt (1921, 1923). More recent reviews include Levi (1961), Shear (1967), Saaristo (1978, 2006), Heimer (1982), Coddington (1990), and Sierwald (1990). The morphology and nomenclature of araneid palps was discussed in detail by Grasshoff (1968, 1973), that of linyphiid palps by Merrett (1963), and of pholcid palps by Huber (1994, 1995, 1996), and Uhl et al. (1995). In addition, recent taxonomic revisions and cladistic analyses have discussed palpal homologies in theridiids and many related spider families (e.g., Hormiga 1994a, b, 2000; Scharff and Coddington 1997; Griswold et al. 1998, 1999, 2005; Agnarsson 2003, 2004, 2005, 2006a, b, c; Agnarsson & Kuntner 2005; Agnarsson et al. 2006, 2007; Agnarsson & Zhang 2006; Kuntner 2005, 2006, 2007; Miller 2007).

Despite this work, theridiid palpal sclerite names (homologies) are unstable. Levi (1953–1972) and Levi & Levi (1962) used a mostly consistent nomenclature for sclerites, but disavowed more general homologies despite using names broadly applied in other families (Levi 1961). Heimer (1982) homologized theridiid sclerites to those in other spider families. Heimer & Nentwig (1982) and Heimer (1986) proposed a detailed theory on the evolution of the theridiid "paracymbium" and Heimer (1986) also discussed the homology of the median apophysis. Saaristo (1978) discussed theridiid palpal morphology in detail suggesting several novel hypotheses, and Bhatnagar & Rempel (1962) described the ontogeny of the *Latrodectus* palp (probably *hesperus*, although identified as "*curacaviensis*"). All these authors disagreed among themselves about homology of palpal sclerites in theridiids in particular and araneoids in general. Most recently, Saaristo (2006) proposed yet another novel scheme of palpal homologies (one proposed without reference to a phylogeny), based on retraction of some, but not all, of his earlier views (see Saaristo 1978) and some apparent misinterpretations of both Coddington (1990) and Agnarsson (2004).

The diversity of views has hindered understanding the phylogenetic relationships among theridiids (see Agnarsson 2004) and other spiders (Coddington 1990; Griswold et al. 1998), and perhaps because of this instability, authors of recent taxonomic papers on theridiids avoid classic palpal sclerite names. For example, Knoflach (1991–2002), Knoflach & Thaler (2000), Knoflach & van Harten (2000, 2001), and Knoflach & Pfaller (2004) label theridiid tegular sclerites consistently and imply homologies within theridiids, but use names like tegular apophysis I, II and III to avoid interfamilial homologies.

Many theridiids have relatively complex palps and diverse palpal conformations (Agnarsson 2004). The worst problems are the sclerites borne on the distal segment of the bulb (tegulum); of which Orbiculariae commonly have three, or sometimes four or more. The plesiomorphic theridiid condition is four sclerites (Agnarsson 2004), but some taxa have five, and many others three, two, or only one. Three names (embolus, conductor, median apophysis) are applied to sclerites in most spider families, while a multitude of other names are variously applied. Of these, only the embolus is not problematic; it contains the ejaculatory duct and conveys sperm to the female. Others, such as the radix, conductor, theridiid tegular apophysis, median apophysis, paramedian apophysis, suprategulum, conductor II, etc., are contentious.

Despite the bleak history of theridiid palpal nomenclature, we nevertheless present yet one more attempt at a durable system of names and homologies. We illustrate 60 theridiid species, belonging to 29 out of the 87 currently recognized genera (Platnick 2006; Agnarsson 2000, 2006a), representing all theridiid subfamilies and the known range of palpal morphologies. We use a new method (Agnarsson & Coddington unpublished ms.) to evaluate quantitatively primary homology hypotheses implied by different criteria of homology in order to propose a less arbitrary and more parsimonious explanation of palpal elements than have previous studies. We use the same method to compare our results to four previous hypotheses of theridiid homologies (Levi 1953–1972; Saaristo 1978; Coddington 1990; Agnarsson 2004) and we discuss the evolution of theridiid palps in a phylogenetic framework.

METHODS

Test of character homology.—Classical criteria for homology include topology, func-

tion, special similarity (similarity in fine detail), and ontogeny (e.g., de Beer 1971; Rieppel 1994, 2001; Hall 1995; Brigandt 2003). Although one of the most detailed studies of spider palpal ontogeny concerned *Latrodectus* (Bhatnagar & Rempel 1962), their study was not comparative, so that ontogeny cannot be compared across Theridiidae.

This study, therefore, is limited to topology, special similarity, and function. We use a new method (Agnarsson & Coddington unpublished ms.) that derives primary homology hypotheses under each criterion in turn but which then assesses them under those criteria not used in their formation. Obviously, primary homologies suggested by topological similarity may differ from those suggested by function or special similarity. The preferred set of homologies is that least contradicted by, or most congruent with, all criteria. Each criterion either supports, contradicts, or is neutral about any homology hypothesis. The method is quantitative in that support or agreement is scored as "1," contradiction as "0," and inapplicability as "–," and these values are summed (or averaged) to reach a conclusion.

Figure 1 presents a didactic example in which two taxa each have three sclerites, provisionally named *r1–3* and *a1–3*, with differing functions (F1–F3), shapes (round or hexagon), and colors (white or black). Taking topology first, it implies that *r1* = *a1*, *r2* = *a2*, and *r3* = *a3*. Sclerite *r1* differs from *a1* in color, *r2* from *a2* in function, and *r3* from *a3* in function and color, for a total of 4 differences. Taking function next, it implies *r1* = *a1*, *r2* = *a3*, and *r3* = *a2*. Sclerite *r1* differs from *a1* in color, *r2* from *a3* in topology, shape, and color, and *r3* from *a2* in topology and shape, for a total of 6 differences. Taking similarity last, it implies *r1* = *a3* (the only black, round sclerite), *r2* = *a2*, and *r3* = *a1*. Sclerite *r1* differs from *a3* in topology and function, *r2* from *a2* in function, and *r3* from *a1* in topology and function, for a total of 5 differences. In this case, topology is preferred because it requires fewer hypothesized changes. Note that special similarity here offers two points of comparison, shape and color, whereas topology and function offer only one each. Similarity, therefore, counts "more" than topology or function, and one might wish to give each criterion equal weight by averaging the points of comparison for special similarity prior to comparison with the other criteria (the equal weights approach). On the other hand, one could argue that complex homologies have more points of comparison and therefore deserve greater weight, so that all conflicts should simply be summed (the parsimony approach). The results of both points of view are presented here. In this didactic example, topology is preferable under both approaches (Agnarsson & Coddington unpublished ms.).

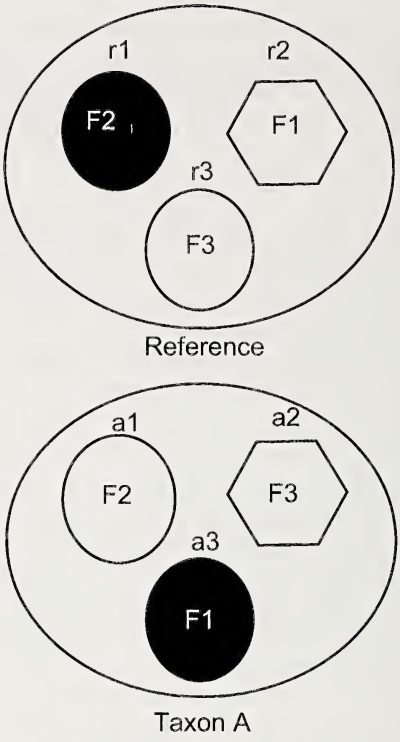


Figure 1.—Two taxa each have three sclerites, but their homologies are ambiguous. They perform three different functions, indicated as F1–F3; occur in various relative positions (topology), and differ in color and shape (black or white, round or hexagon; special similarity). Depending on which criterion is primary, different primary homology hypotheses result. Under topology, *r1* = *a1*, *r2* = *a2*, and *r3* = *a3*; under function, *r1* = *a1*, *r2* = *a3*, and *r3* = *a2*; under special similarity, *r1* = *a3*, *r2* = *a2*, and *r3* = *a1*. See text and Agnarsson and Coddington (in press) for explanation.

Another complication is unrestrained hypotheses of loss of one sclerite and gain of

another. Strictly speaking, an author might argue that *any* difference between two structures justifies the supposition that the one has been lost and the other gained, even in the face of many "similarities." Under the "gain/loss" approach such similarities are interpreted as convergences. Although not illustrated in Fig. 1, we attempt to constrain such an approach by counting the loss and gain each as one step, and each "convergence" between the lost and gained sclerites as an additional step. If transformation were preferred to loss/gain, such similarities would have required no explanation, and therefore count against the loss/gain hypothesis. For example, Saaristo (1978), Coddington (1990), and Agnarsson (2004) regarded the "third" tegular apophysis in Theridiidae as at least one novel sclerite, but Levi (1953–1972) called it a "radix," presumptively homologous to the araneid radix. The former authors therefore incur costs for hypothesizing a new sclerite, whereas Levi incurs costs only when topological, functional, or detailed attributes of the araneid and theridiid radices differ. We also freely admit that it is often impossible to know exactly what prior authors were thinking when they used classical sclerite names in Theridiidae. Our inferences in Table 1, although our best guess as to what those authors intended, are primarily to show how these logical procedures may resolve the problem of palpal sclerite homologies in Theridiidae (Tables 1, 2).

Abbreviations and conventions.—References to figures published elsewhere are listed in lowercase type (fig.); references to figures in this paper are capitalized (Fig.). Anatomical abbreviations appear in Appendix A.

Taxon choice.—Agnarsson (2004) analyzed theridiid phylogeny at the generic level using a matrix of 61 terminals (8 outgroup genera, 31 theridiid genera) and 242 characters, of which 88 (36%) pertained to the palpal organ. Based on these results (Fig. 2, see also Arnedo et al. 2004), we chose exemplars of 29 genera (16 represented by their type species) from across (and beyond) the cladogram, to represent theridiid palpal diversity for the purposes of this paper (see Appendix B, Table 1, and Figures 4–200). For simplicity, a portion of those were selected for analysis using the new method (Table 1); the inclusion of the remainder does not alter the results (Agnarsson & Coddington unpublished ms.). To the best of our knowledge, omitted genera do not present dramatically different palpal configurations but seem to fit in the schema proposed here. Nevertheless, rare genera not covered by Agnarsson (2004) or, indeed, still undiscovered theridiids could change these results in the future. For the complete list of material examined in this study, see Agnarsson (2004), and Appendix B.

Figure 3 is a schematic "groundplan" of theridiid palps, representing the sclerites most commonly found in these spiders (Agnarsson 2004; Knoflach 2004). This groundplan facilitates discussion of phylogenetically important elements of the theridiid palp, and also serves as a reference against which primary homology hypotheses are compared (see "reference" in Fig. 1).

Specimen examination.—Specimens were examined under a Wild M-5A dissecting microscope. Male palps were immersed in concentrated KOH (~1 g/ml) for about one minute and then transferred to distilled water where rapid expansion of hematodochae took place in less than one minute (see Coddington 1990, modified from Shear 1967). In theridiids full expansion often requires unlocking the MA from the cymbium, and occasionally re-immersion in KOH. Artificial expansion of palps greatly facilitates understanding of palpal morphology (Coddington 1990), although it is a poor technique to understand how palps function (Huber 1993). In many cases, palps must be dissected to understand their anatomy. After examining the expanded palp, removal of the embolus (and sometimes other sclerites) facilitated examination of the tegulum and tegular sclerites residing behind or beneath the embolus. Sketches were made of preparations mounted as described in Coddington (1983) using both dissecting and compound microscopes equipped with camera lucida. For SEM examination, specimens were cleaned ultrasonically for one minute and then transferred to 100% ethanol overnight. The specimens were then dissected, and either critical point or air-dried. Specimens were glued to round-headed rivets using an acetone solution of polyvinyl resin, and sputter coated. All drawings were rendered in Adobe Photoshop, and plates were composed with Adobe Illustrator.

Table 1.—Results of the method outlined in Fig. 1 and the text, as applied to three problematic theridiid palpal sclerites: median apophysis (MA), theridioid tegular apophysis (TTA), and conductor (C) with topology as the primary criterion. Similar tables were compiled for function and special similarity and are summarized under the SS and FNC columns in Table 2. Scores are given for topology (TOP), special similarity (SS) and function (FNC) as secondary criteria. Dashes are inapplicables; question marks are unknowns. Special similarity includes three points of comparison: flexible or fused tegular connection (Cxn), sperm duct presence or absence (Dct), and membranous or sclerotized texture (Tex), which three scores are averaged under SS for each sclerite under the equal weights point of view (see text). The strict gain/loss point of view (see text) is tabulated in the G/L column. As the primary criterion, topology naturally does not conflict with itself as a secondary criterion (all scores = 1, or agreement) but it conflicts with function for the TTA and C, and with special similarity for MA and C. Subtotals by taxon (averages for TOP, SS, FNC, and G/L) and counts of conflict for parsimony (PAR) appear at right; grand totals are counts or averages of raw scores under each sclerite and are carried forward to Table 2.

	MA							TTA						
Taxon	TOP	Cxn	Dct	Tex	SS	Fnc	G/L	TOP	Cxn	Dct	Tex	SS	Fnc	G/L
Achaearanea tabulata	—	—	—	—	—	—	0	—	—	—	—	—	—	0
Achaearanea tepidariorum	—	—	—	—	—	—	0	—	—	—	—	—	—	0
Achaearanea trapezoidalis	—	—	—	—	—	—	0	—	—	—	—	—	—	0
Ameridion sp.1	1	1	0	1	0.67	1	1	1	1	1	1	1.00	0	0
Ameridion sp.2	1	1	0	1	0.67	1	1	1	1	1	1	1.00	1	0
Anelosimus eximius	1	1	0	1	0.67	1	1	1	1	1	1	1.00	?	0
Anelosimus vittatus	1	1	0	1	0.67	1	1	1	1	1	1	1.00	0	0
Argyrodes argyrodes	1	1	1	1	1.00	1	1	1	1	1	1	1.00	1	0
Argyrodes elevatus	1	1	1	1	1.00	1	1	1	1	1	1	1.00	1	0
Carniella schwendingeri	1	1	1	1	1.00	1	1	—	—	—	—	—	—	0
Coleosoma floridanum	1	1	0	1	0.67	1	1	1	1	1	1	1.00	1	0
Enoplognatha ovata	1	1	1	1	1.00	1	1	1	1	1	1	1.00	0	0
Enoplognatha sp.	1	1	1	1	1.00	1	1	1	1	1	1	1.00	0	0
Episinus angulatus	1	1	1	1	1.00	1	1	1	1	1	1	1.00	1	0
Episinus maculipes	1	1	1	1	1.00	1	1	1	1	1	1	1.00	1	0
Euryopis flavomaculata	1	1	1	1	1.00	1	1	1	1	1	1	1.00	0	0
Latrodectus geometricus	1	1	1	1	1.00	1	1	1	1	1	1	1.00	?	0
Neospintharus trigonum	1	1	1	1	1.00	1	1	1	1	1	1	1.00	1	0
Phoroncidia americana	1	1	1	1	1.00	1	1	1	1	1	1	1.00	0	0
Selkirkiella sp.	1	1	1	1	1.00	1	1	1	1	1	1	1.00	?	0
Steatoda americana	1	1	1	1	1.00	1	1	1	1	1	1	1.00	1	0
Styposis selis	1	1	1	1	1.00	1	1	1	1	1	1	1.00	0	0
Theridion cochise	1	1	0	1	0.67	1	1	—	—	—	—	—	—	0
Theridion frondeum	1	1	0	1	0.67	1	1	1	1	1	1	1.00	1	0
Theridion varians	1	1	0	1	0.67	1	1	1	1	1	1	1.00	1	0
Theridula emertoni	—	—	—	—	—	—	0	—	—	—	—	—	—	0
Theridula opulenta	—	—	—	—	—	—	0	—	—	—	—	—	—	0
Thymoites nr. *prolatus*	1	1	0	1	0.67	1	1	1	1	1	1	1.00	1	0
Grand Totals														

RESULTS AND DISCUSSION

Test of character homology.—Table 2 shows that topology outperforms similarity and function (columns TOP, SS, FNC) in accounting for theridiid palpal diversity whether assessed under the equal weights or parsimony approach; hypotheses based on topology are globally most congruent. Topology is fully congruent with some other criterion for each sclerite in all taxa. For example, median apophysis topology agrees with function (locking the palp in the cymbium) and two similarities (texture and membranous attachment to the tegulum), but a second similarity (presence of a duct) is highly incongruent. If duct presence is used as a primary criterion, two sclerites would be recognized (corresponding to locking apophyses A and B of Saaristo (1978)). The two would be topologically and functionally identical, and phylo-

Table 1.—Extended.

C							Subtotals by Taxon						
TOP	Cxn	Dct	Tex	SS	FNC	G/L	TOP	SS	FNC	G/L	PAR	G/L	PAR
1	1	1	0	0.67	1	1	1.00	0.67	1.00	0.33	3	0.83	1
1	1	1	0	0.67	1	1	1.00	0.67	1.00	0.33	3	0.83	1
1	1	1	0	0.67	1	1	1.00	0.67	1.00	0.33	3	0.83	1
1	1	1	1	1.00	0	1	1.00	0.89	0.33	0.67	4	0.96	3
1	1	1	1	1.00	1	1	1.00	0.89	1.00	0.67	2	0.96	1
1	1	1	1	1.00	?	1	1.00	0.89	1.00	0.67	2	0.96	1
1	1	1	1	1.00	0	1	1.00	0.89	0.33	0.67	4	0.96	3
1	1	1	1	1.00	1	1	1.00	1.00	1.00	0.67	1	1.00	0
1	1	1	1	1.00	1	1	1.00	1.00	1.00	0.67	1	1.00	0
—	—	—	—	—	—	0	1.00	1.00	1.00	0.33	2	1.00	0
1	1	1	1	1.00	1	1	1.00	0.89	1.00	0.67	2	0.96	1
1	1	1	?	1.00	0	1	1.00	1.00	0.33	0.67	3	1.00	2
1	1	1	1	1.00	0	1	1.00	1.00	0.33	0.67	3	1.00	2
1	1	1	1	1.00	1	1	1.00	1.00	1.00	0.67	1	1.00	0
1	1	1	1	1.00	1	1	1.00	1.00	1.00	0.67	1	1.00	0
—	—	—	—	—	—	0	1.00	1.00	0.50	0.33	3	1.00	1
1	1	1	1	1.00	?	1	1.00	1.00	1.00	0.67	1	1.00	0
1	1	1	1	1.00	1	1	1.00	1.00	1.00	0.67	1	1.00	0
1	1	1	1	1.00	0	1	1.00	1.00	0.33	0.67	3	1.00	2
1	1	1	0	0.67	?	1	1.00	0.89	1.00	0.67	2	0.96	1
1	1	1	?	1.00	1	1	1.00	1.00	1.00	0.67	1	1.00	0
1	1	1	0	0.67	0	1	1.00	0.89	0.33	0.67	4	0.96	3
1	1	1	1	1.00	1	1	1.00	0.83	1.00	0.67	2	0.92	1
1	1	1	1	1.00	1	1	1.00	0.89	1.00	0.67	2	0.96	1
1	1	1	1	1.00	1	1	1.00	0.89	1.00	0.67	2	0.96	1
1	0	1	1	0.67	0	1	1.00	0.67	0.00	0.33	4	0.33	2
1	0	1	1	0.67	0	1	1.00	0.67	0.00	0.33	4	0.33	2
1	1	1	1	1.00	1	1	1.00	0.89	1.00	0.67	2	0.96	1
							1.00	0.92	0.77	0.58	66	0.92	31

genetically the loss of one would take place at the same instance as the origin of the other (Fig. 201). Similarly, function would make both topology, connection to the tegulum, and texture quite variable for the conductor and theridiid tegular apophysis. Under the topology rule, the conductor is consistently membranous.

We think this proposed homology scheme (Fig. 3, see also Figs. 4–200) is clearly more logical for theridiids than others hitherto proposed. While we have used classical names and hence implied testable interfamily homology hypotheses, we have not yet extended our test to other araneoid families. To effectively homologize sclerites (e.g., across Orbiculariae), a similarly detailed study is needed for each family. One difficulty in comparing theridiids with related families is that the theridiid bulb connects differently in

Table 2.—Results of the logic of Table 1 as applied to four prior analyses of theridiid palpal homologies (A04 = Agnarsson 2004; C90 = Coddington 1990; L62 = Levi 1953–1973; S78 = Saaristo 1978) and for all three primary criteria (TOP, FCN, SS). The column TOP carries forward the grand totals of Table 1. Either as mean performance under all criteria and accounting for gain/loss hypotheses (Grand mean) or simple step counting (parsimony) topology (TOP) as applied by Agnarsson (2004) outperforms other criteria and previous homology hypotheses.

Secondary criterion	Study or primary criterion						
	A04	C90	L62	S78	TOP	SS	FCN
Topology	1.00	0.61	0.68	0.67	1.00	0.93	0.77
Similarity	0.92	0.86	0.72	0.70	0.92	0.94	0.79
Function	0.77	0.48	0.61	0.41	0.77	0.75	1.00
Gain/Loss	0.58	0.58	0.86	0.31	0.58	0.52	0.59
Grand mean	0.82	0.63	0.72	0.52	0.82	0.78	0.79
Parsimony	66	129	121	181	66	74	87

the alveolus. In theridiids the subtegulum attaches mesobasally to the cymbium, but in the outgroups it attaches centrally. Therefore the orientation of the palpal bulb differs (e.g., the embolus appears to be proximal in the outgroups), but ventral-apical in theridiids. Nevertheless, the origin of the embolus is roughly opposite the fundus in all taxa considered here. Outgroup "theridiid tegular apophyses" and conductors are topologically and functionally similar to the theridiid condition as well.

The araneoid "median apophysis" is problematic. None of the primary homology criterion applied here to Theridiidae clearly corroborates any prior nomenclature of the median apophysis. As defined by other workers, the median apophysis topology varies across families, although theridiids are similar to araneids, which formed the basis for Comstock's (1910) nomenclature. Special similarity and function also differ. Difference in function is not surprising because our results suggest that it is the median apophysis homolog that forms part of the uniquely theridiid bulb-cymbium lock mechanism. These results show, yet again, that median apophysis nomenclature across spiders is inconsistent, and it remains to be seen if the sort of logic used here can improve the situation.

Composition and evolution of the male theridiid palp.—This study examined representatives of 29 theridiid genera, about 33% of the 87 currently recognized theridiid genera (Platnick 2006; Agnarsson 2000, 2006a). Palpal organs of 14 further genera are illustrated in Agnarsson (2004) and Knoflach (2002) (see also Agnarsson 2003, 2005, 2006a, b; Agnarsson & Kuntner 2005; Miller & Agnarsson 2005). These 43 genera include all common, species-rich theridiid genera and thus represent the vast majority of theridiid diversity, while the majority of the omitted genera are small (20 monotypic, 13 with 2–3 species, and 8 with 4–8 species). We therefore feel that our results, summarized below, apply broadly to theridiids.

The male palp consists of six segments: the coxa, trochanter, femur, patella, tibia, and the tarsus modified for sperm transmission. Most of these segments may bear modifications that are phylogenetically informative (Figs. 4–200). The palpal femur, for example, is elongated in some theridiid genera (Figs. 111, 112) and the patella also is rarely elongated (Fig. 112). However, we focus our discussion on the tibia and especially the tarsus, forming the cymbium and the palpal bulb. The reader should refer to Coddington (1990) for further discussion on ontogeny and homology of palpal elements. An overview of the theridiid palp and its landmarks is given in Figure 3.

Tibia: The male palpal tibia is typically a simple, quasi-cylindrical, segment broadening somewhat towards the tip. In theridiid relatives such as linyphioids, nesticids, and synotaxids, the tibial rim (the long edge of the tibial tip) that faces the dorsum of the cymbium is inconspicuous (Figs. 189, 190, 195–198; see also Griswold 2001, fig. 140A) and irregularly hirsute. Theridiid tibiae are characteristically modified into a cup-like segment, with a broadened distal tip (Figs. 3, 4, 12, 16, 17, 29, 50, 57, 66–69, 71, 72, 75, 76,

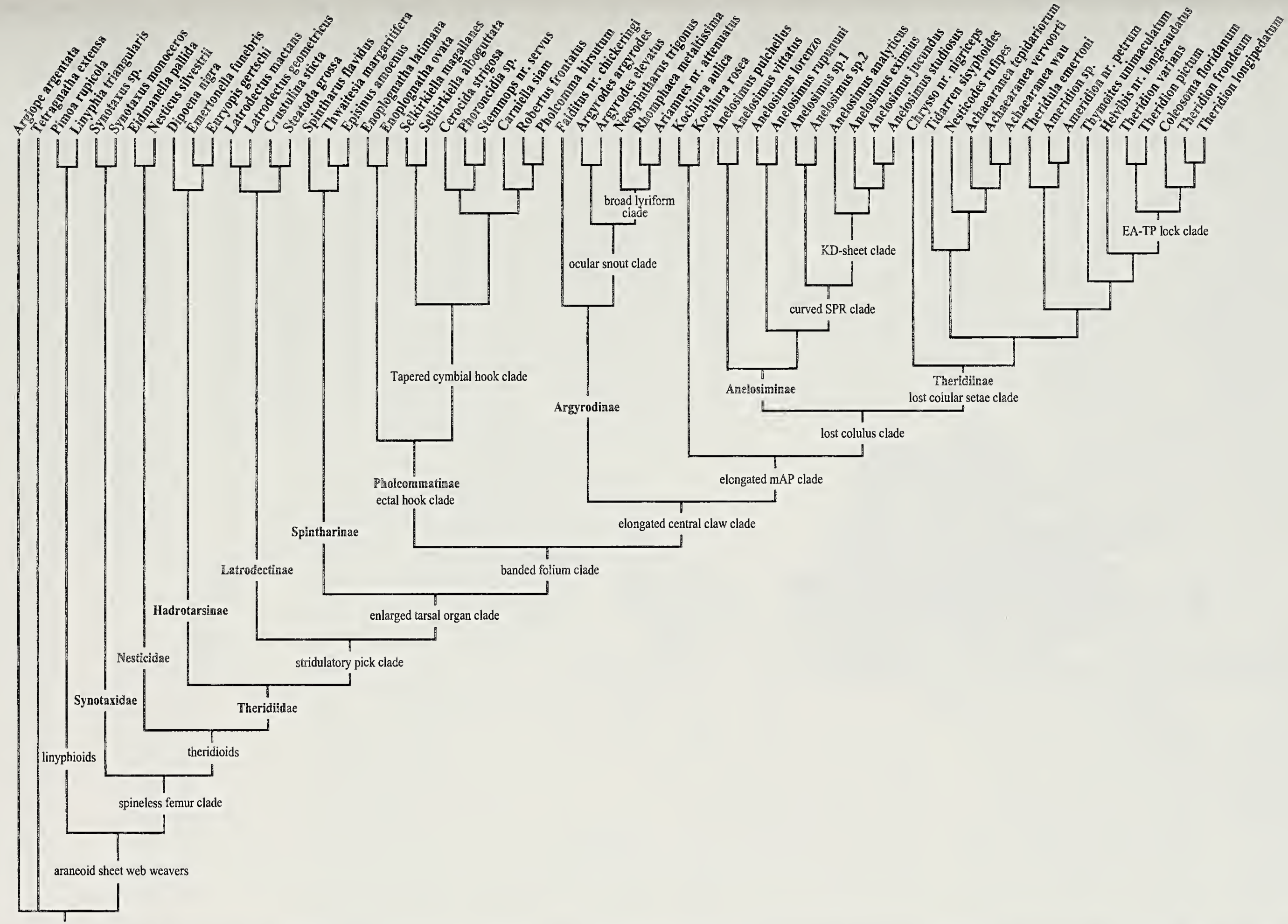


Figure 2.—Cladogram of Theridiidae (reproduced from Agnarsson 2004) labeled with subfamily and informal clade names, some of which refer to characters of the male palp. This cladogram forms the basis for the taxon choice in this study and is used to evaluate evolutionary hypotheses of palpal elements.

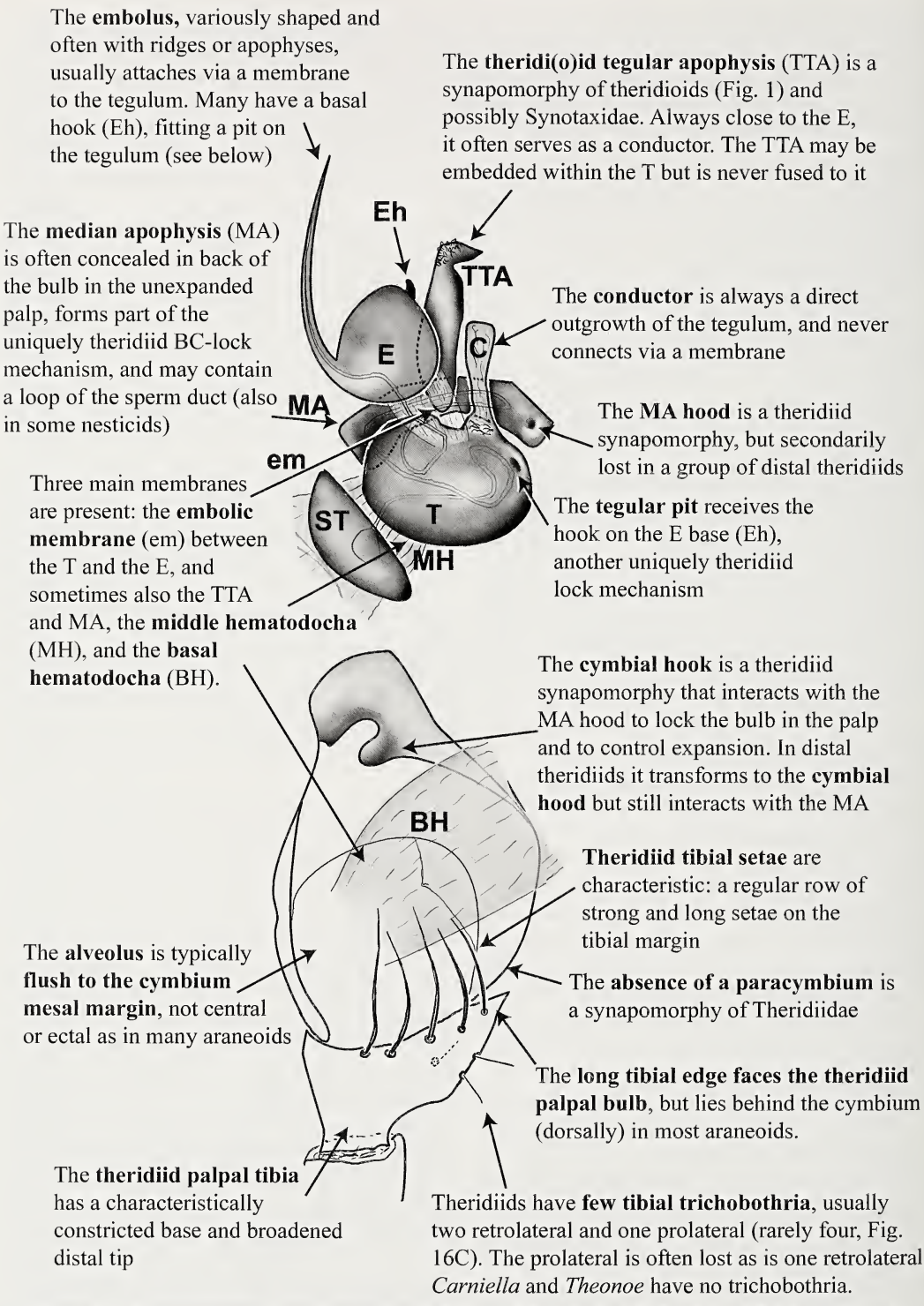
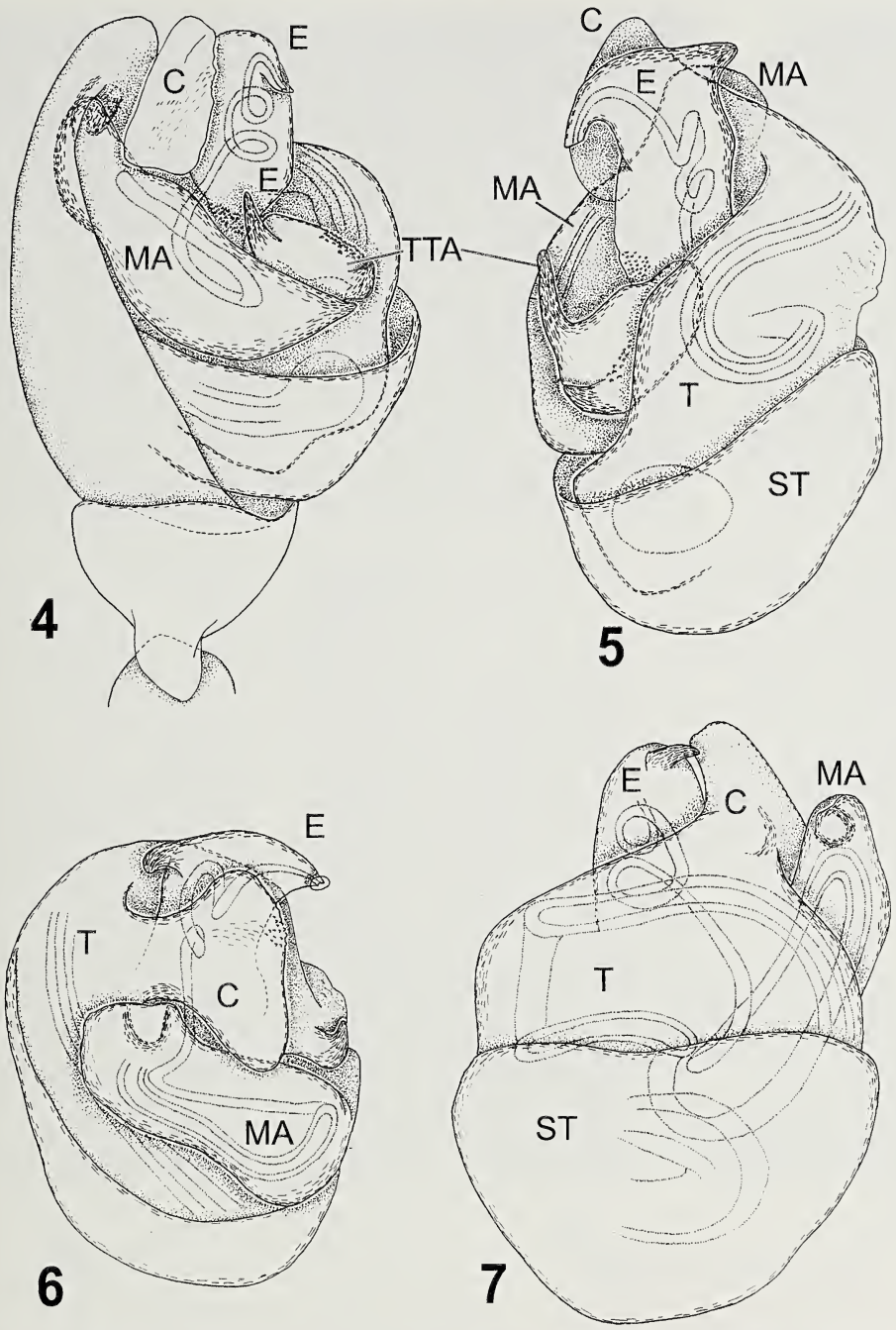


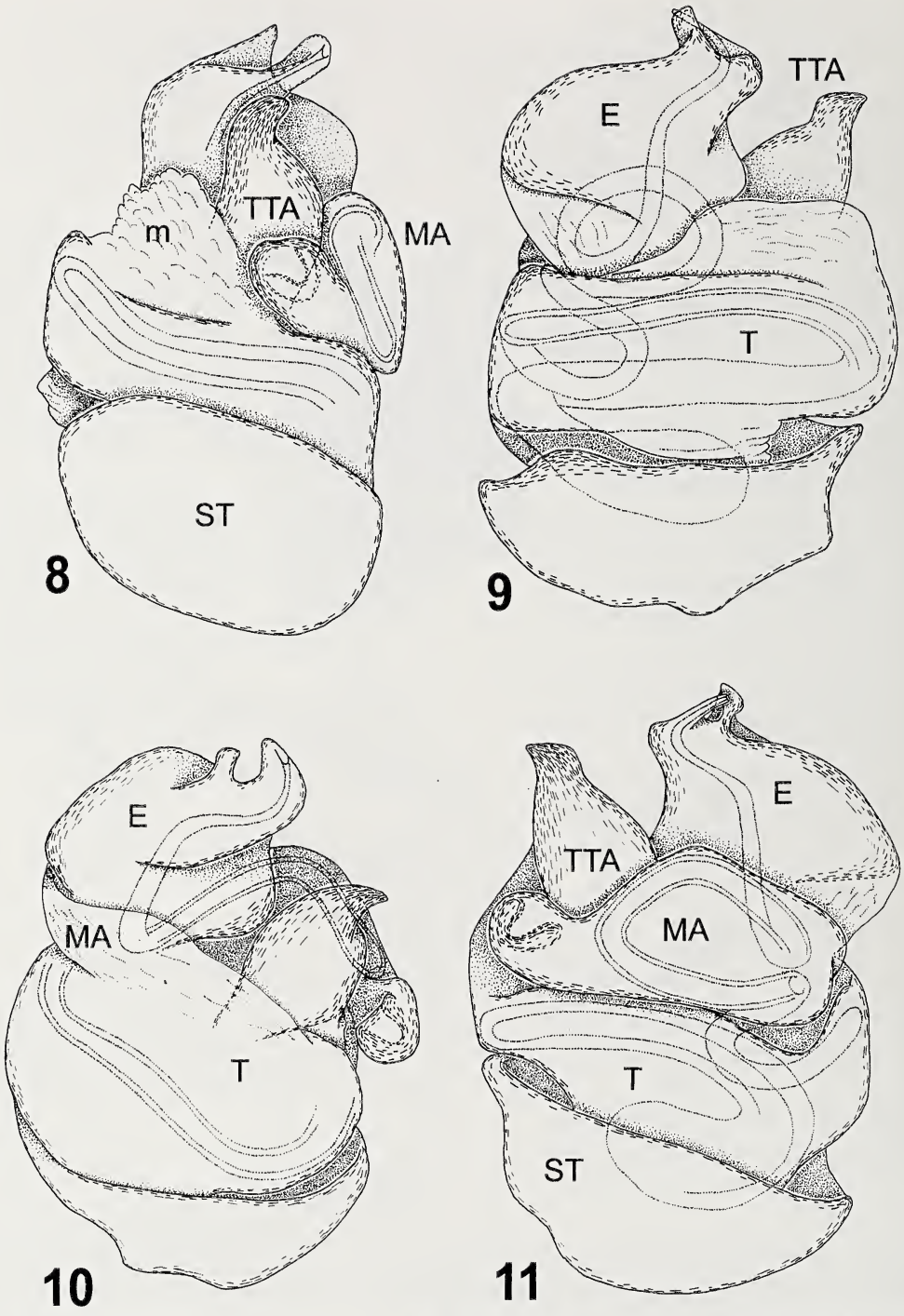
Figure 3.—Landmarks and descriptions of the major features and sclerites of the theridiid palp.



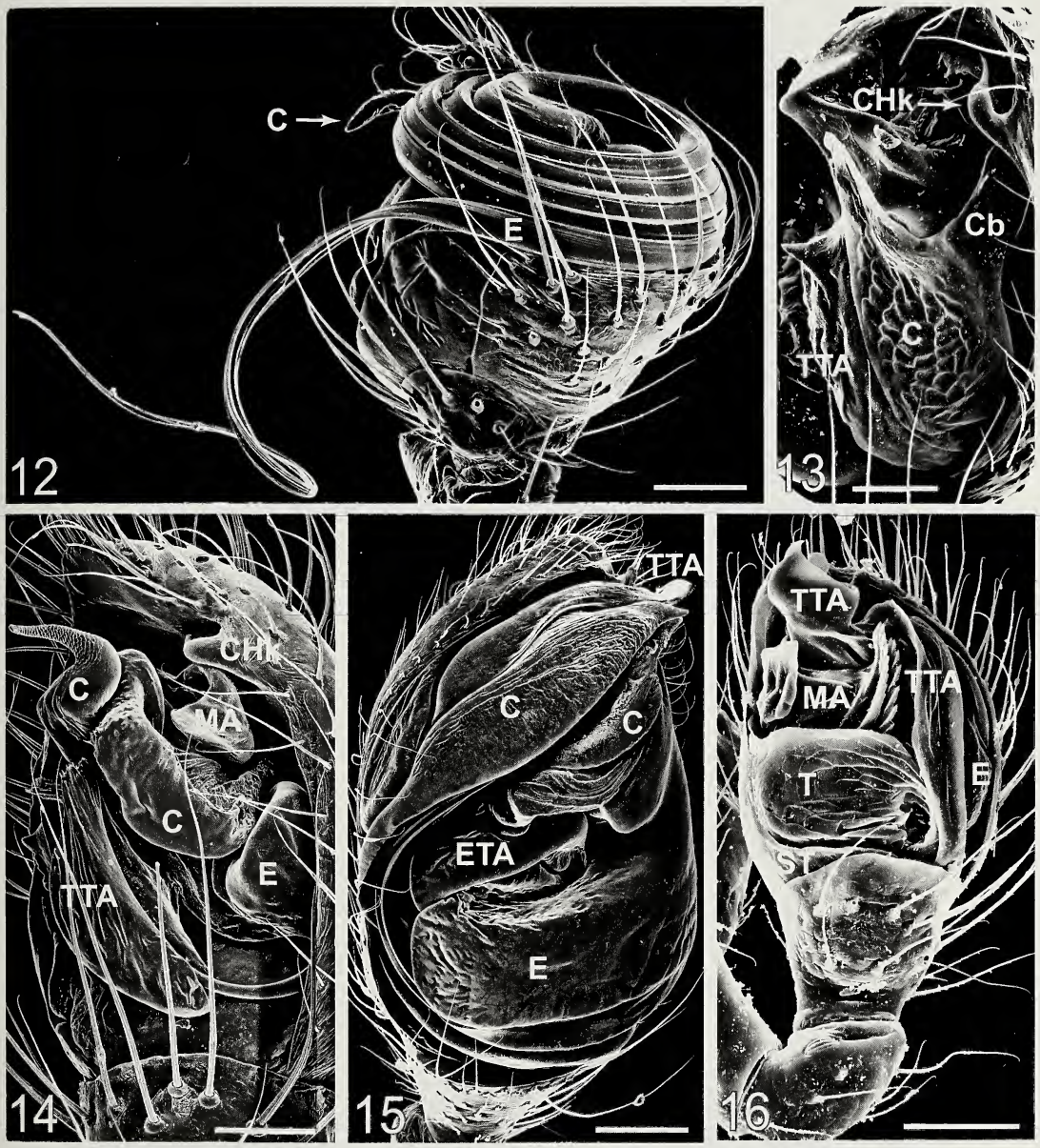
Figures 4–7.—*Dipoena melanogaster*. 4, male palp mesal; 5, bulb removed from cymbium, ectal; 6, apical; 7, dorsal; note loops of sperm duct within T, MA and E.

80, 87, 88, 92, 96, 97, 100, 104, 105, 107–109, 111–113, 131, 140, 146, 150, 157, 161, 162, 166, 170) (see also Agnarsson, 2004). The distinct tibial rim thus formed always faces the palpal bulb in the cymbium. The rim, furthermore, carries a highly regular row of strong and long, usually serrated setae (Figs. 3, 12, 14, 100, 104, 105, 107–109).

The number and distribution of tibial trichobothria is also phylogenetically informative. Agnarsson (2004) found that the reduction to two retrolateral trichobothria (versus

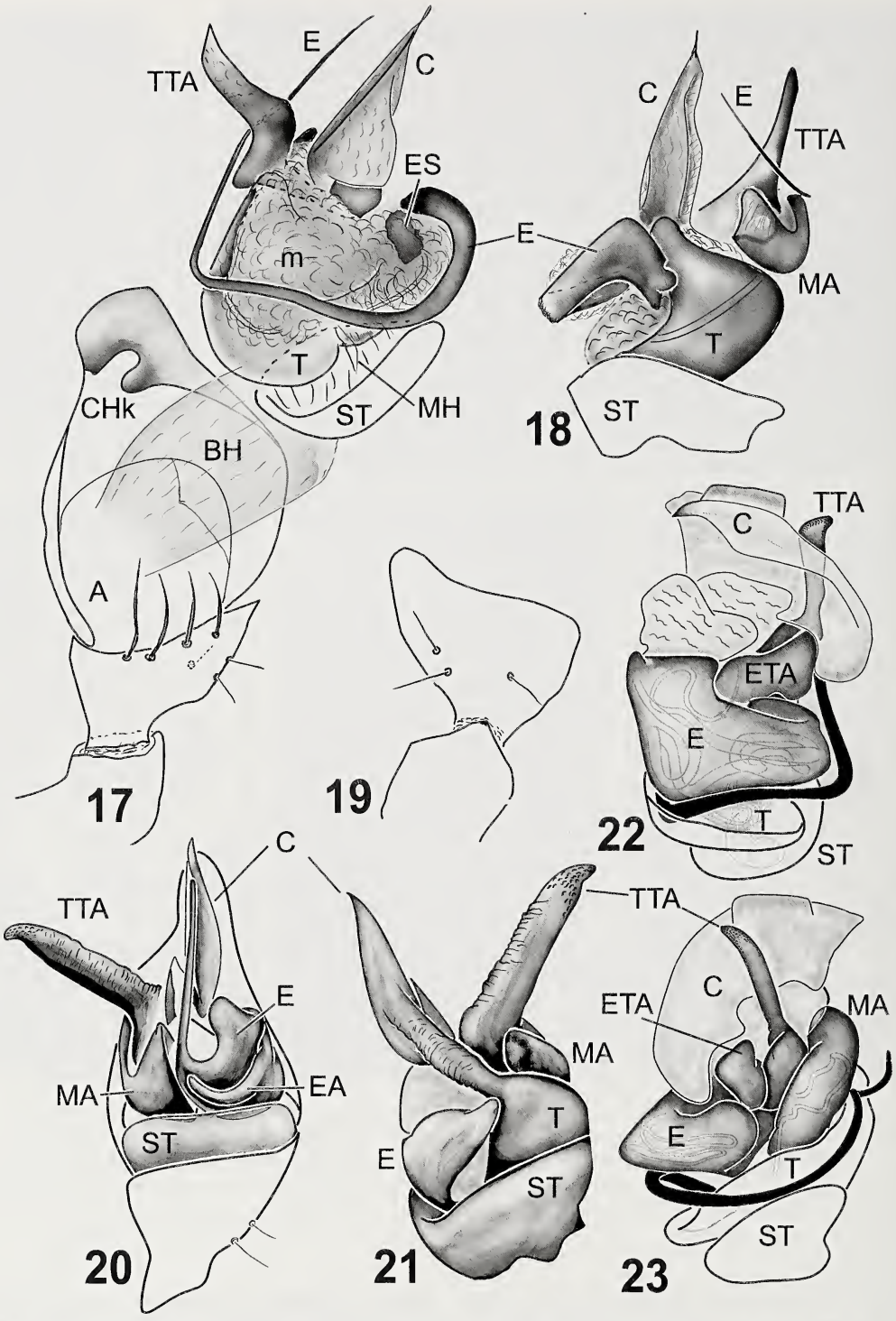


Figures 8–11.—*Euryopis flavomaculata*. 8, bulb removed from cymbium, mesal-dorsal; 9, ectal; 10, apical-dorsal; 11, ventral; conductor absent, note loop of sperm duct within MA.

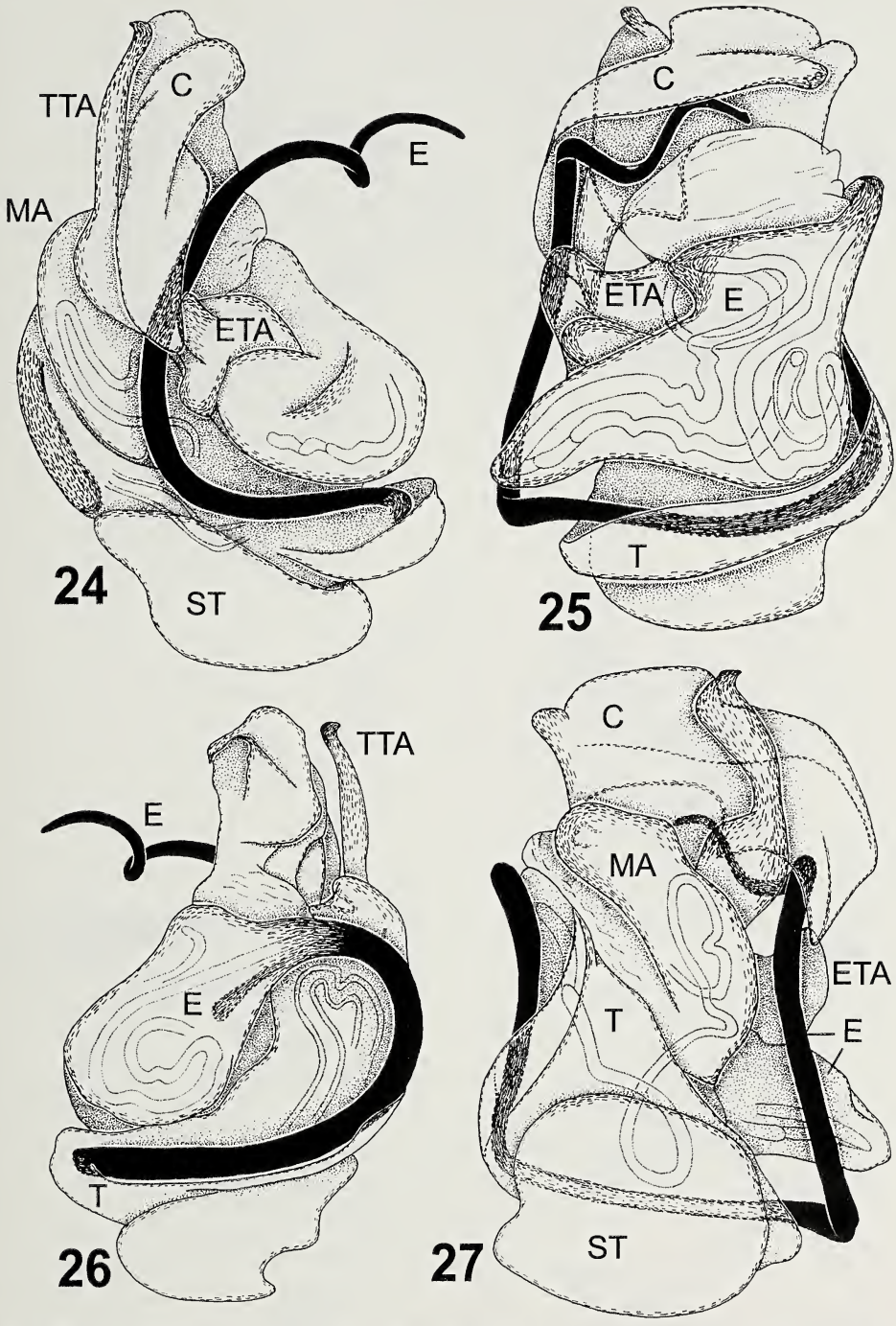


Figures 12–16.—12, *Latrodectus geometricus* C.L. Koch 1841; 13, *Selkirkiella* sp. note the bent and sharp tipped cymbial hook, a synapomorphic condition for Pholcommatinae, the tight juxtaposition of C and TTA is a synapomorphy of *Selkirkiella*; 14, *Enoplognatha ovata*; 15, *Episinus maculipes* Cavanna 1876, the huge and complexly folded C is a synapomorphy of Spintharinae; 16, *Styposis selis* Levi 1964, the ectal E with tip inside a TTA groove suggests affinities with Pholcommatinae. Scale bars: 12, 14, 15 = 100 μm; 13, 16 = 50 μm.

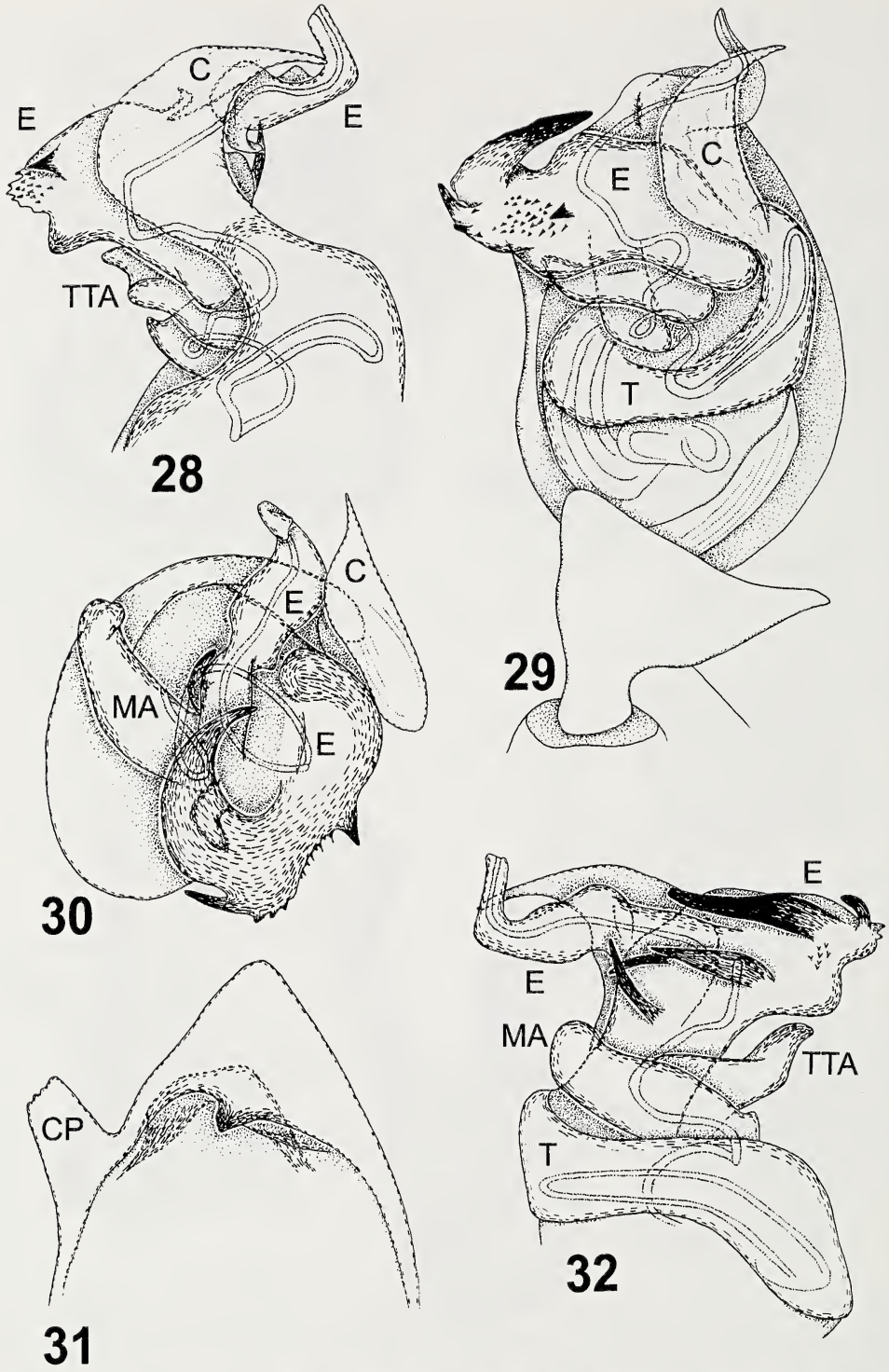
three or more in outgroups, Fig. 195) characterizes the spineless femur clade (*Synotaxus* plus the theridioid lineage, also reduced in cyatholipids, see Griswold 2001). Typically theridiids have two retrolateral and one prolateral trichobothria (Figs. 3, 19, 71). Independent reductions to one retrolateral trichobothrium are synapomorphies for Theridiinae and a clade within Pholcommatinae (Fig. 104). Absence of tibial trichobothria unites the pholcommatines *Carniella* (Fig. 50) and *Theonoe* (Figs. 96, 97). The loss of the prolateral trichobothrium is an unambiguous theridiine synapomorphy.



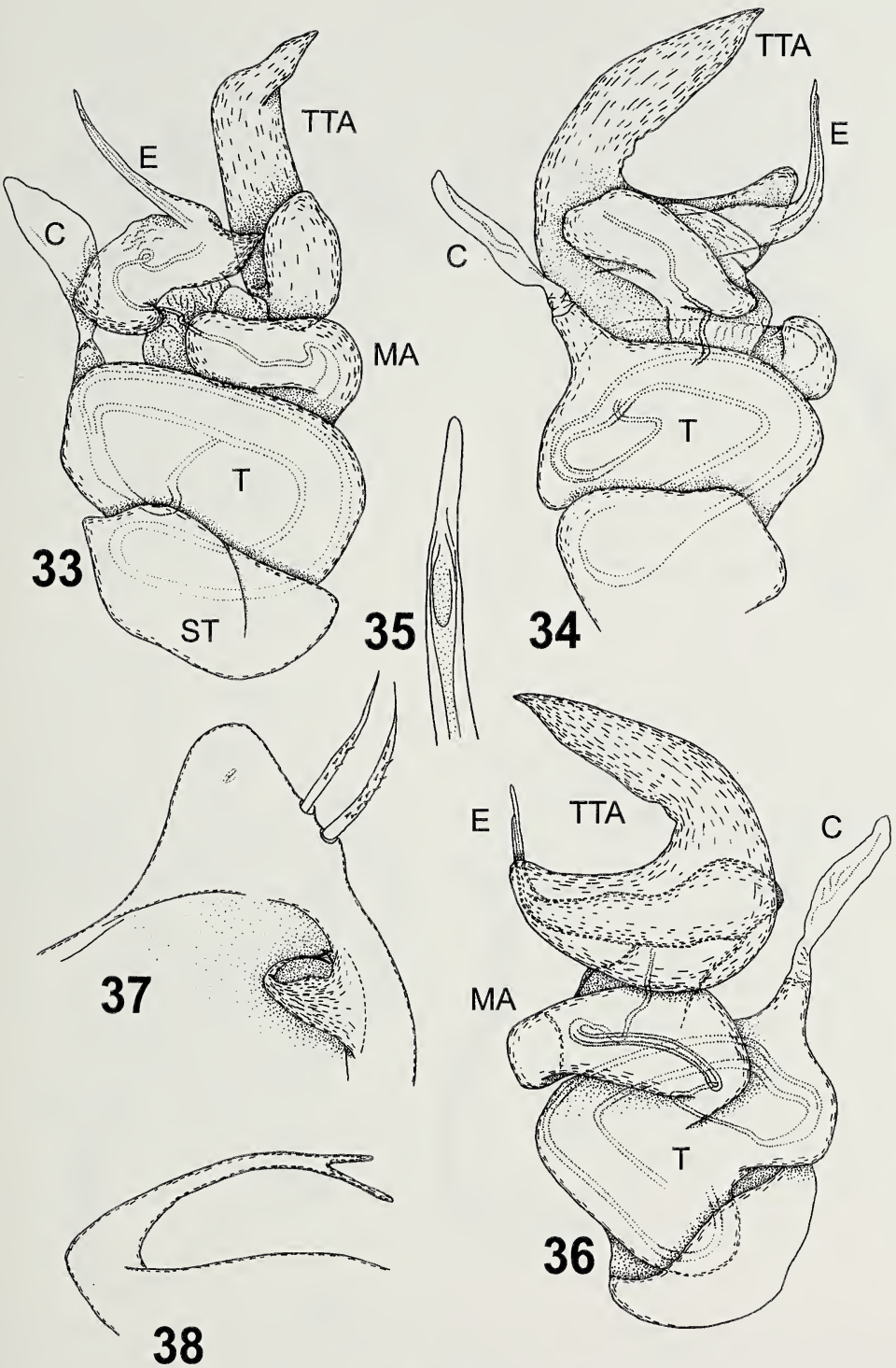
Figures 17–23.—17–19, *Steatoda americana* (Emerton 1882). 17, male palp ventral expanded; 18, bulb dorsal, removed from cymbium; 19, tibia dorsal view; 20, 21, *Steatoda albomaculata* (De Geer 1778), 20, palp ventral; 21, bulb removed from cymbium, dorsal view (redrawn from Knoflach 1996a); 22, *Episinus angulatus*, bulb removed from cymbium, ventral (redrawn from Knoflach 1993b), 23, mesal. 22, 23 reproduced from Agnarsson (2004) with permission from Blackwell Publishing.



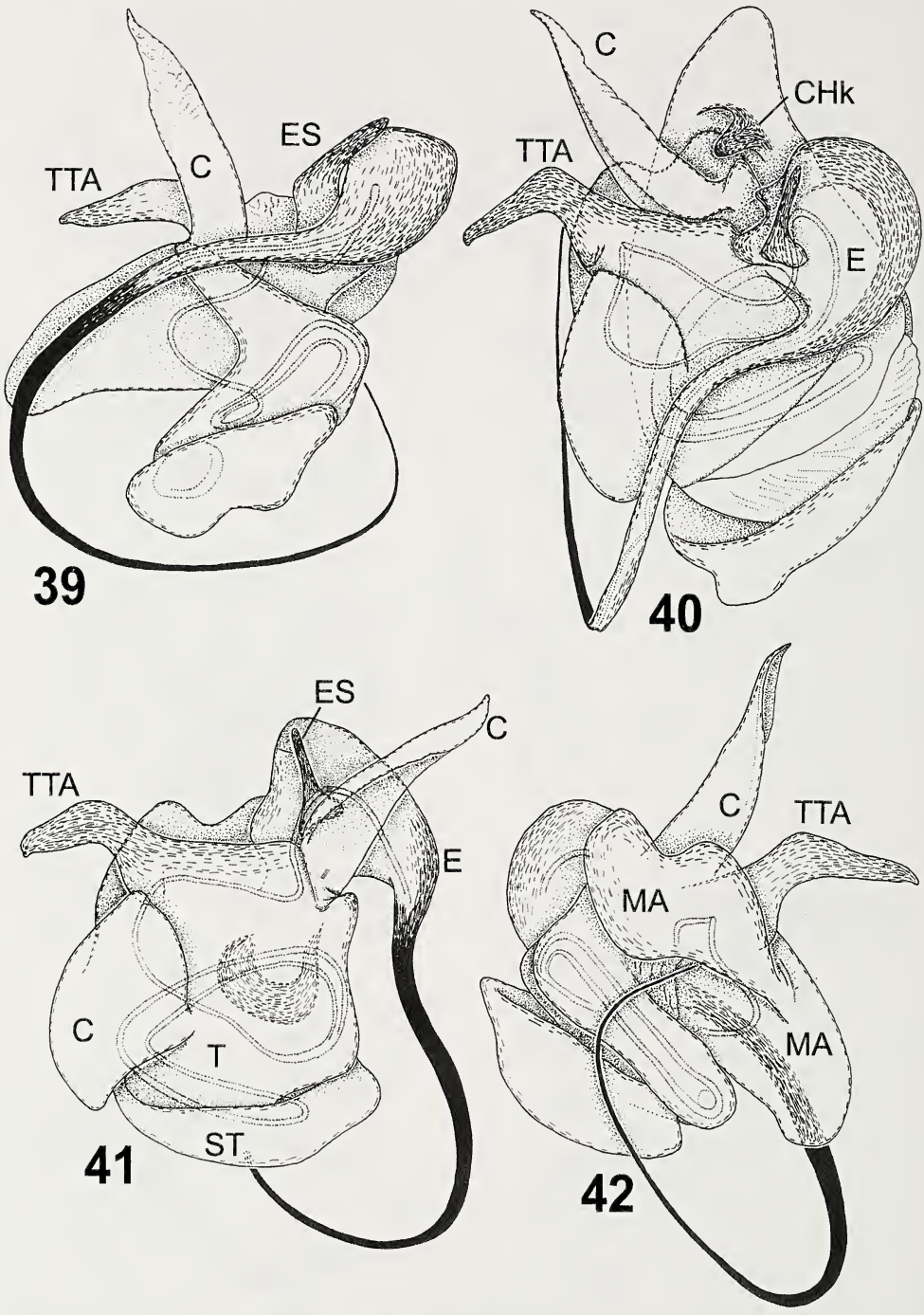
Figures 24–27.—*Episinus truncatus*. 24, bulb removed from cymbium, mesal; 25, ventral; 26, ectal; 27, dorsal; a third tegular sclerite ETS is present; note convoluted sperm duct within E and loop within MA; ventral tegulum conducts a part of the distal E.



Figures 28–32.—*Crustulina guttata*. 28, distal bulb, ectal; 29, palp expanded, ventral; 30, bulb removed from cymbium, apical; 31, cymbium, ventral; note large, mesal cymbial process; 32, bulb removed from cymbium, mesal-dorsal; embolus bears numerous processes.

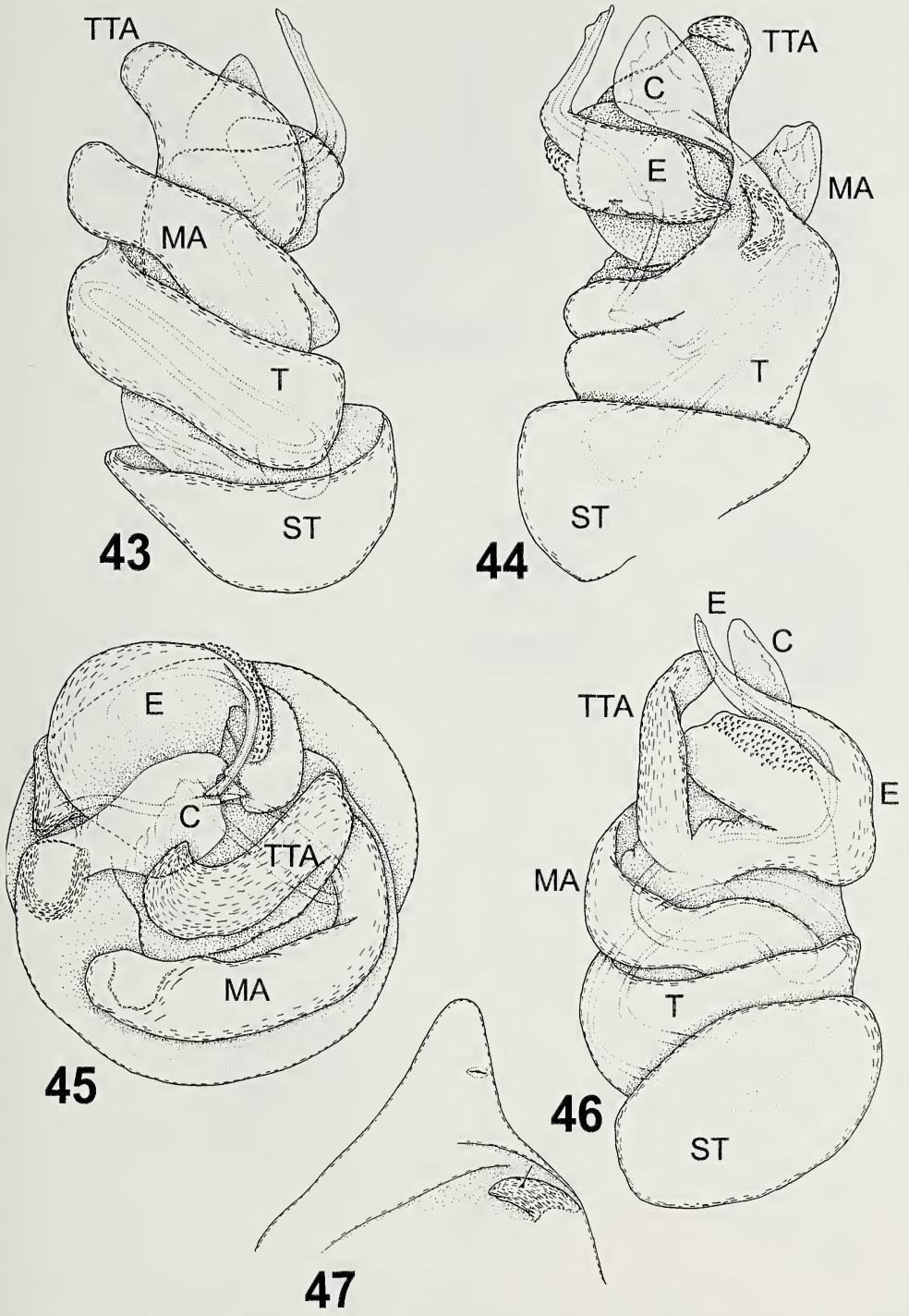


Figures 33–38.—*Steatoda bipunctata*. 33, bulb slightly expanded and removed from cymbium, ectal; 34, dorsal; 35, ventral; sperm duct narrows when leaving T, then widens within MA and again becomes constricted when passing to E; 36, tip of embolus; 37, tip of cymbium, ventral; 38, tip of cymbial hook.

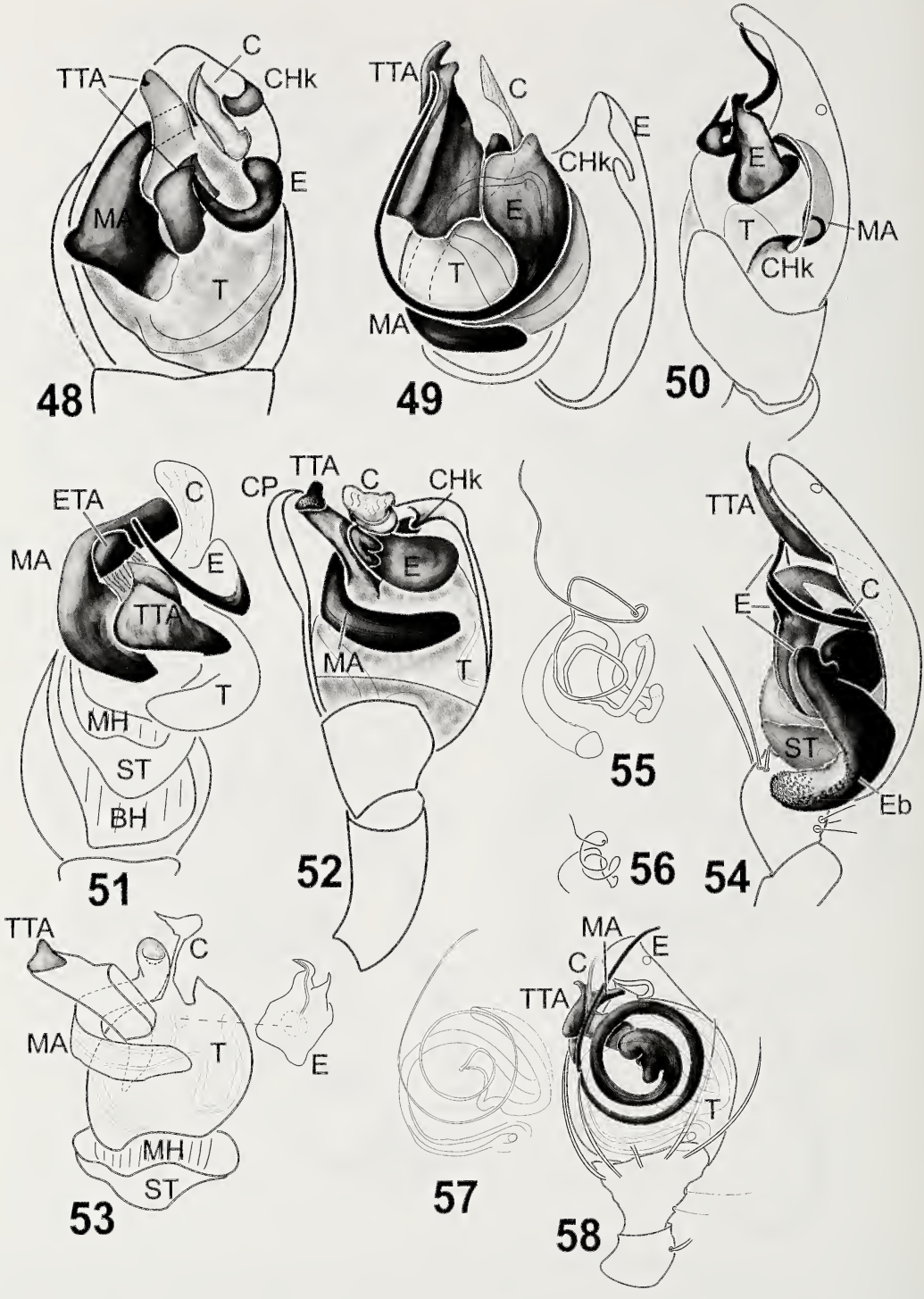


Figures 39–42.—*Steatoda phalerata* (Panzer 1801). 39, bulb removed from cymbium, ectal; 40, apical-ventral; 41, mesal; 42, ventral.

Cymbium: Comstock (1910) defined the cymbium as the basal portion of the tarsus expanded to protect and partially surround the genital bulb. In more recent use, the cymbium refers to the entire entelegyne palpal tarsus (e.g., see Grasshoff 1968, p. 38, 39, fig. 33; Ledoux & Canard 1981, p. 12), which we follow here. Theridiid cymbial shape varies and



Figures 43–47.—*Steatoda triangulosa*. 43, bulb removed from cymbium, mesal; 44, ectal-dorsal; 45, apical; 46, ventral; 47, tip of cymbium, ventral; note tegular pit in 44 close to conductor, into which an embolar process articulates.


C
TTA
CHk
E
MA
T
48
TTA
C
E
CHk
E
T
MA
49
E
T
MA
CHk
50
ETA
C
MA
E
TTA
T
MH
ST
BH
51
CP
TTA
C
CHk
E
MA
T
52
55
56
TTA
E
C
ST
Eb
54
TTA
C
MA
T
MH
ST
53
MA
E
C
TTA
T
57
58

is phylogenetically informative. A distal cymbial process is present in *Argyrodes* (Figs. 52, 60), *Crustulina* (Fig. 31), *Theonoe minutissima* (O.Pickard-Cambridge 1879) (Figs. 96, 97), and some *Achaearanea* species (Fig. 118). The cymbium extends well beyond the alveolus in some taxa (Figs. 14, 20, 50, 58, 96, 100, 105, 118, 193, 194), and a pronounced incision of the mesal margin of the cymbium characterizes most *Anelosimus* species (Fig. 62) (see Agnarsson 2005, 2006b; Agnarsson & Kuntner 2005; Agnarsson & Zhang 2006).

Paracymbium: The araneoid paracymbium is an "apophysis arising from the base of the cymbium" (Comstock 1910, p. 175). This retrolateral, proximal process on the cymbium (Figs. 190–192, 195–198) has long been recognized as an araneoid synapomorphy (Coddington 1986, 1990; Hormiga et al. 1995; Griswold et al. 1998). In some taxa, the paracymbium articulates to the cymbium (Linyphiidae, e.g., *Linyphia triangularis* (Clerck 1757), Fig. 196), whereas in others it is rigidly fixed (e.g., Araneidae, *Araneus diadematus* Clerck 1757).

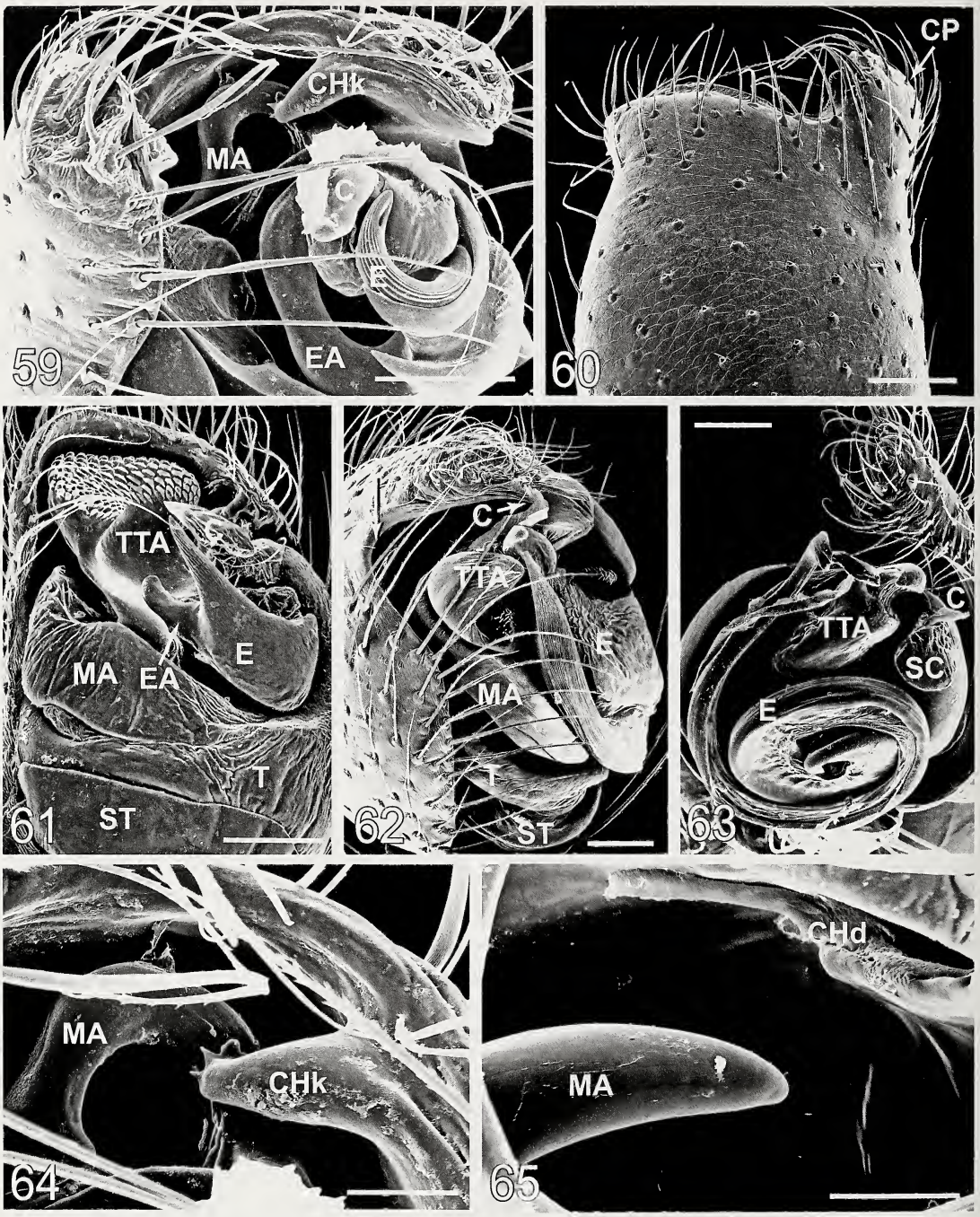
Theridiid cymbia usually lack a basal apophysis (Figs. 3, 4, but see 50), but have a distal apophysis that forms one half of the cymbium-bulb locking mechanism (Figs. 3, 13, 14, 17, 31, 37, 38, 47, 52, 59, 63, 68, 79, 80, 84, 88, 92, 97, 173–182), or a functionally identical cymbial pocket in the same place (Figs. 65, 105, 106, 113, 117, 124, 129, 142, 183–188).

Some authors regarded this process as the transformed homolog of the araneoid paracymbium (e.g., Levi & Levi 1962; Shear 1967; Wunderlich 1978; Heimer 1982, 1986; Heimer & Nentwig 1982; Coddington 1990; Forster et al. 1990; Knoflach 1996b; Levy 1998). Coddington (1990), for example, argued that "transformation" of one structure into another (one step) was, in general, a more parsimonious and efficient explanation than complete loss of a plesiomorphic feature and gain of an apomorphy (two steps). The theridiid locking mechanism between a tegular sclerite and the cymbium is an unusual example of a morphological function being assessable even in preserved material. Influenced by Heimer's work on the interaction between the araneoid paracymbium and tegular sclerites, and Bhatnagar & Remple's (1962) demonstration of profound morphological displacements of apophyses during palpal ontogeny, he proposed homology of the theridiid distal cymbial apophysis or notch with the araneoid paracymbium via transformation, rather than loss of the plesiomorphic paracymbium and gain of the novel locking mechanism. This view presumes both topological and detailed morphological change. It argues that both are cymbial apophyses that never cooccur (Agnarsson 2004), and that both may interact with palpal tegular sclerites of the palpal bulb during natural expansion of the palp (Heimer & Nentwig 1982; Huber 1993; Knoflach 1998, 2004; Agnarsson 2004; Knoflach & Pfaller 2004).

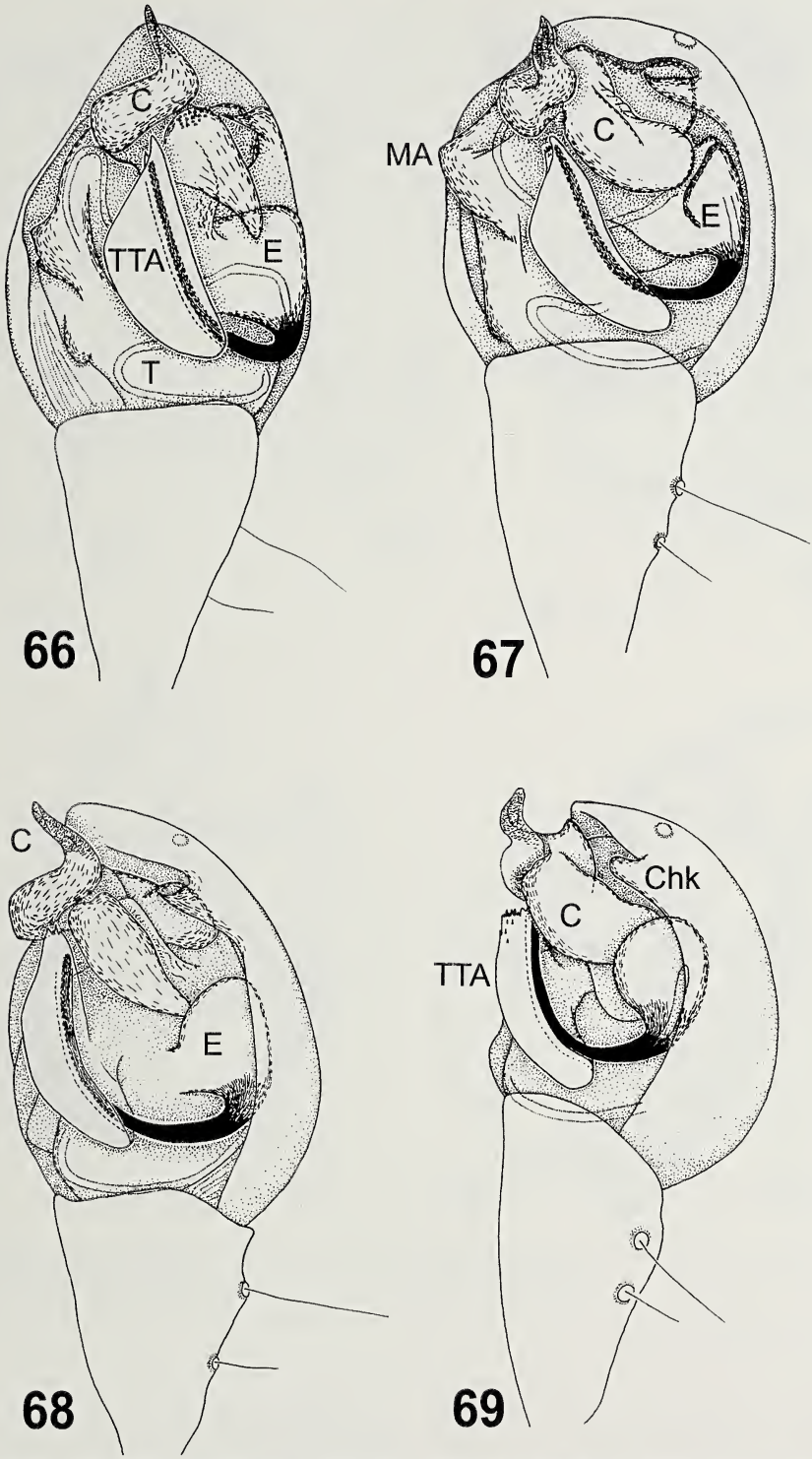
Others regard the araneoid paracymbium as lost and the theridiid feature as a novelty (Saaristo 1978; Griswold et al. 1998). Heimer (1982, 1986) and Heimer & Nentwig (1982, p. 289) envisioned the transformation of the paracymbium from the nesticid type: "In plesiomorphic Theridiidae (e.g., *Robertus* O.P.-C., 1879) the paracymbium is distally transferred but maintains its function. It conducts the median apophysis which glides at its ventral side and fixes it. A further reduction of paracymbium and median apophysis shortens the distance the median apophysis must be moved. Finally, the paracymbium is modified

←

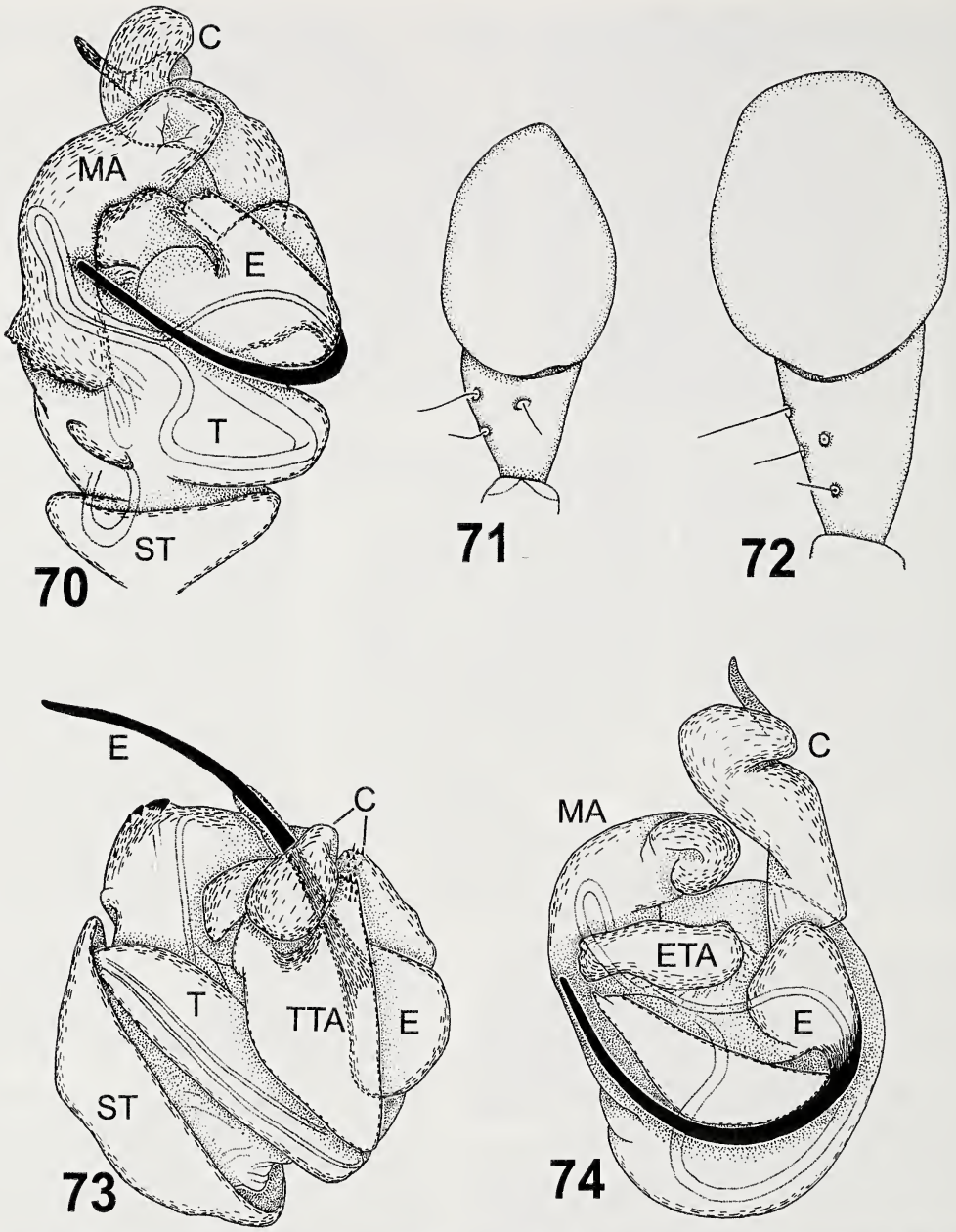
Figures 48–58.—48, *Enoplognatha gemina* Bosmans & van Keer 1999, palp ventral (redrawn from Levy 1998; sub *E. mandibularis* (Lucas 1846)); 49, *Phoroncidia americana* (Emerton 1882), palp loosened from cymbium, ectal (redrawn from Levi & Levi 1962); 50, *Carniella schwendingeri* Knoflach 1996, palp ectal (redrawn from Knoflach 1996b); 51, *Enoplognatha* sp. expanded; 52, 53, *Argyrodes argyrodes* (Walckenaer 1842) (redrawn from Saaristo 1978). 52, ventral; 53, schematic of bulb removed from cymbium and E removed from T; G-I, *Anelosimus vittatus* (C.L. Koch 1836). 54, palp ectal; 55, sperm duct trajectory, see Agnarsson (2004) for nomenclature and discussion; 56, schematic look at duct loops; 57, 58, *Anelosimus* sp. 57, palp ventral; 58, sperm duct trajectory. 50, 57, 58 reproduced from Agnarsson (2004) with permission from Blackwell Publishing.



Figures 59–65.—59, 60, 64 *Argyrodes elevatus* Taczanowski 1873 male palp. 59, apical; 60, dorsal; 61, *Neosphintharus trigonum* (Hentz 1850), palp ventral; 62, 63, *Anelosimus eximius*. 62, apical view of mesal side, note strong cymbial incision, an *Anelosimus* synapomorphy; 63, apical view of ventral side, showing clearly the C coming out of the base of the SC; 64, hooked bulb to cymbium lock system; 65, *Anelosimus* sp. hooded BC-lock system. Rather than representing independent lines of evolution the hooded system is derived from the hooked one (see Fig. 201). Scale bars: 59–63 = 100 μm; 64, 65 = 50 μm.

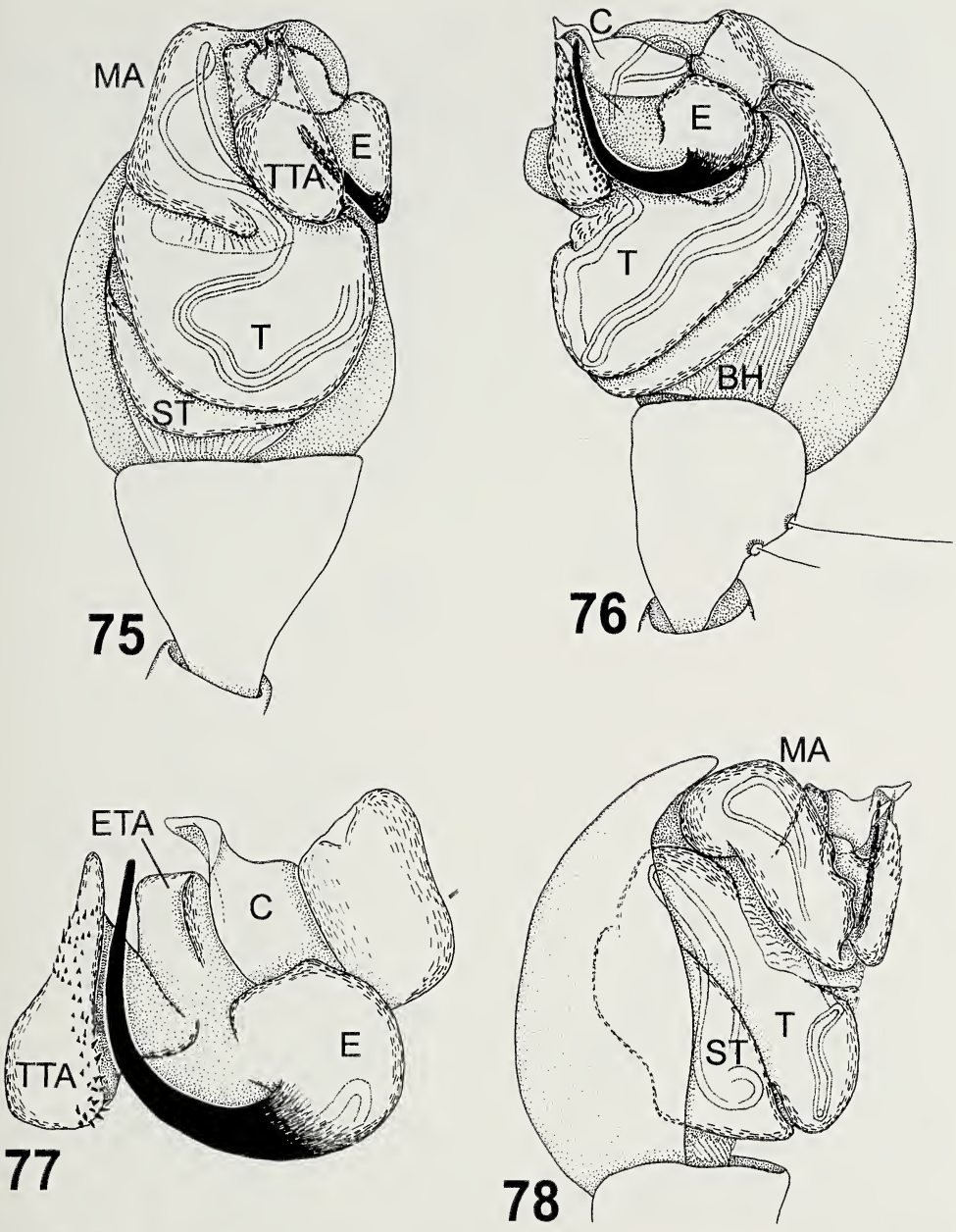


Figures 66–69.—66, 68, *Enoplognatha ovata*. 66, ventral; 68, ectal; 67, 69, *E. latimana*. 67, ventral; 69 ectal; note TTA conducting E in the unexpanded palp.



Figures 70–74.—70, 71, 73, 74, *Enoplognatha ovata*. 70, 73, 74, bulb slightly expanded and removed from cymbium; 70, ventral; 71, cymbium and tibia, dorsal; 72, *Enoplognatha latimana*, cymbium and tibia dorsal; 73, "naturally" expanded, ventral-mesal, note embolus shifted into furrow of conductor; 74, apical, note third tegular sclerite.

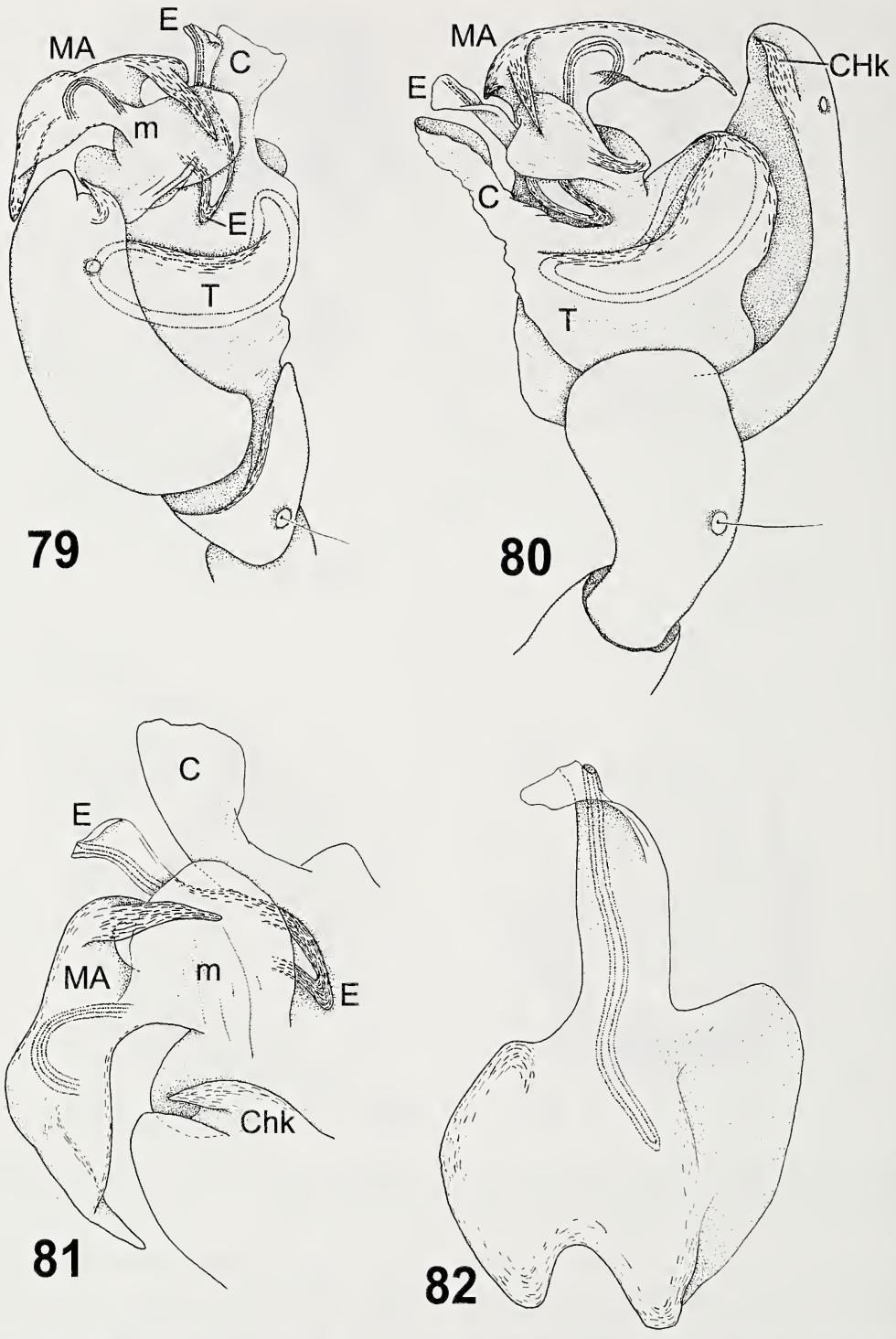
up to a degree in which no free sclerite of the cymbium can be found. Now the median apophysis is fastened in pocket-like deepenings at the inside of the cymbium during the fixation of the palp. . . . Genera with this modified palpal mechanism are e.g. *Episinus* Latreille, 1809, *Theridion* Walckenaer, 1805, and *Dipoena* Thorell, 1869." The view that the theridiid cymbial hook is not homologous to the paracymbium, on the other hand, is based on difference in position, shape, and function. Saaristo (1978, p. 112) criticized the hypothesis on topological grounds: "This [homology of the theridiid cymbial process with



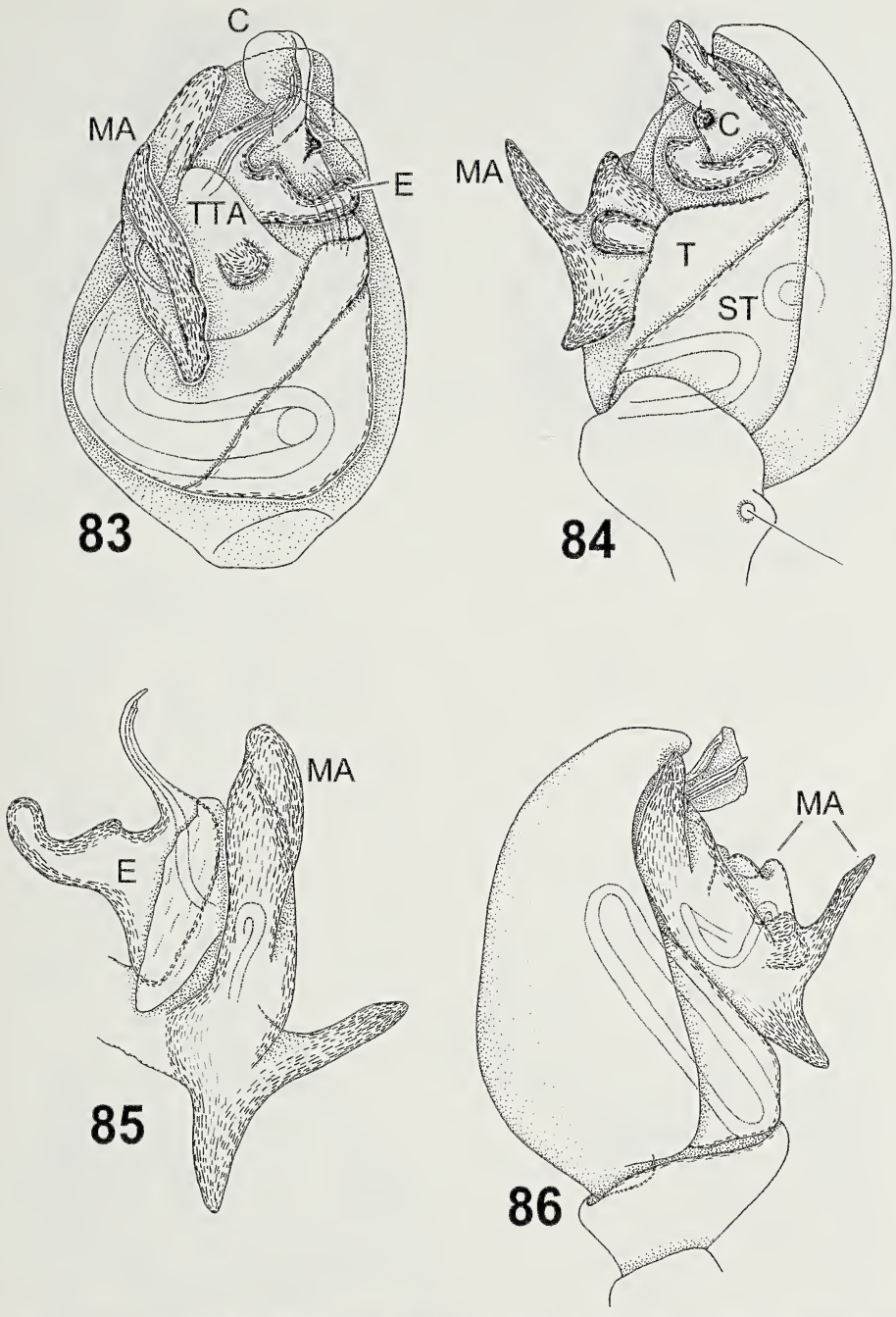
Figures 75–78.—*Enoplognatha thoracica*. 75, 76, 78, male palp slightly expanded, ventral, ectal, mesal. 77, distal palpal sclerites, ectal.

the paracymbium] must be an error, because the cymbial hook lies near the tarsal organ and distally to it, whereas in araneids and linyphiids the paracymbium is far from the tarsal organ and proximal to it." Although the theridiid process is usually very different from araneoid paracymbia, in others its position and shape are somewhat similar (compare *Carniella* (Fig. 50) to the synotaxid *Synotaxus waiwai* Agnarsson 2003 (Fig. 191)). Saaristo's (1978) criterion of topological relation to the tarsal organ does not, furthermore, apply to all taxa. The paracymbial hook of *Carniella*, for example, is proximal to the tarsal organ and far away from it (Fig. 50).

Phylogenetic analysis treating the theridiid hook as a transformed araneoid paracymbium or as different, independent characters both re-

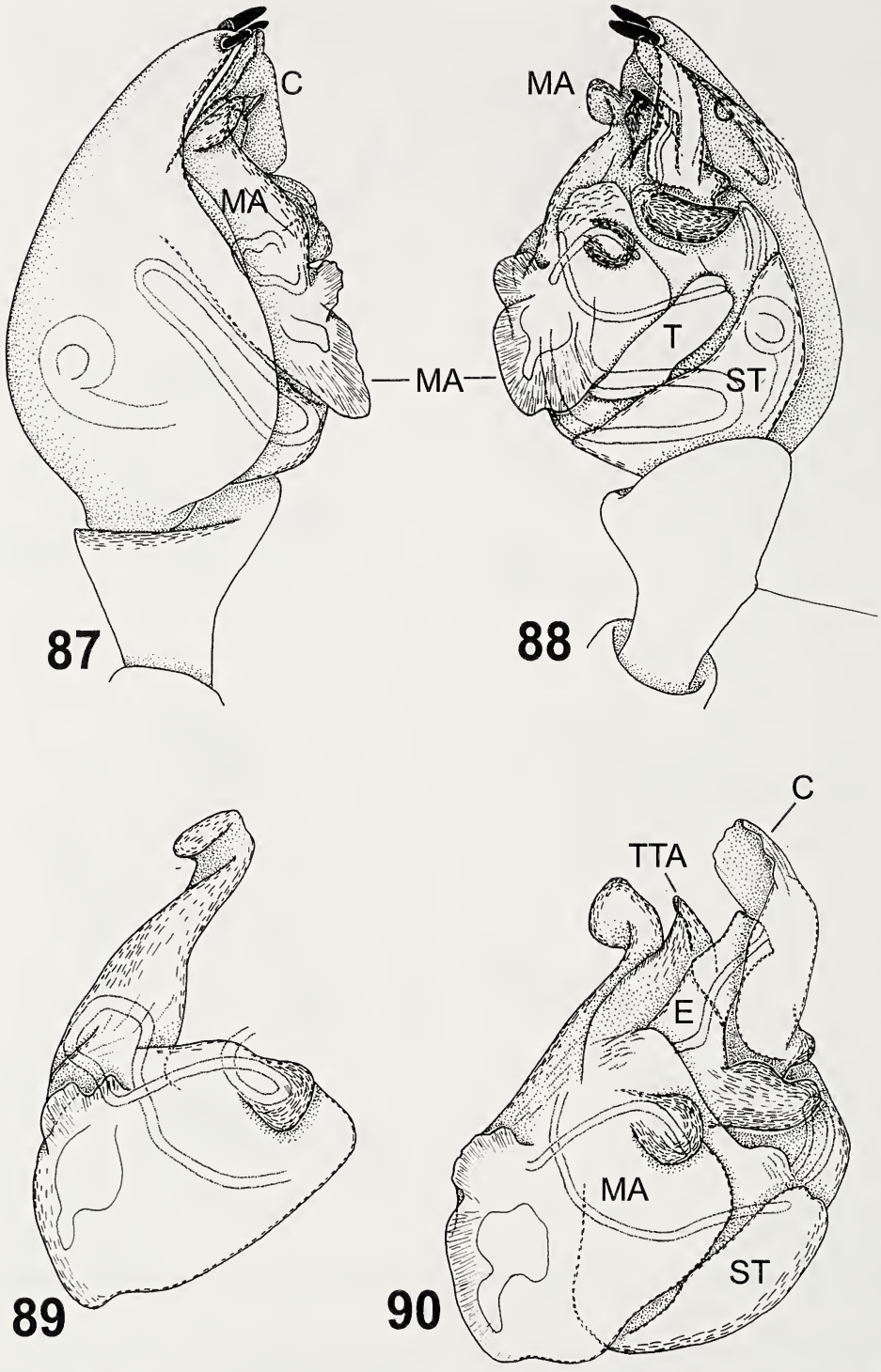


Figures 79–82.—*Robertus neglectus*. 79, 80, male palp expanded, ectal-apical, ectal; 81, distal sclerites, ectal; only one tegular apophysis present; 82, embolus.

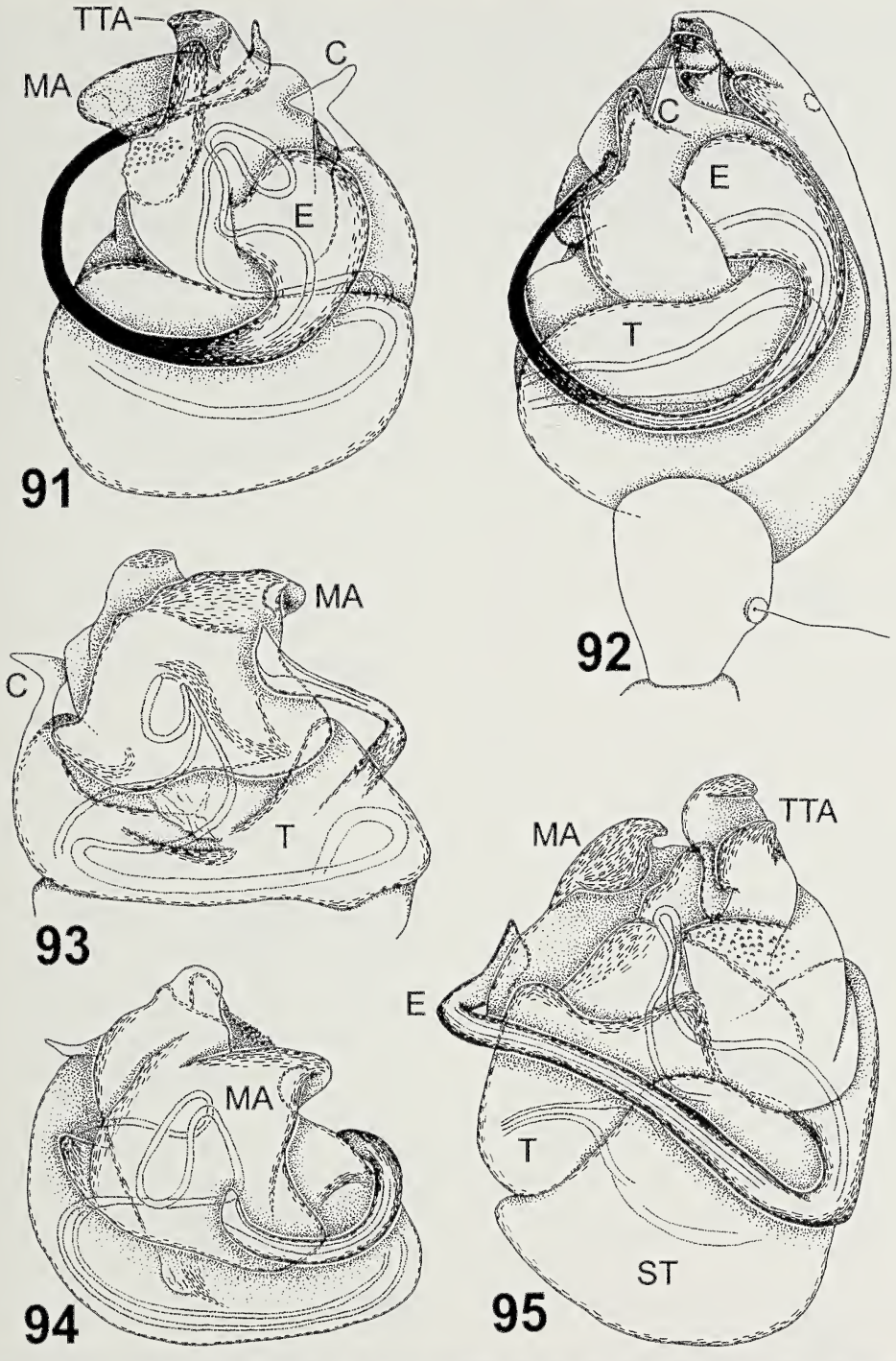


Figures 83–86.—*Robertus scoticus*. 83–85, male palp, ventral, ectal, mesal; 86, distal palpal sclerites.

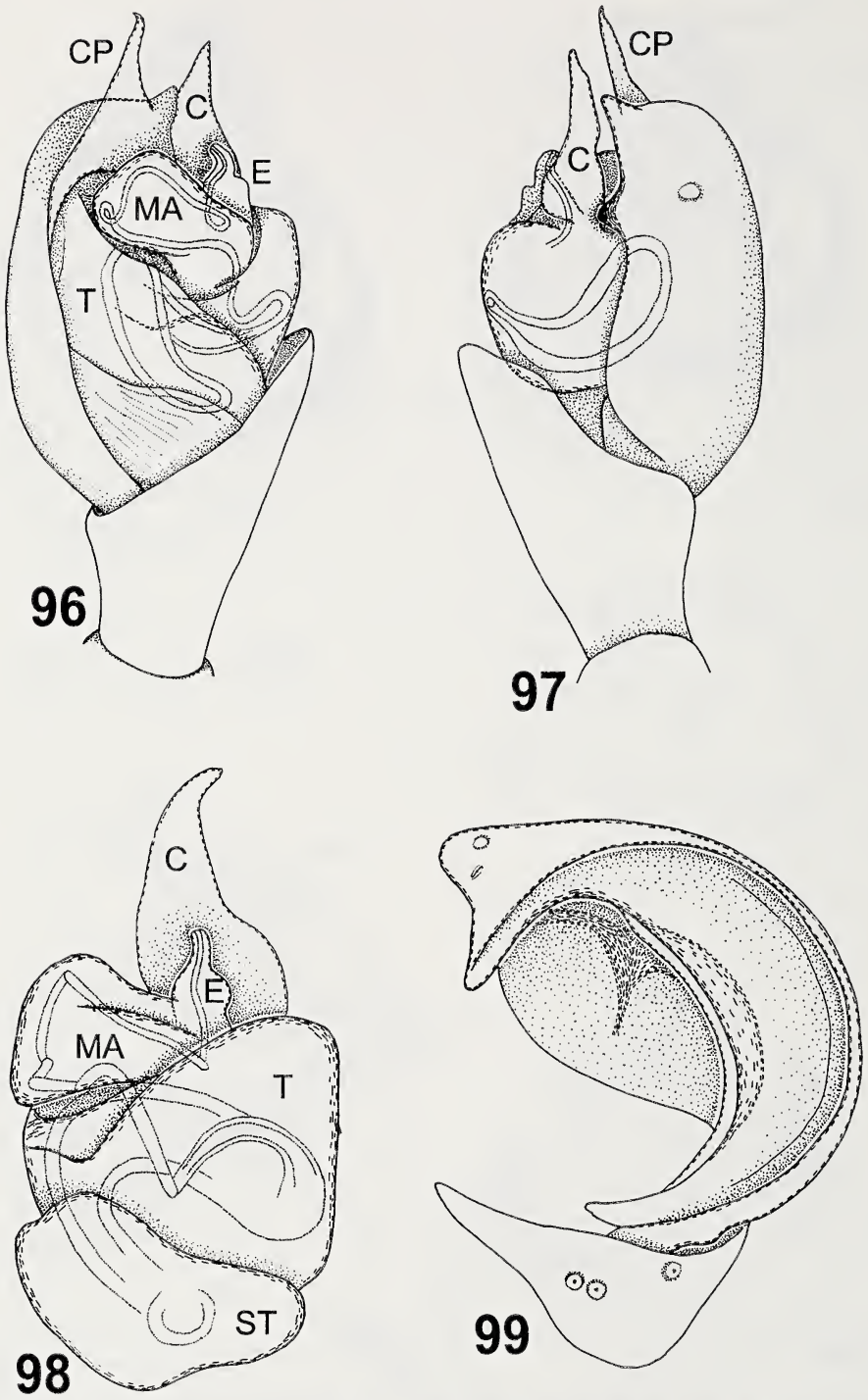
sult in same topology (Figs. 2, 201; Agnarsson 2004), which refutes Heimer & Nentwig's speculation about the primitive theridiid condition (as well as the basal position of *Robertus*). Instead, the primitive condition is a knob distally inside the cymbium locking the MA "securely" (e.g., *Dipoena*, Fig. 176). The more paracymbium-like hook on the cymbial margin (e.g., in *Carniella*, Fig. 50) and *Robertus* (Fig. 182) is derived. *Robertus* is a pholcommatine, for which the rather loose connection between the cymbial hook and the MA is synapomorphic. The pronounced basal paracymbium in the outgroups (e.g., *Pimoa*,



Figures 87–90.—*Robertus ungulatus*. 87, 88, male palp, mesal, ventral; 89, median apophysis; 90, distal palpal sclerites; two tegular apophyses present.



Figures 91–95.—*Pholcomma gibbum*. 91, bulb slightly expanded and removed from cymbium, ectal; 92, ventral; conductor hyaline, slender and apparently without guiding function, whereas MA and TTA show a broad groove, which presumably supports the embolus; 93–95, distal bulb, removed from cymbium, 93, mesal-dorsal; 94, apical-dorsal; 95, caudal-ventral; note loop of sperm duct within MA.



Figures 96–99.—*Theonoe minutissima*. 96, 97 male palp, ventral-mesal, ectal; 98, bulb, ventral; note distal process of cymbium and distinct constriction of sperm duct within T; embolus and median apophysis probably fused; 99, *Kochiura aulica*, cymbium ectal, largely excavated.

Fig. 195; *Linyphia*, Fig. 196; *Synotaxus*, Fig. 197; and *Nesticus*, Fig. 198) and the plesiomorphic distal cymbial knob in hadrotarsids and basal theridiids, such as latrodectines (Fig. 17), have little in common beyond being parts of the cymbium. Because the homology hypothesis fails the criteria of position, special similarity, and function, the gain-loss interpretation receives support using our method, and is preferred on the phylogeny (Fig. 201). The theridiid cymbial hook is unique to theridiids, and the araneoid PC has been lost in hadrotarsids and theridiids (Saaristo 1978; Griswold et al. 1998; Agnarsson 2004). Of course, transformation is currently an "elastic" concept; any amount of change can be packed into a single cladistic step.

Griswold et al. (1998) and Agnarsson (2004) used the terms "theridiid cymbial hook" and "theridiid cymbial hood," which we follow. Other names (apart from PC) applied to this structure include "cymbial tooth" (Bhatnagar & Rempel 1962), "cymbial pit" (Saaristo 1978), and "distal hook" (Griswold et al. 1998).

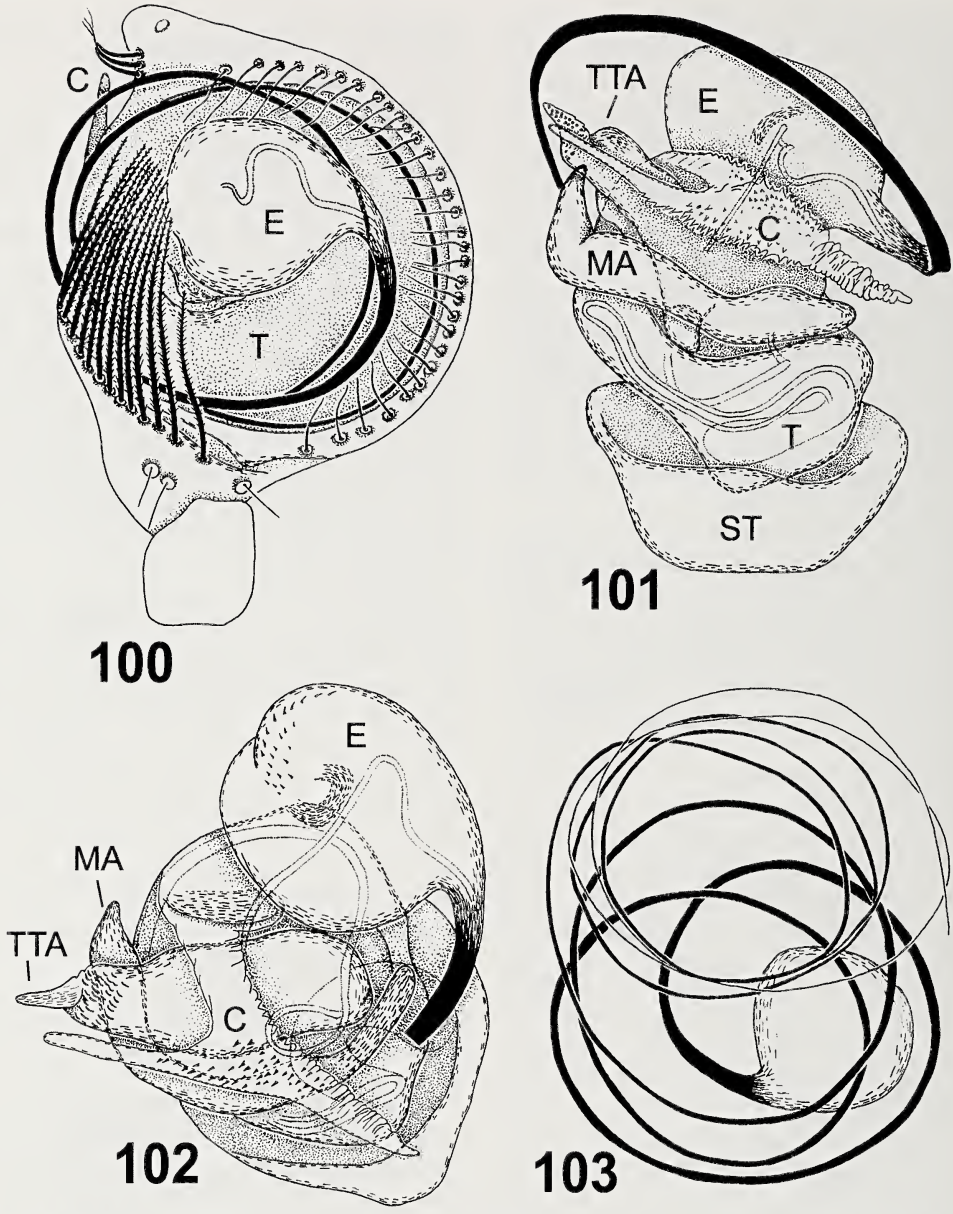
Theridiid bulb-cymbium lock mechanism: The theridiid BC-lock mechanism is unique to and universal among theridiids (highly modified in *Paratheridula* and *Theridula*). In it the median apophysis (usually the sclerite most flexibly attached to the tegulum) interacts with the theridiid cymbial process to lock the bulb in the palp (Levi 1961) as it expands (Knoflach 1998; Knoflach & van Harten 2000), or even in the unexpanded palp (Coddington 1990). It takes two forms: (1) Theridiid "cymbial hook"; and (2) "Theridiid cymbial hood." The hook (Fig. 3) is the primitive condition, and it engages a distal pit on the median apophysis (Figs. 3, 59, 64). Alternatively, the median apophysis may simply lodge under the process and the MA distal pit is sometimes indistinct or absent. In the theridiid cymbial hood condition, the cymbial hook has apparently been submerged into the cymbium (Figs. 65, 129). Saaristo (1978, p. 112) considered the cymbial hook and hood to be unrelated and independent, making a clear distinction between the two: "Levi (1961) did not realize that this coupling of bulbus and cymbium is accomplished in two entirely different ways, which possibly represent two main evolutionary lines in Theridiidae. They are here referred to as locking systems A and B." Phylogenetic analysis, in addition to topology, rejects Saaristo's distinction (Fig. 201); the hook is plesiomorphic with respect to the hood. The same conclusion has been reached by Heimer (1982), Forster et al. (1990), and most recently, by Saaristo (2006) himself. In contrast to the situation with the araneoid paracymbium, the hook and hood are topologically, morphologically, and functionally similar. The transformation required is plausible, in part due to extant intermediates, such as *Anelosimus*, where the hood is formed on the cymbial margin by thin cuticle. In other cladistically distal theridiids this hood is further away from the margin, but of the same form. A gain-loss scenario requires the simultaneous loss of the hook and gain of the hood while they are topologically identical and serve the same function.

Some hadrotarsids, latrodectines, and spintharines have a "hood-like" groove beneath the cymbial hook (see also discussion of Forster et al. 1990 about *Thwaitesia*). The presence of a hook and hood simultaneously might seem to fail Patterson's (1982) test of conjunction. However, the phylogeny rejects the homology of this groove to the hood because it is absent in the "hood lock clade" sister taxon. The conjunction test, as pointed out by de Pinna (1991), actually indicates homoplasy rather than decisively refuting homology. That homoplasy is here more parsimoniously attributed to the "sub hook groove" being a unique feature, not homologous to the hood found in the hood lock clade (Agnarsson 2004).

When present, the MA interacts with the cymbium via the locking mechanism. In a few taxa the MA has either been lost or has fused with the embolus (Figs. 96–98, 109, 119, 121–123, 125–128). In this case the basal portion of the embolus (or the fused embolus-median apophysis complex) assumes this function.

Alveolus: The alveolus is the cymbial cavity in which the genital bulb rests (Figs. 3, 17). Plesiomorphically the alveolus is usually central or ectal in the cymbium. Its placement flush on the mesal side of the cymbium is synapomorphic for theridiids (Fig. 1; see also Agnarsson 2004, fig. 92).

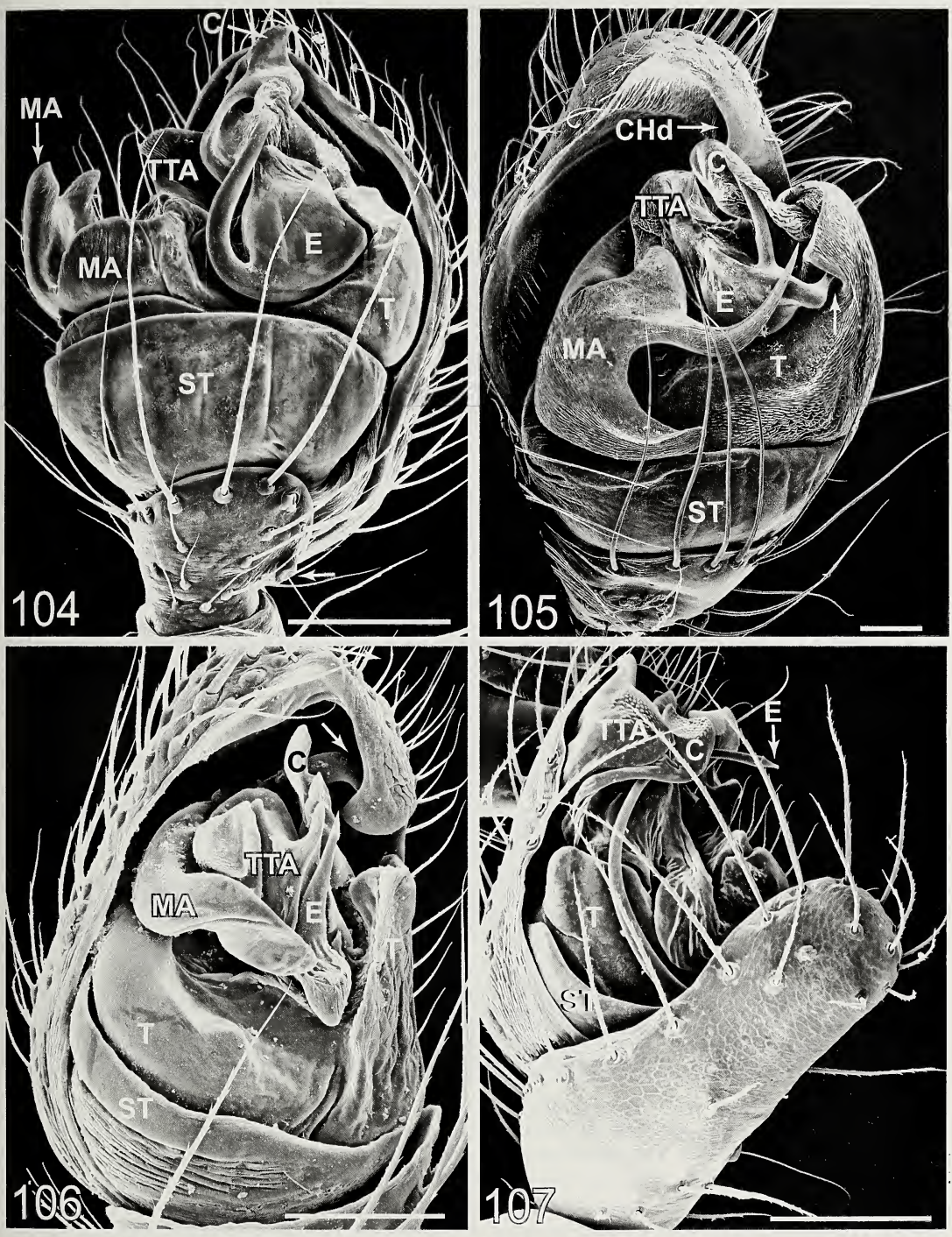
Basal haematodocha: The membranous basal haematodocha connects the alveolus to the subtegulum (Figs. 3, 17, 29, 51, 76, 120).



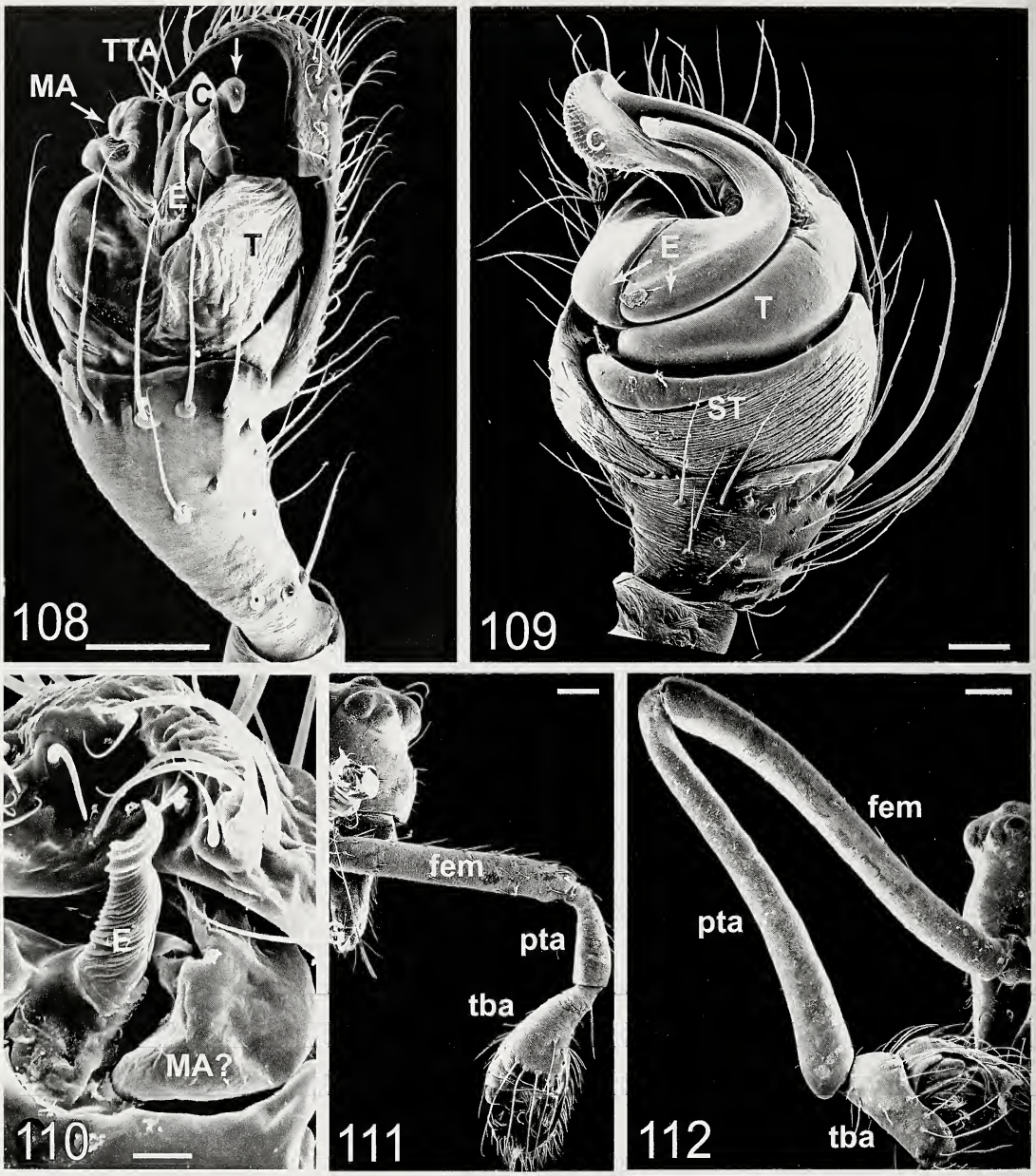
Figures 100–103.—*Kochiura aulica.* 100, male palp, ectal; 101, 102, bulb expanded, dorsal, apical; modified tibial and cymbial setae support the embolus, whereas conductor is comparatively inconspicuous; 103, embolus separated from palp, coiling up in spirals; its remarkable length measures three times the male's total body length.

It inflates during copulation and artificial expansion of the palp.

Petiole: In distant outgroups, the petiole is a large and prominent sclerite in the wall of the basal hematodocha (e.g., lycosoids, Sierwald 1990). In araneoids, it is usually small or even absent. Bhatnagar & Rempel (1962, p. 476) described a petiole in *Latrodectus* as "A distinct sclerite lodged within the hematodocha on the ectal side of the genital-bulb . . . this sclerite has no articulation with the subtegulum. . . . In the expanded bulb, the petiole appears as an extended, flat, heavy sclerite." According to our observations, theridiids generally lack a petiole, although a small, indistinct, and lightly sclerotized region



Figures 104–107.—Male palps ventral; 104, *Theridion varians* Hahn 1833, note furcated MA, typical of *Theridion* and relatives; 105, *Theridion frondeum* Hentz 1850, note also tegular pit (arrow) involved in a lock mechanism with E via a embolic apophysis (see also Avilés et al. (2006, fig. 4) for SEM photographs of the closely related *T. nigroannulatum*); 106, *Ameridion* sp. like all theridiines with a hooded BC-lock system (arrow); 107, *Thymoites* nr. *prolatus* (Levi 1959), the grossly enlarged tibial rim is shared with some other *Thymoites*. All scale bars = 100 μm.



Figures 108–112.—108, *Ameridion* sp., arrow indicates tip of MA; 109, *Achaearanea tepidariorum*, like most other *Achaearanea* the TTA has been lost. Note the seam in the embolus (or possibly the fusing point between the E and MA, see discussion); 110, *Theridula opulenta* (Walckenaer 1842), among the simplest palps of theridiids, the TTA has been lost (independently from the loss in *Achaearanea*, see Fig. 201), the C is also absent, while a membranous connection exists between the T distally and cymbium. This membrane is possibly a homolog of the MA; 111, *Ameridion* sp., note elongated palpal femur; 112, *Thymoites* nr. *prolatus*, here not only the femur, but also the palpal patella is grossly elongated. Scale bars: 108, 109, 111, 112 =100 μm; 110 = 10 μm.

within the basal haematodocha in some taxa may be homologous to the petiole. At the very least, the sclerite is difficult to see and rarely mentioned or drawn in species descriptions of theridiids and other araneoids. Its distribution remains little known.

Subtegulum: The subtegulum is the ring-like sclerite that forms the base of the bulb, and connects to the alveolus via the basal haematodocha and to the tegulum via the middle haematodocha. It contains the fundus of the sperm duct (Fig. 3).

Sperm duct: Comstock labels the sperm duct "receptaculum seminis," a term more usually reserved for the female sperm storage organ; we prefer the term sperm duct or spermophore. The sperm duct consists of three distinct parts: first, the proximal end of it, the fundus, is enlarged so as to form a pouch; second, the intermediate portion, the reservoir, is a large coiled tube occupying the middle division of the genital-bulb; third, the terminal portion constitutes the ejaculatory duct; this is the slender tube traversing the apical division of the bulb (Comstock 1910, p. 163). In theridiids, the fundus is normally adjacent or fused to the subtegular wall. The so-called reservoir (a functional term that may not be appropriate since the fundus may be the main reservoir) spirals and sometimes switchbacks through the tegulum. The ejaculatory duct occupies the length of the embolus and opens at its tip.

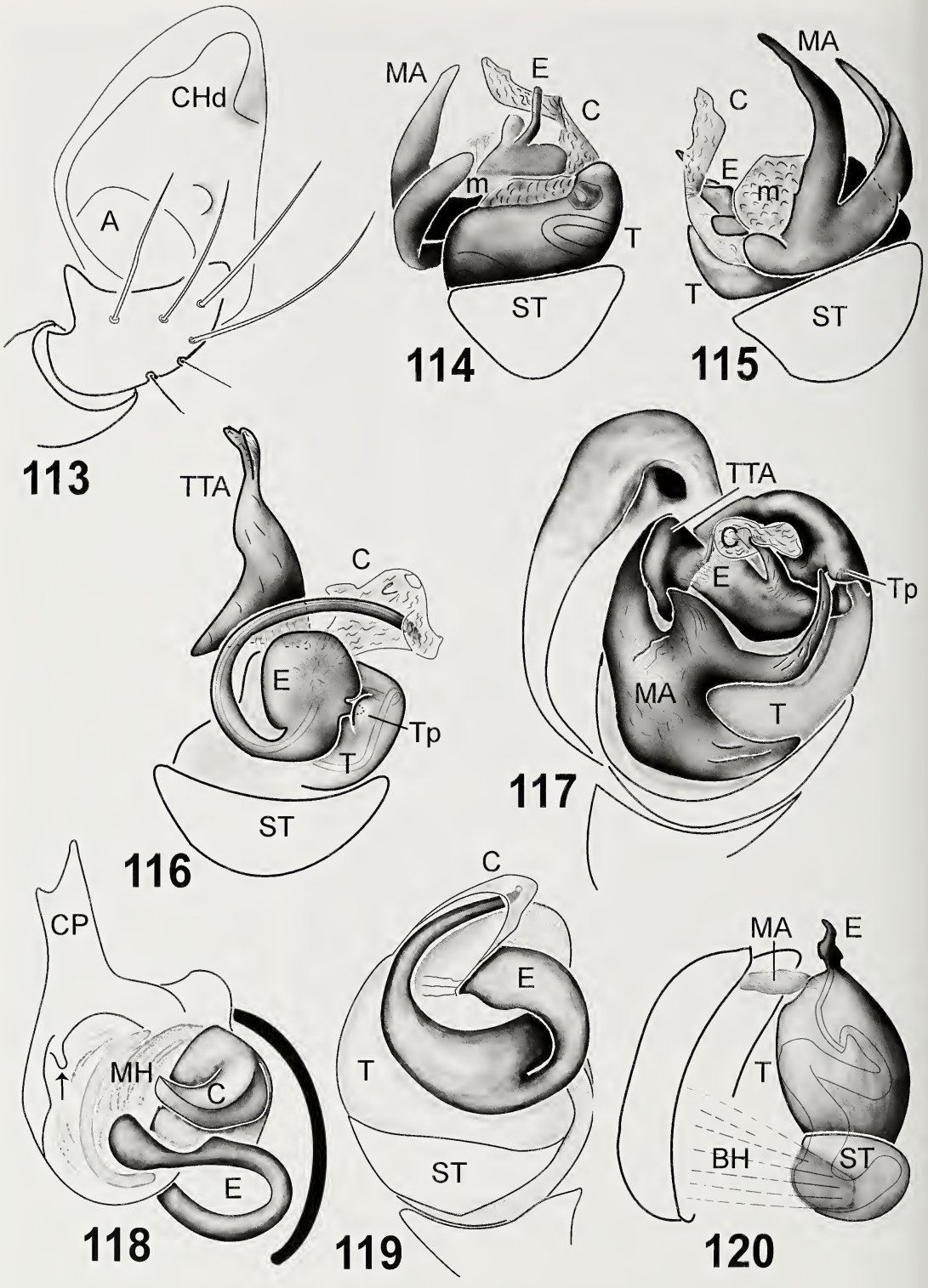
Sperm duct trajectory: Primitively the sperm duct spirals simply in the tegulum (Comstock 1910; Coddington 1990). In many araneoids, however, the sperm duct trajectory (hereafter referred to as SDT) is moderately complex to very complex with numerous loops and switchbacks (Figs. 55–58; also 4–11, 24–27, 29, 33, 34, 36, 39–46, 96, 101, 102, 121, 123, 125–128, 134–138, 145–148, 165–168, 169). Coddington (1986) homologized individual loops and switchbacks in theridiosomatids and suggested the SDT could be an important new character system in spider systematics. This system was used by Agnarsson (2004); however, most other recent phylogenetic analyses of spiders have not looked at STD in detail. Hormiga et al. (1995), for example, identified the presence of switchbacks as a synapomorphy of higher araneoids, but did not attempt to make further specific homology statements. Griswold (2001) describes the variation found within cyatholipids, but does not include it in his phylogenetic analysis.

The SDT varies greatly between theridiid genera, but within genera and species is often quite constant. At least some switchbacks and loops are consistent enough to homologize across theridiid genera (Agnarsson 2004). In some cases, differences in the sperm duct trajectory even define species groups within a genus (Agnarsson & Kuntner 2005).

Tegulum: The tegulum forms the middle part of the bulb, contains most, or all, of the sperm duct reservoir, and bears all remaining palpal sclerites. Some sclerites are fused to the tegulum and some articulate to it via a membrane. In some species, a tegular pit (Fig. 3) is present into which the base of the embolus, or an embolic apophysis, fits. This constitutes another locking mechanism that presumably also affects palpal expansion.

Embolus-tegulum membrane: The theridiid embolus typically articulates to the tegulum via a narrow membrane, which is traversed by the sperm duct on its way to the embolus tip. This membrane has been called the distal haematodocha, but that term was originally applied to one between the embolus and the radix and/or stipes in some araneids (Comstock 1910, p. 177; Hormiga et al. 1995, character 36; Scharff & Coddington 1997). The embolus-tegulum membrane is apparently homologous in tetragnathids, nephilids, and araneids, but is independently derived in linyphiids. There it is quite different because the "column" separates the entire embolic division from the tegulum (Hormiga et al. 1995; Griswold et al. 1998). Despite the discovery of an embolus-tegulum membrane in theridiids (previously coded as absent [Hormiga et al. 1995; Griswold et al. 1998]), these three similar features apparently all arose independently. The name theridioid embolus-tegulum membrane therefore seems appropriate. Apparently the same membrane usually connects to the MA and TTA (Fig. 17). However, the distal membranes are hard to interpret and apparently the MA and TTA are either connected to the tegulum via their own membranes, or they are closely associated and share a membrane, which then broadly attaches to the tegulum (Figs. 163, 164).

Median apophysis: Comstock (1910, p. 172) described and named the MA: "arising


CHd
A
113
MA
E
C
m
T
ST
114
MA
C
E
m
T
ST
115
TTA
C
E
Tp
T
ST
116
TTA
C
E
Tp
MA
T
117
CP
MH
C
E
118
C
E
T
ST
119
MA
E
T
BH
ST
120

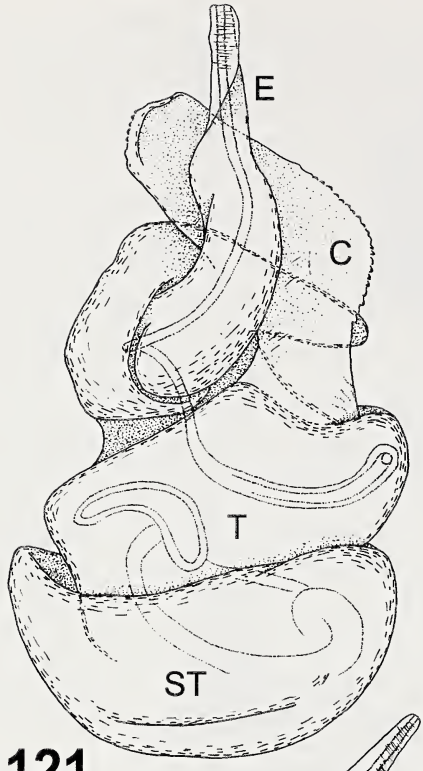
within the distal margin of the tegulum there is an appendage. . . . this is the *median apophysis.* In many spiders this appendage is very conspicuous and to it have been applied several names. In fact in several instances a writer has applied different names to this part in his description of different genera." The situation in theridiids has been no less confused than Comstock described for spiders in general. Being an "appendage of the tegulum" does not set it clearly aside from other sclerites that arise from the tegulum, and theridiids usually have three besides the embolus. Other definitions of the MA broadly agree that it is a distinct mesal process of the tegulum, typically, but not invariably, connected flexibly to the tegulum via a membrane (Lehtinen 1967; Shear 1967; Coddington 1986; Sierwald 1990; Griswold 1993). It is generally true in spiders that if a bulb has two apophyses, the "conductor" is usually close to the E, and if it has only one tegular apophysis, it is also usually close to the E. For that reason, Griswold et al. (1998) made the heuristic decision to consider the MA as "the second tegular process in araneoids, once the conductor has been accounted for." Of course, if taxa lose the C rather than the MA, such an approach will fail. Hormiga (1994a) considered a small tegular knob of pimoids (see Fig. 195) the homolog of the araneid MA, based on similarity criteria, but a similar knob in cyatholipids has been interpreted as being the C (Griswold 2001), where the MA is presumed absent (following the "conductor first" rule). Linyphiids are considered to lack both MA and C, yet many linyphiid tegula bear various lobes (Fig. 196) that have received new names (Hormiga 2000). Examples include the "mynoglenine tegular process" found in the mynoglenine linyphiid genera *Haplinis* and *Novafroneta* (Hormiga 1994b, fig. 5B) and the suprategulum present in most linyphiids. An effort to deflate linyphiid sclerite names has been made by Miller (2007, and Miller & Hormiga 2004).

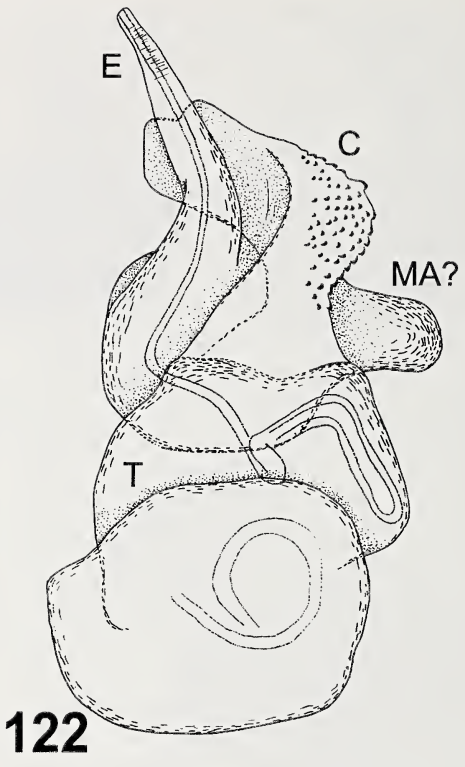
Correctly identifying non-embolic tegular apophyses is daunting, especially the MA versus conductor if only one is present. The MA and C seem to be intimately associated in their ontogeny (Bhatnagar & Rempel 1962; Coddington 1990). Its topology is fairly consistent: the MA is usually positioned on the mesal side of the tegulum (often retrolaterally) towards the center or the base in the tegulum, further away from the embolus than is the conductor. The MA is generally the sclerite that interacts with the araneoid paracymbium. This description conforms closely to most of Comstock's (1910) use of MA. Secondly, our emphasis here is to provide internally consistent terminology (across theridiids), so that if what we call a MA in theridiids turns out to be something else, at least that nomenclatural change should apply to all sclerites so labeled here; the homology of this tegular apophysis among theridiids themselves is strongly corroborated.

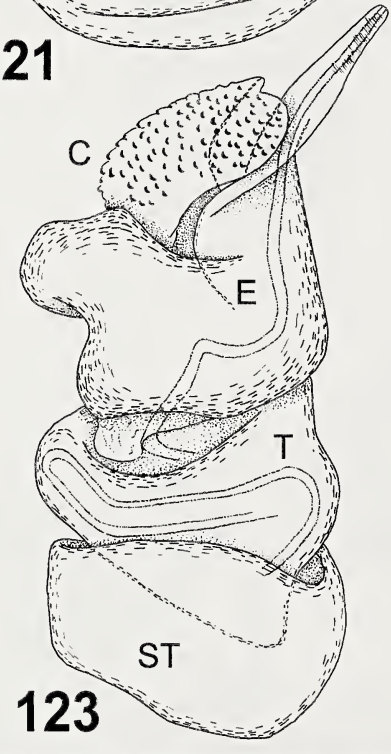
The MA of theridiids has been a particularly great source of confusion. Myriad names have been applied to the structure we now term the MA in theridiids; examples include: "locking apophysis A" (Saaristo 1978, p. 113, fig. 126), "locking apophysis B" (Saaristo 1978, p. 119, fig. 193), "theridiid tegular apophysis" (Coddington 1990, p. 41, fig. 76), "tegular apophysis I" (Knoflach 1997, p. 134, fig. 4), and "radix" (Levy 1998, p. 33, fig. 47), to name a few. In addition, most of the sclerites of the theridiid palp have at one time or another been labeled MA. Griswold et al. (1998), for example, studying *Steatoda grossa* (C. L. Koch, 1838), labeled an apophysis of the embolus as MA (their figure 16C), while the large and conspicuous MA is itself miss-

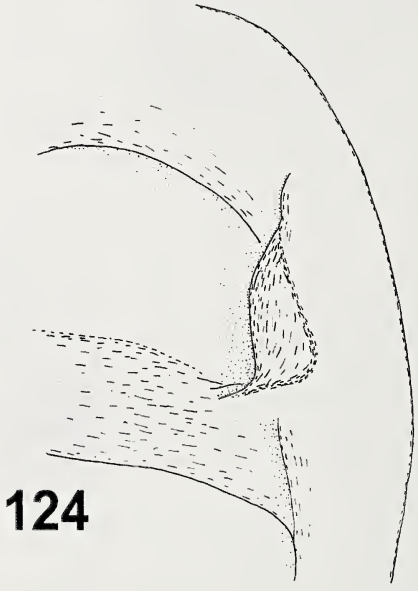
←

Figures 113–120.—113–115, *Theridion cochise* Levi 1963 dissected palp. 113, cymbium; 114, bulb ventral, absence of TTA and flat based E are shared with some other *Theridion*, e.g.,*T. grallator* Simon 1900; 115, bulb dorsal; 116, *Coleosoma floridanum* Banks 1900, schematic drawing of a dissected palp, MA is present, but not shown; 117, *Theridion frondeum*, palp ventral; 118, *Achaearanea trapezoidalis* (Taczanowski 1873), the type species of *Achaearanea*, uniquely among theridiines, has a hooked BC-lock system (arrow); 119, *Achaearanea tabulata* Levi 1980 (redrawn from Knoflach 1991), like most *Achaearanea* lacks TTA; the MA is either lost as well or fused with the embolus; 120, *Theridula emertoni* Berland 1920 (redrawn from Levi & Levi 1962), lacks a conductor, TTA is also absent. The tegulum is distally attached to the cymbium via a membranous sclerite, most likely the MA.

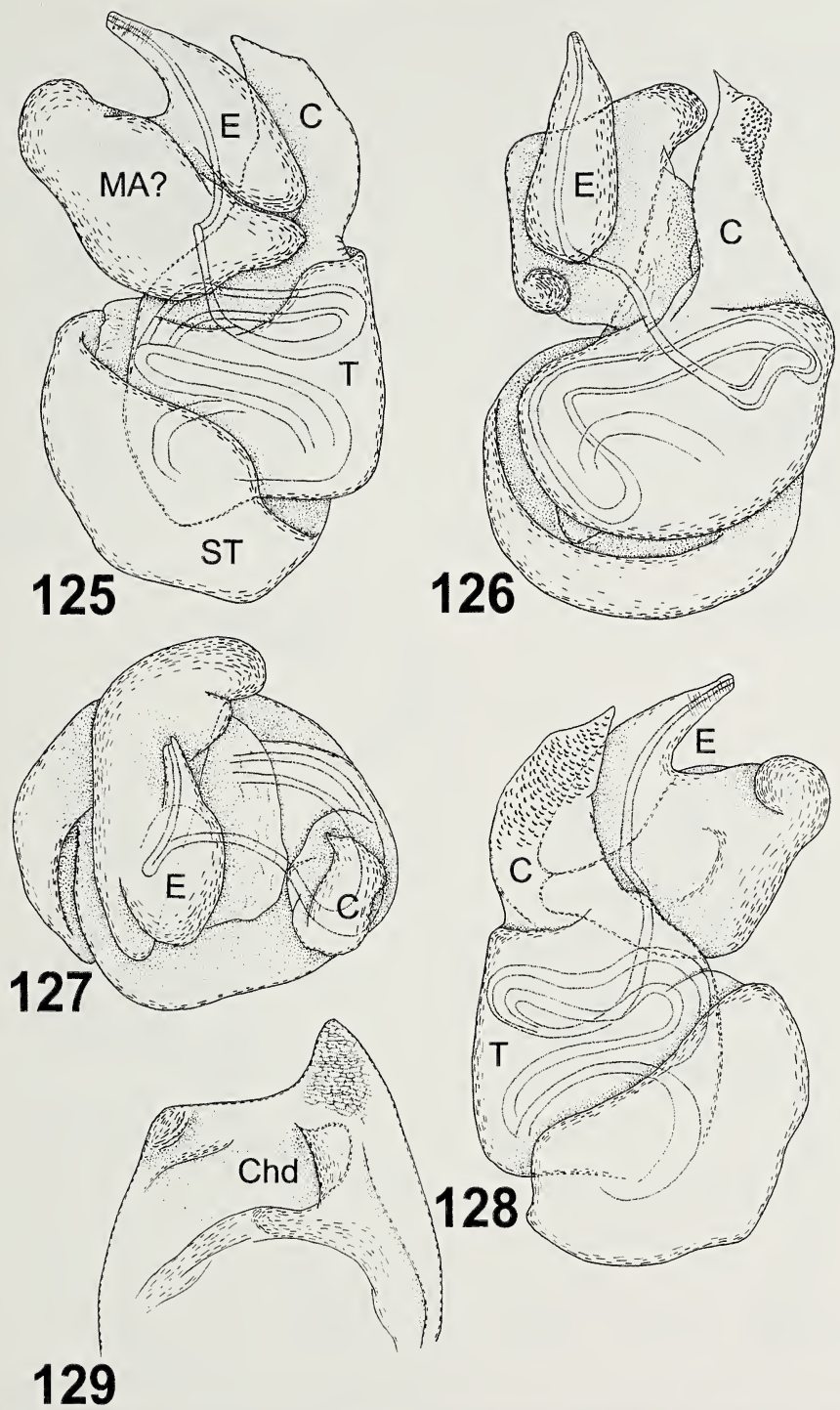




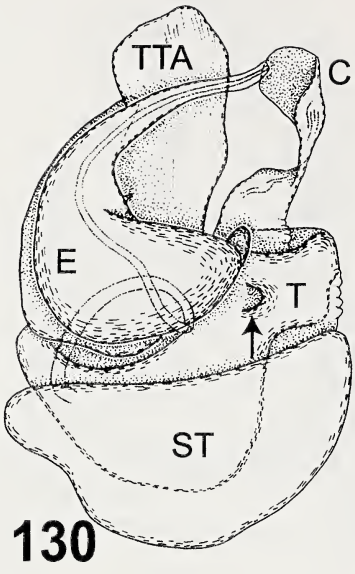


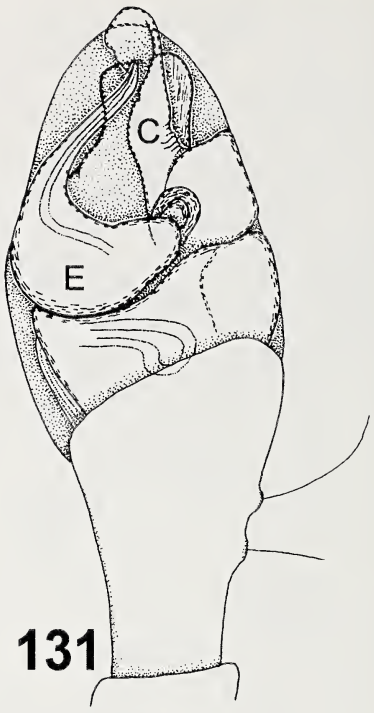


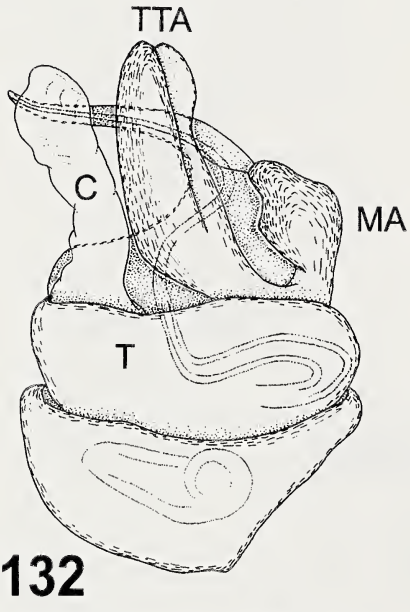
Figures 121–124.—*Achaearanea lunata*. 121–123, bulb slightly expanded and removed from cymbium, ventral, ectal, mesal; the TTA has been lost; it is uncertain whether the MA has been fused with the embolus, or lost, in which case the embolus base interacts with the BC-lock system; 124, distal ectal margin of cymbium in ventral view with cymbial hood.

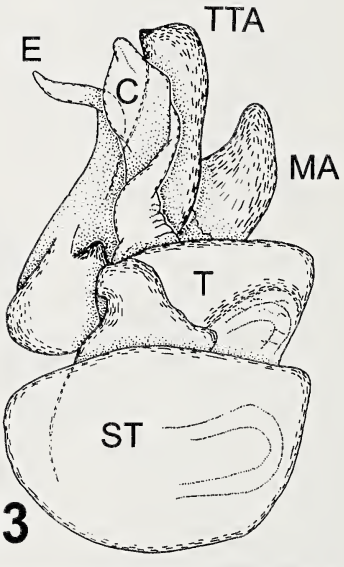


Figures 125–129.—*Achaearanea riparia*. 125–127, bulb slightly expanded, ventral, mesal, ectal-dorsal; 128, apical; conductor with scaly surface as present in many *Achaearanea* species. 129, distal cymbium in ventral view, with protruding tip.

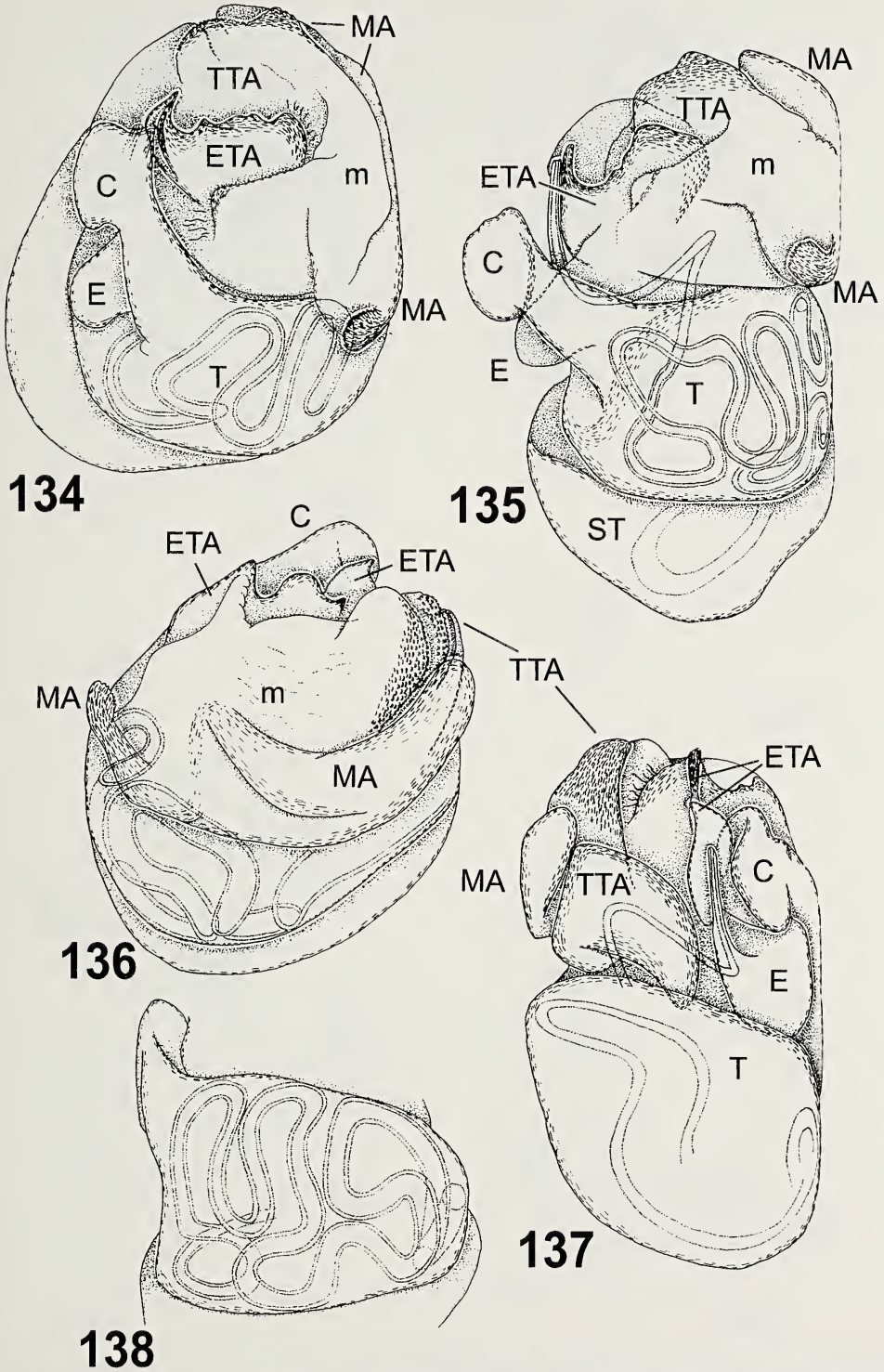




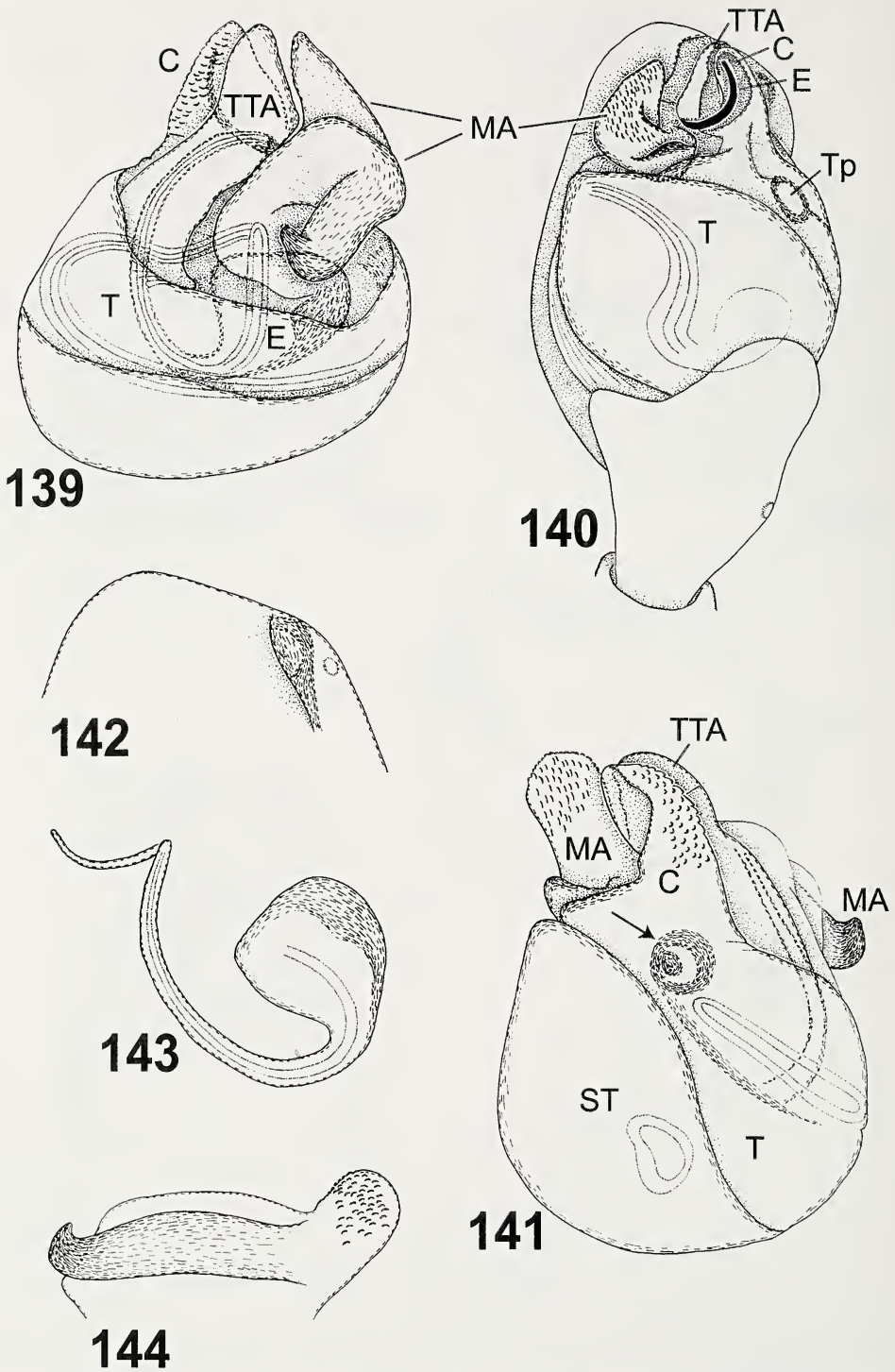




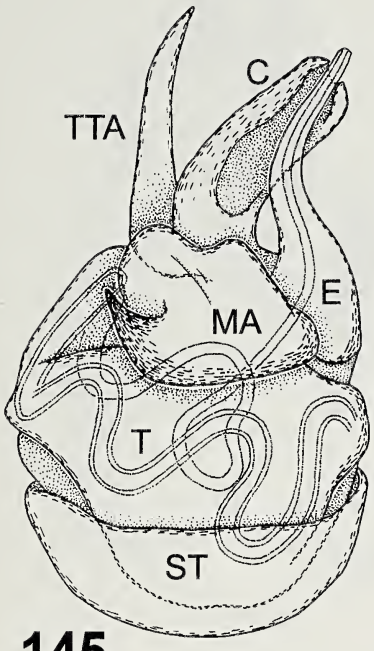
Figures 130–133.—*Keijia tincta*. 130, 132, 133, bulb slightly expanded, ventral, ectal, dorsal; 131, male palp, ventral; both tegular apophyses present; note tegular pit and corresponding embolar process (arrow).

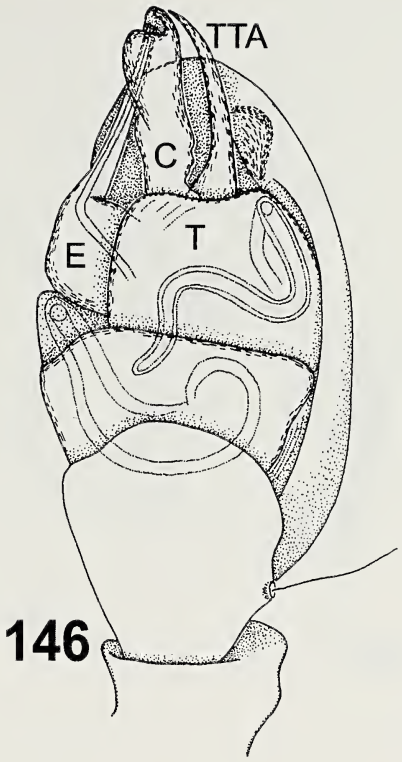


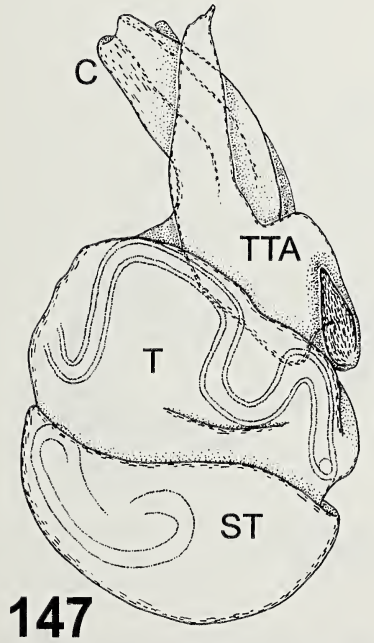
Figures 134–138.—*Neottiura bimaculata*. 134–137, bulb removed from cymbium and slightly expanded, apical-ectal, ectal, apical-dorsal, ventral; three tegular apophyses present, which are complexly folded and connected by a large membrane; 138, tegulum, dorsal; note convoluted course of sperm duct within T.

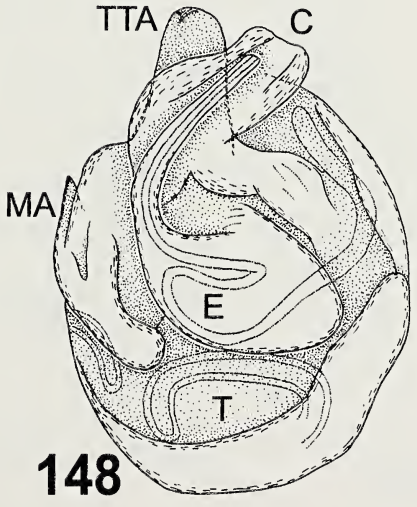


Figures 139–144.—*Rugathodes bellicosus*. 139, 141, bulb removed from cymbium and slightly expanded, dorsal, ectal; both tegular apophyses present; 140, male palp in ventral view; 142, distal cymbium in ventral view with cymbial hood; 143 embolus removed from bulb. 144, median apophysis, mesal. Embolus submerged deeply into tegulum, only its tip being free, accompanied by the conductor; its articulation into the tegular pit also inside but visible through tegulum (arrow).

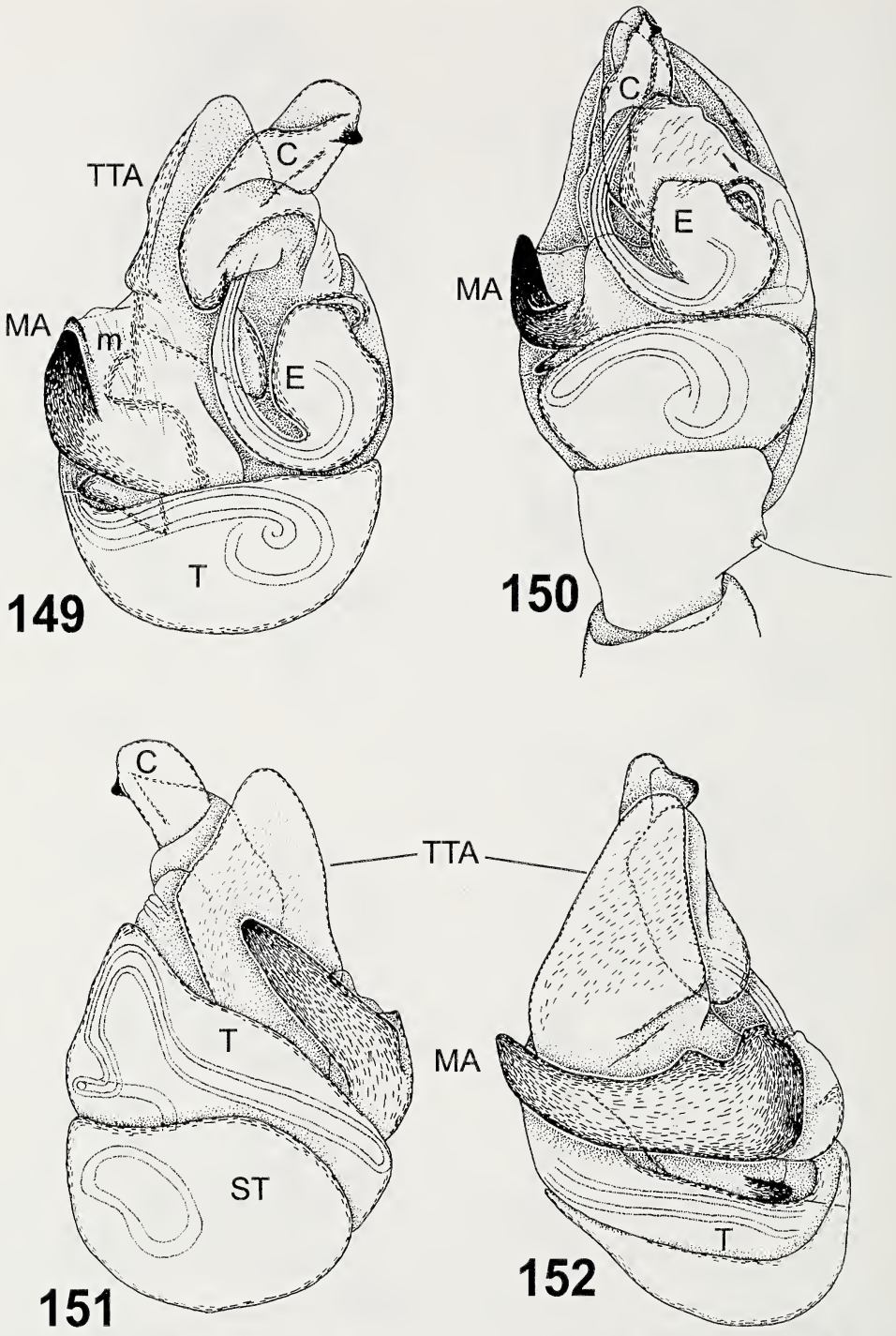




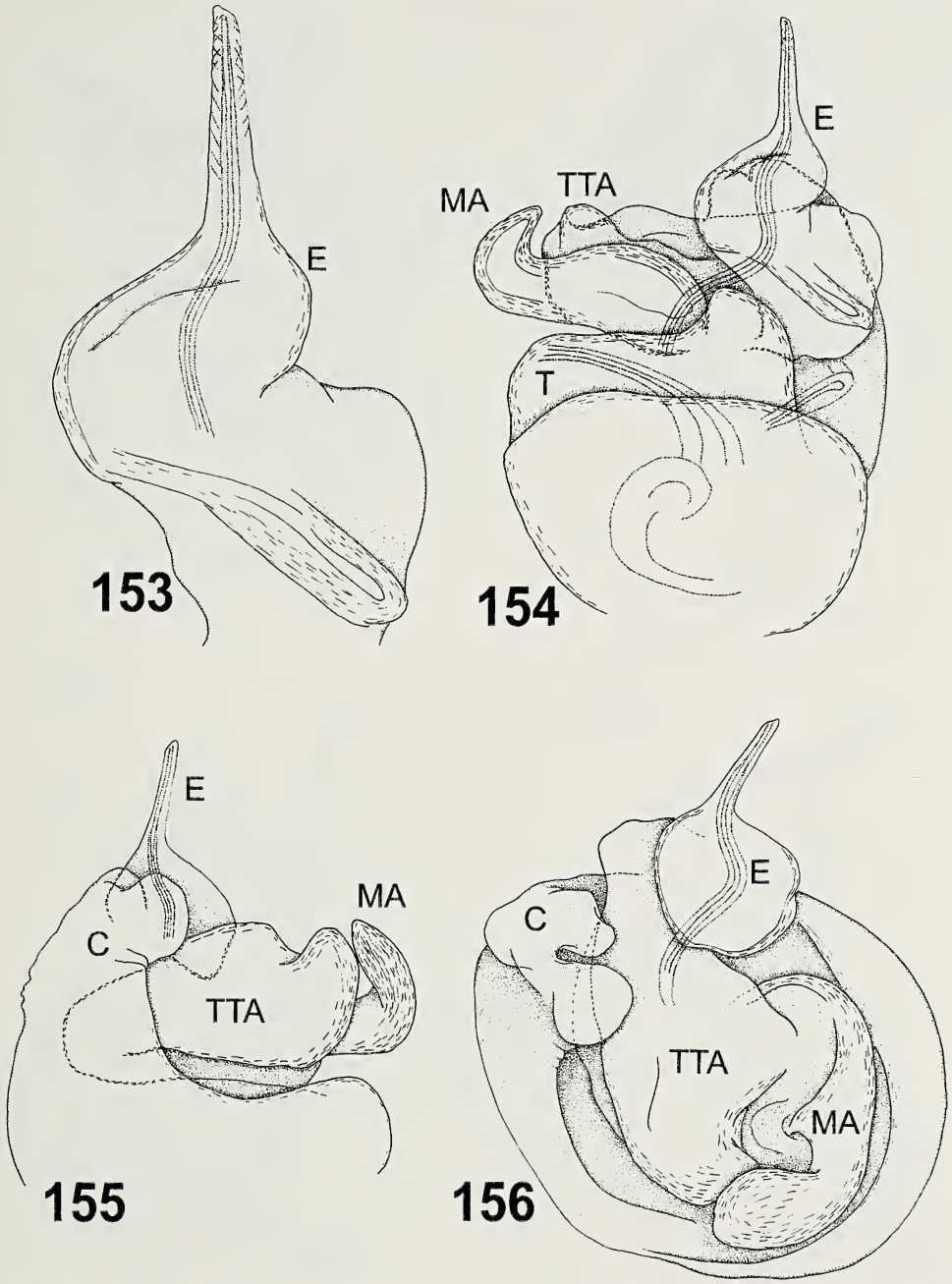




Figures 145–148.—*Simitidion simile*. 145, 147, 148, bulb removed from cymbium and slightly expanded, mesal, dorsal, apical-ventral; sperm duct forms numerous coils within tegulum; 146, male palp, ectal; both tegular apophyses present, connected by a membrane.



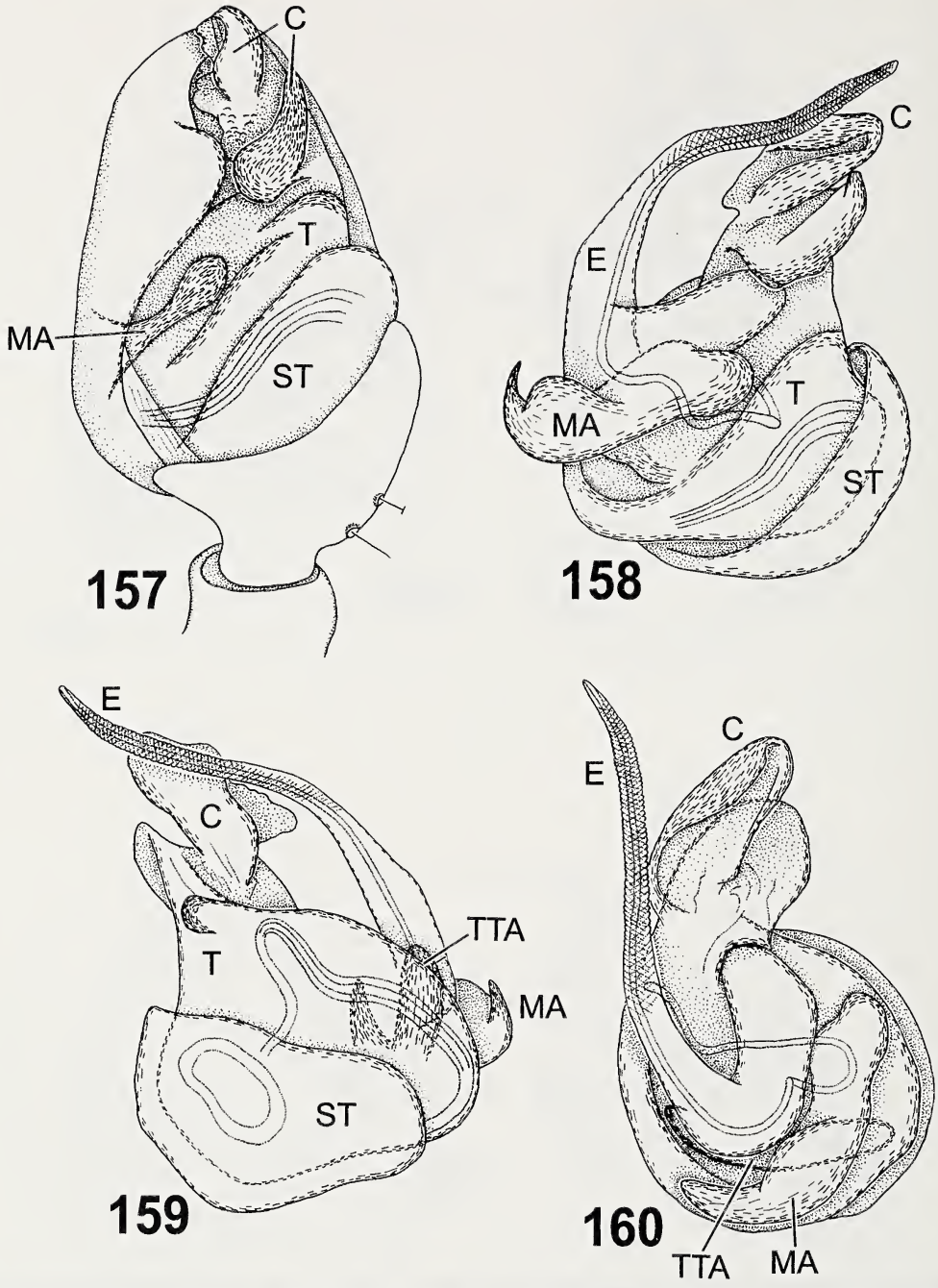
Figures 149–152.—*Theridion pictum.* 149, 151, 152, bulb removed from cymbium and slightly expanded, ventral, dorsal, mesal; both tegular apophyses present, both without sperm duct; conductor with broad, short channel supporting the embolus; scales on TTA indicate contact to the female epigynum; 150, male palp, ventral; arrow points to tegular pit.



Figures 153–156.—*Theridion conigerum*. 153, embolus, ventral; distal part corrugated; 154, 156, bulb removed from cymbium and slightly expanded, ventral, apical-mesal; 155, distal bulb, dorsal; conductor lobe-like, forming a fold.

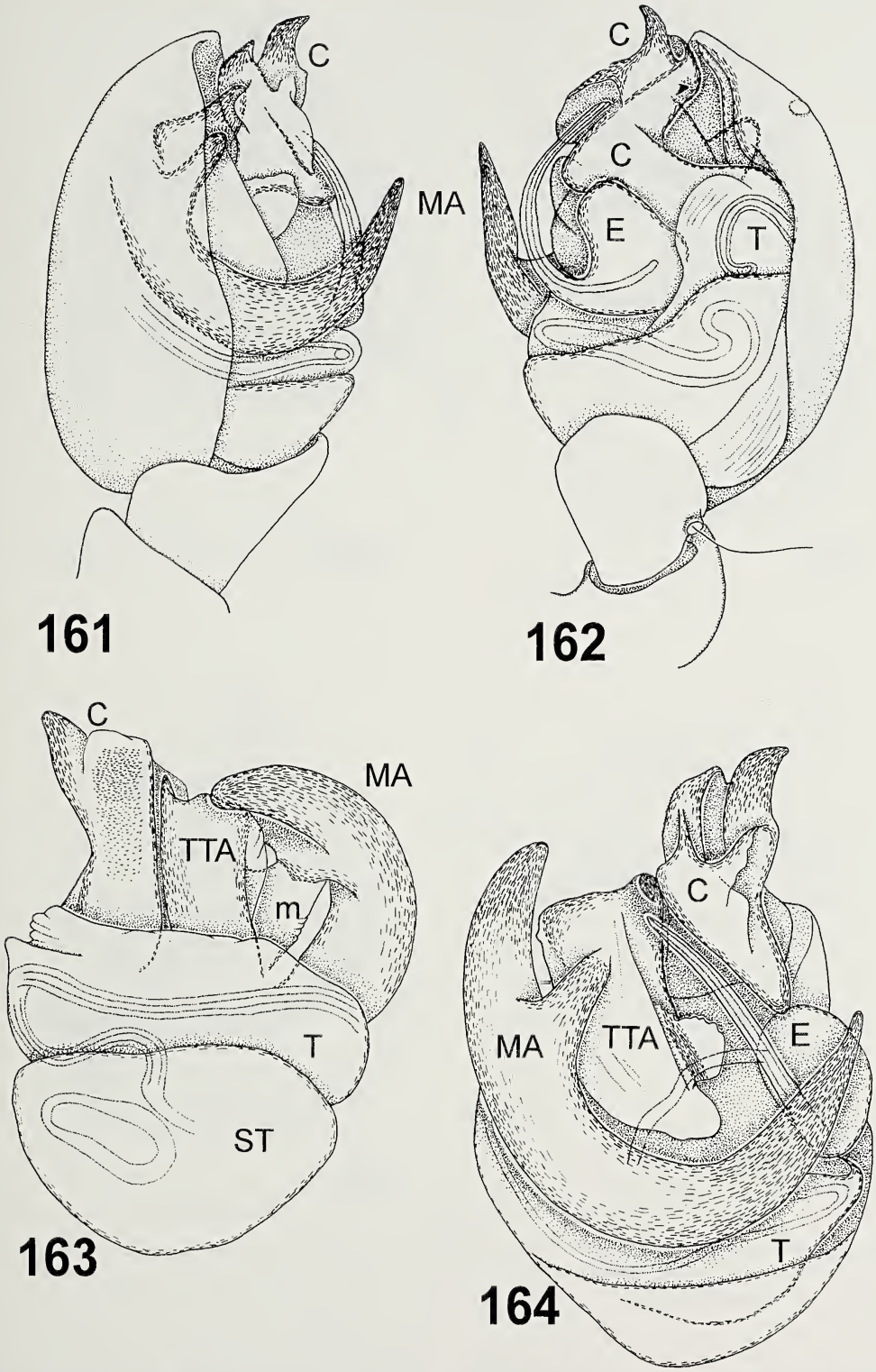
ing from the drawing. Saaristo (1978, 2006) maintained that MA was not present in theridiids at all and furthermore that the apophysis interacting in the lock mechanism was not homologous across theridiids (his locking apophysis A and B). Coddington (1990) agreed with Saaristo's second point, but not his first, using the terms TTA for his laA and MA for his laB.

Coddington (1990) and Sierwald (1990) paid particular attention to the theoretical basis for homology in their consideration of pal-

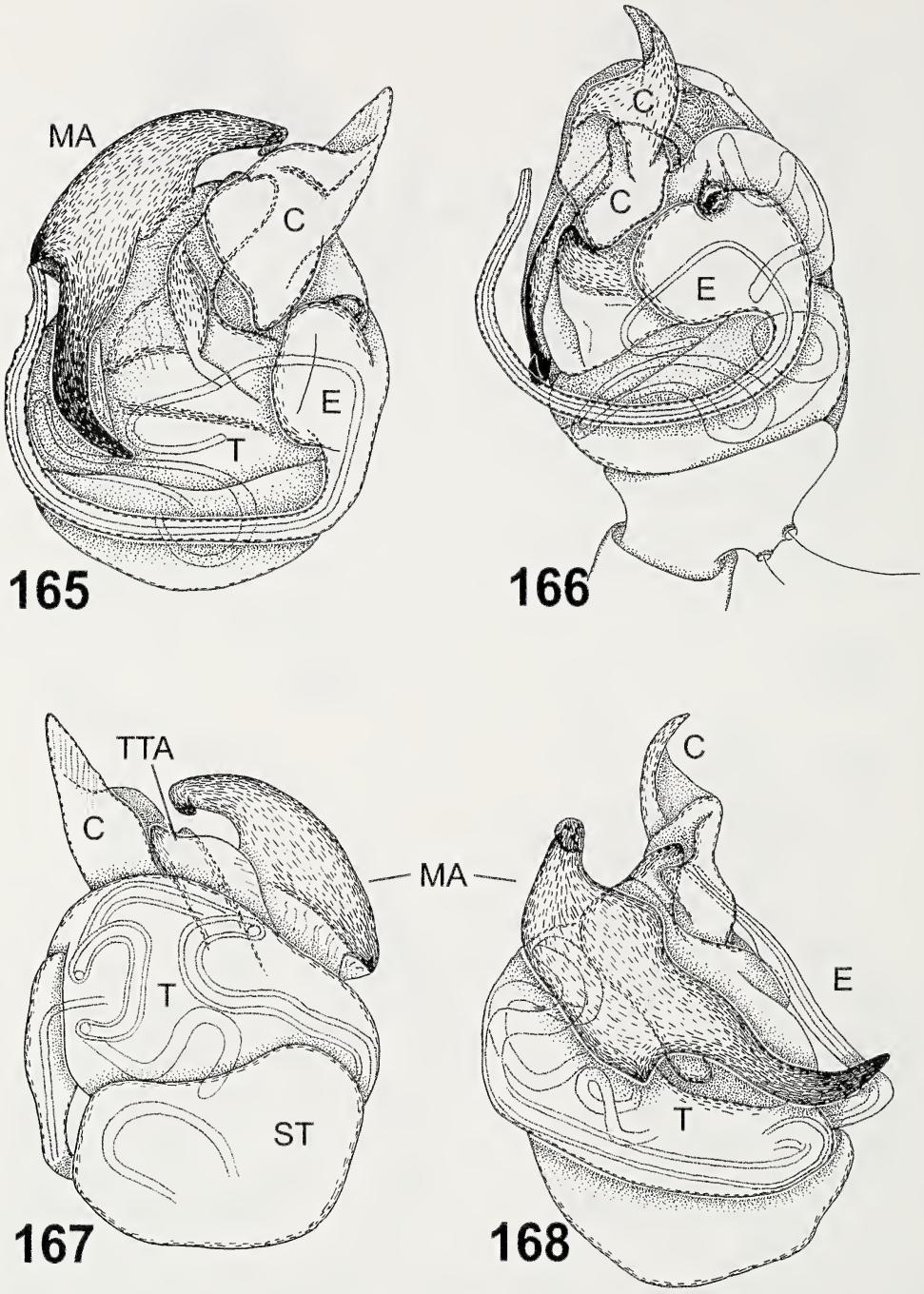


Figures 157–160.—*Theridion ohlerti*. 157, male palp, ventral; embolus hidden by cymbium; 158–160, bulb removed from cymbium and slightly expanded, ventral, dorsal, apical-mesal; TTA small and submerged into tegulum; distal embolus corrugated; tegulum with distinct lobe close to conductor.

pal sclerites in spiders. Influenced by the theoretical debates of the times (e.g., Nelson 1978; Patterson 1982), Coddington chose ontogeny and the potential for transformation during ontogeny over topology or function as homology criteria for two controversial sclerites. Unusually, ontogeny applied to Theridiidae because of the study of *Latrodectus* "*curacaviensis*" (may have been *hesperus*, see below) by Bhatnagar & Remple (1962). First,

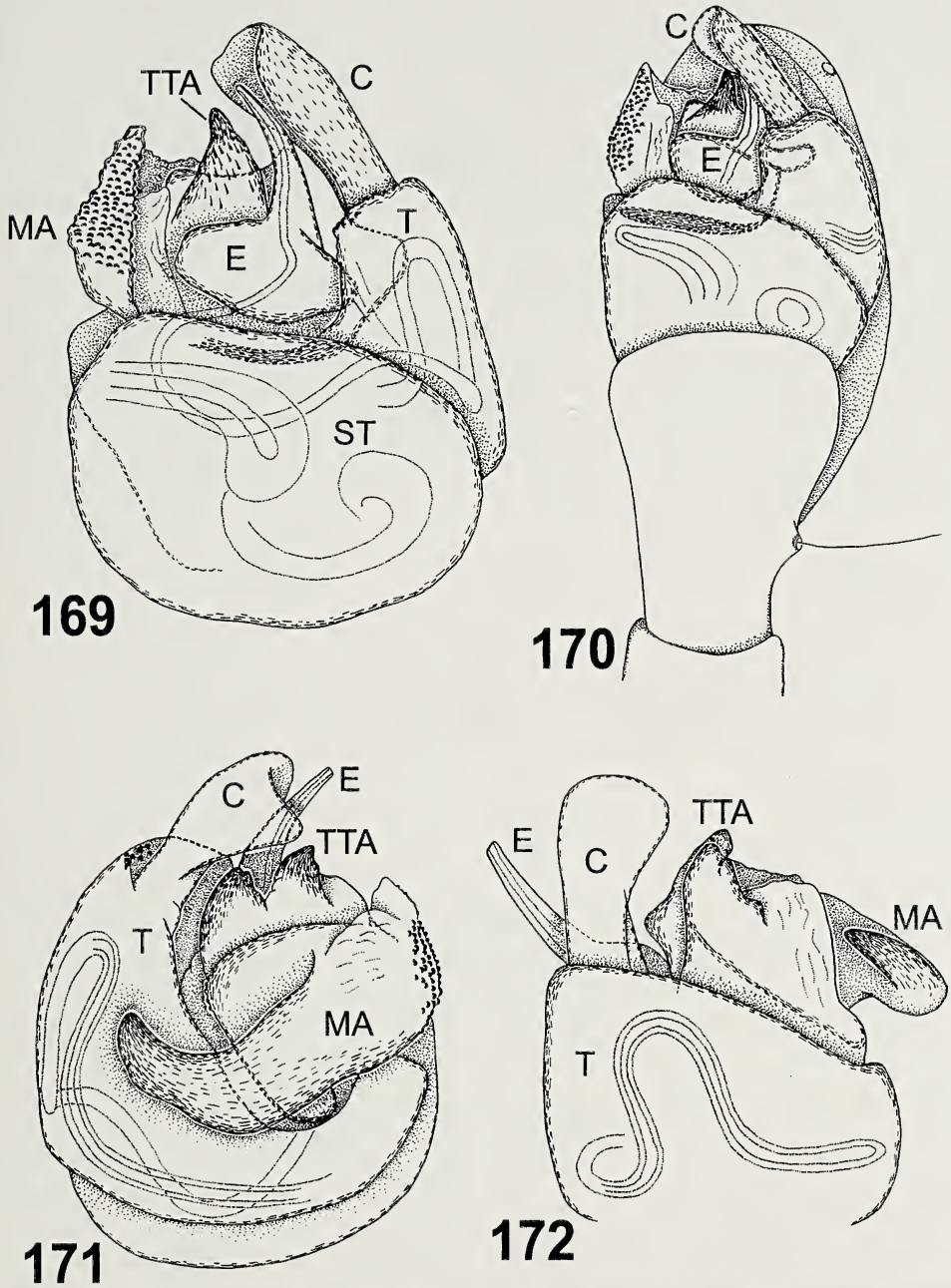


Figures 161–164.—*Theridion petraeum*. 161, 162, male palp, mesal, ectal; 163, 164, bulb removed from cymbium and slightly expanded, dorsal, apical-mesal; conductor bifid, containing a groove and channel for embolus; ventral end of MA typically sickle-shaped.



Figures 165–168.—*Theridion pinastri*. 165, 167, 168, bulb removed from cymbium and slightly expanded, apical-ventral, mesal, dorsal; the TTA is relatively small; 166 male palp, ventral; note articulation between embolus and tegulum.

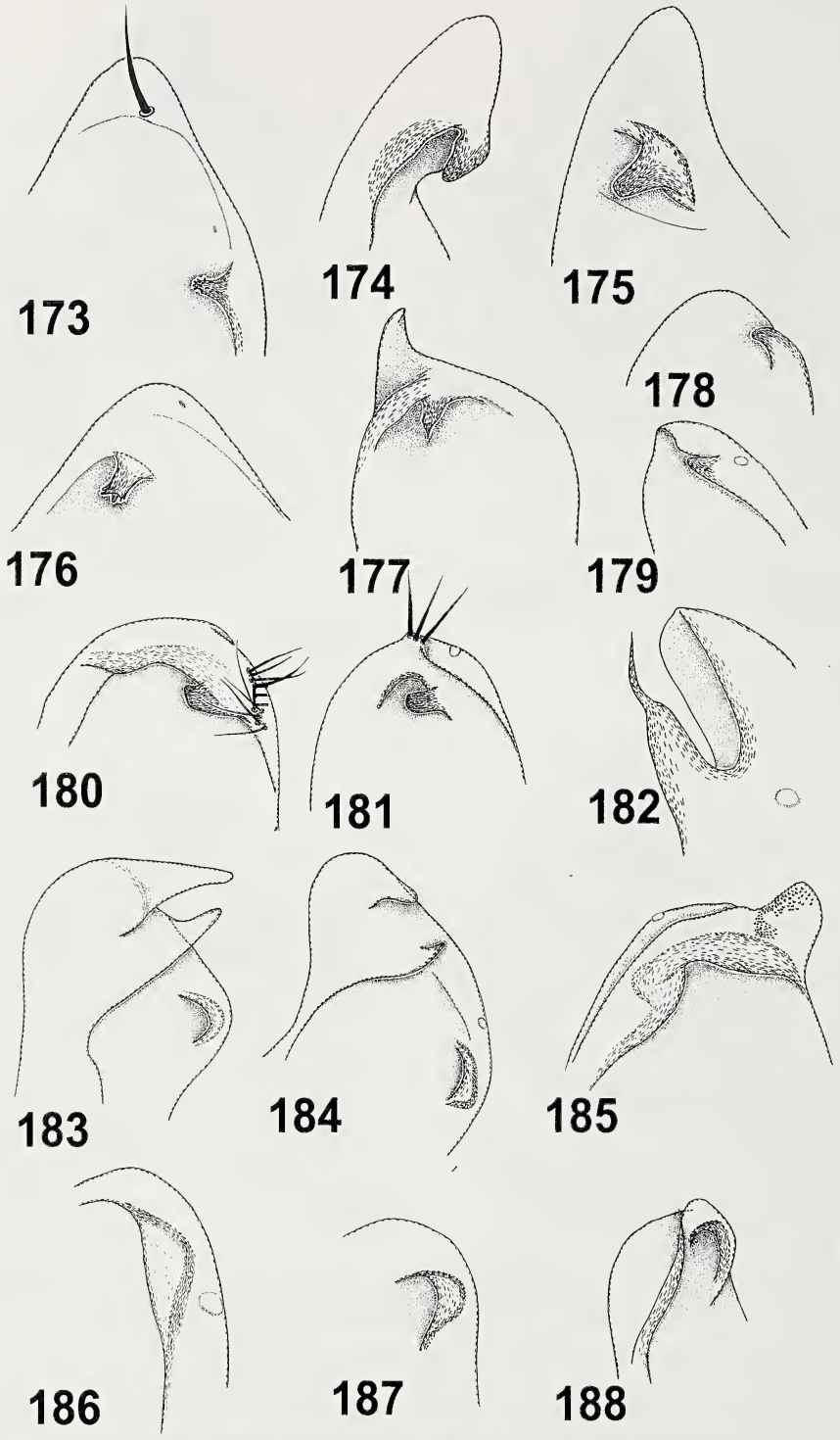
theridiids clearly have an "extra" tegular sclerite beyond those normally present (median apophysis and conductor). One clue was that one of the three theridiid tegular sclerites had a loop of the ejaculatory duct running through it. Outgroup comparison to other spider groups implied that the sperm duct never traverses either the MA or C. Bhatnagar &



Figures 169–172.—*Theridion sisyphium*. 169, 171, 172, bulb removed from cymbium and slightly expanded, ventral, apical-mesal, dorsal; TTA small and bifid; 170, male palp, ventral.

Remple also showed that the MA and C were closely linked ontogenetically, differentiated early from the rest of the palp, and before the invagination of the ejaculatory duct. Coddington therefore concluded that the theridiid sclerite containing a loop of sperm duct was neither MA nor C but something new, which he called the "theridiid tegular apophysis." In our reassessment here, we reach a different conclusion in light of new and more detailed data and analyses (contra Saaristo 2006).

We agree with Levi (see Levi 1961 and Levi's numerous other publications on theridiids (1953–1972)) in considering MA in ther-



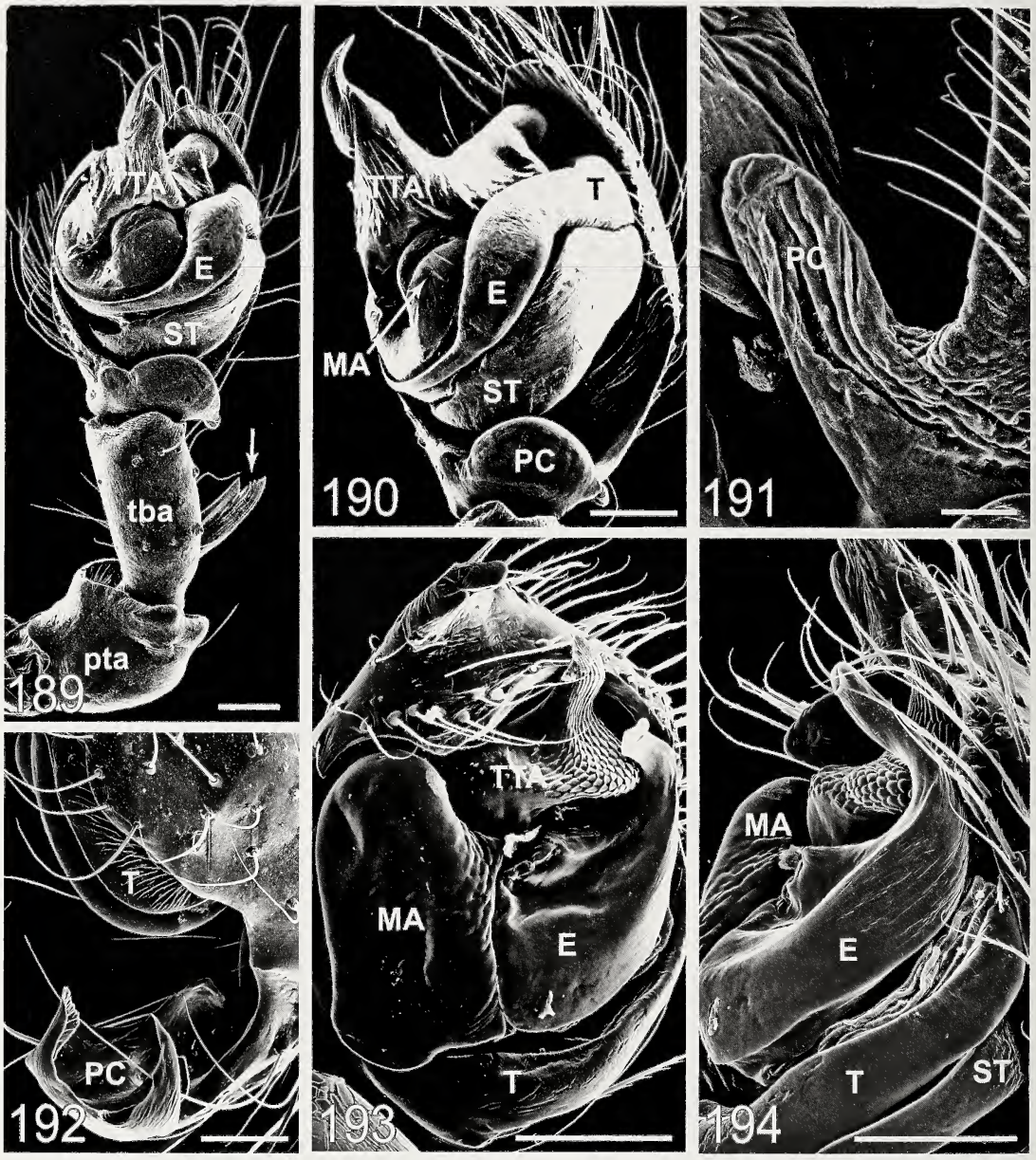
Figures 173–188.—Distal cymbium with cymbial hook, 173–182, and hood, 183–188. 173, *Lasaeola tristis*; 174, 175, *Steatoda phalerata*; 176, *Dipoena melanogaster*; 177, *Euryopis flavomaculata*; 178, 179, *Pholcomma gibbum*; 180, *Episinus truncatus*; 181, *E. theridioides*; 182, *Robertus neglectus*; 183, 184, *Neottiura bimaculata*; 185, *Theridion nigrovariegatum*; 186, *Keijia tincta*; 187, *Simitidion simile*; 188, *Theridion sisyphium*.

idiids as a sclerite positioned retrolaterally on the mesal side of the tegulum (Figs. 4–11, 18, 20–24, 27, 30, 32–34, 36, 42–44, 46, 48–54, 58, 59, 61, 62, 67, 70, 74, 75, 78–81, 83–91, 93–96, 98, 101, 102, 104–106, 108, 114, 115, 117, 132–137, 139–141, 145, 148–152, 154–165, 167–172). It is closely associated with the tegulum, and contains a loop of the sperm duct in the more basal theridiids (Figs. 4–11, 23, 24, 27, 30, 32–34, 36, 42–44, 46, 58, 74, 75, 78–81, 83–91, 93–96, 98). The MA in theridiids is always attached by a membrane to the tegulum (Fig. 3, in some cases the membrane is very narrow, so that the MA appears fused to the tegulum), often sharing a membrane with the TTA and E. The MA is present in most theridiids, it is topologically very consistent across genera, and if present always functions as the sclerite that interacts with the cymbium in the cymbial lock system (Figs. 64, 65). The link to MA in the outgroups is supported by topological similarity (araneid and nesticid MA's are a retrolateral process of the tegulum, Fig. 198), similarity in structure and association with other palpal elements (nesticid MA resemble theridiid MA in shape, and often contains a loop of the sperm duct, as do basal theridiids Fig. 198) and similarity in function (nesticid MA interacts with the cymbium during palpal expansion) (Huber 1993). This outgroup comparison contrasts with Saaristo's (1978, 2006) view that his laA and laB (our MA) are confined to theridiids. Furthermore, contrary to Saaristo (1978, 2006) and Coddington (1990) it is simpler, according to our method, to hypothesize a transformation of the MA structure (loss of sperm duct and MA hood, both of which seem to take place gradually if optimized on a cladogram) than a sudden and drastic topological, structural, and functional shift in this sclerite. In either case, the loss of the sperm duct loop must be accounted for anyway.

As noted, Levi (1961) consistently used the term MA as we suggest here. Knoflach (1991–2002; Knoflach & Thaler 2000; Knoflach & van Harten 2000, 2001; Knoflach & Pfaller 2004) also viewed the structure involved in the lock mechanism as homologous across theridiids, although she hitherto used a neutral term for it (tegular apophysis I).

Conductor: Like the MA, the term "conductor" has not been consistently applied across araneoid palpal sclerites (Griswold et al. 1998), although its usage in theridiids has been fairly consistent. Comstock's definition of the C was, rather atypically and unfortunately, functional: "the conductor . . . [is] easily recognized by its relation to the embolus, which rests upon it . . . " (Comstock 1910, p. 172), but it now seems clear that there is more than one sclerite that can serve as conducting the embolus tip in araneoid spiders (see Lehtinen 1967; Coddington 1990). However, Comstock (1910, p. 176) gave other criteria as well: "The conductor arises at the base of the apical division and is closely connected with the tegulum" and "[it] is easily recognized by its . . . membranous texture" (Comstock 1910, p. 172). Other authors have followed in treating the C as the sclerite most closely associated with the tegulum. Bhatnagar & Rempel (1962, p. 478) showed that in *Latrodectus*: "Histological study indicates its [the conductor's] origin from the median wall of the tegulum". Sierwald (1990, p. 21) described the pisaurid C: "The conductor inserts directly on the tegulum and appears to be a mere extension of the tegular wall . . . immovably attached to and continuous with the tegular wall" and the lycosid C is a "tegular outgrowth of the same texture and color as tegulum" Griswold (1993, p. 10).

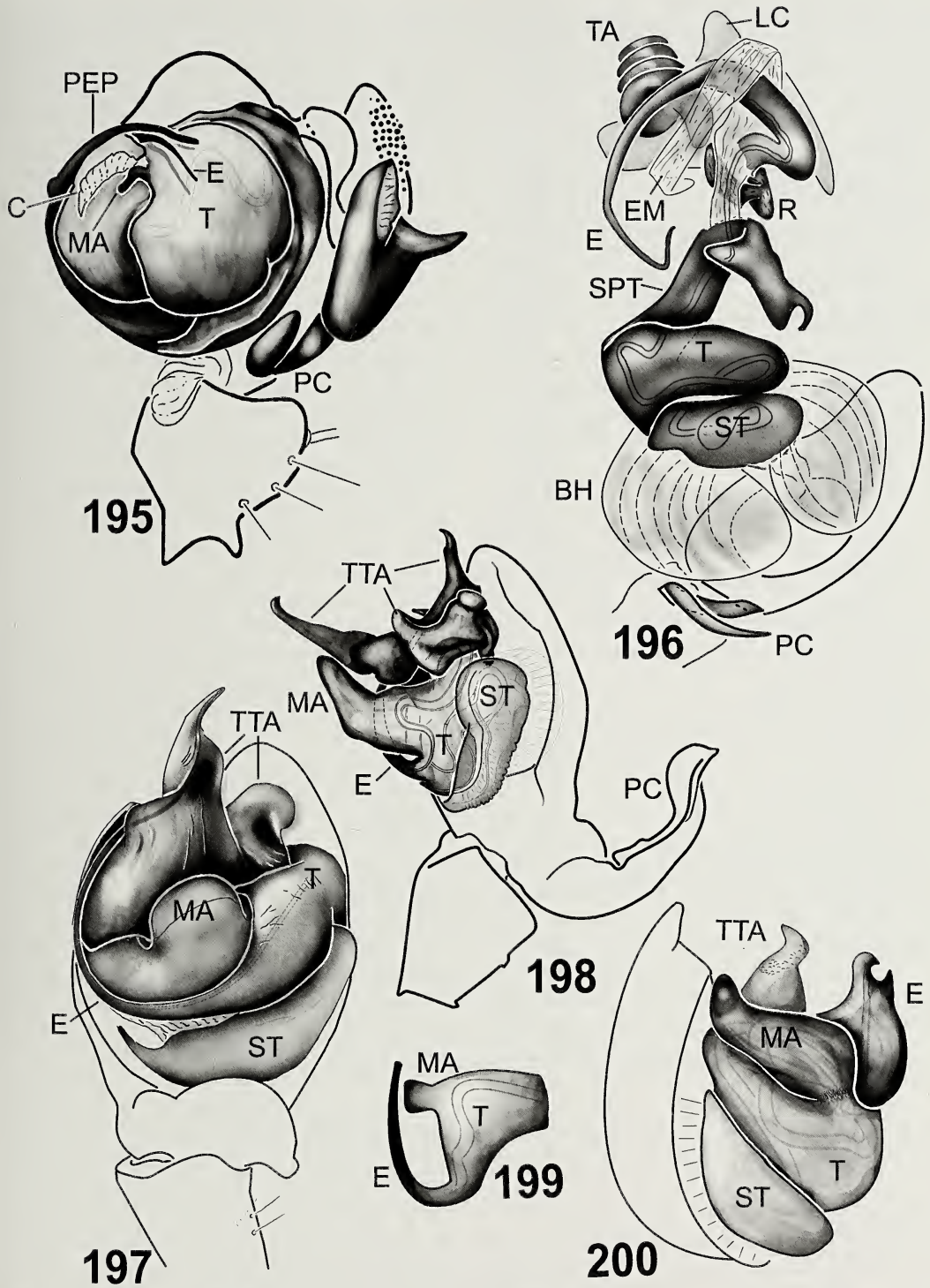
In theridiids, at least two sclerites, the C and the TTA, may perform the act of conducting the E. Many theridiids have a relatively small C, sometimes only vestigial, in which case the TTA serves to "conduct" (or support) the E (Figs. 8, 49, 77, 91–94). This seems also to be the case in nesticids and synotaxids (Figs. 189, 190, 197, 198). The theridiid C is always a direct and immovable outgrowth of the tegulum, lying close to (but behind) the E, centrally or slightly ectally in the palp (Figs. 3, 6, 7, 12, 17, 18, 20–23, 28–30, 33, 34, 36, 39–41, 44, 45, 74, 79–81, 83, 84, 88, 90, 91–93, 97, 101, 102, 105, 107, 109, 114, 115, 121, 122, 125–128, 130–135, 141, 146, 148, 149, 155, 156, 158–160, 162, 163, 165, 166, 169–172). The C is often membranous or of the same texture as the tegulum, but sometimes heavily sclerotized and rugose (e.g., *Achaearanea lunata* (Clerck 1757) and *A. tepidariorum* (C. L. Koch 1841), Figs. 109, 122, 123). In *Anelosimus* the tegulum has a sclerotized area, or a separate outgrowth at the base of the C, the subconductor (see below).



Figures 189–194.—189, 190 *Synotaxus monoceros* (Caporiacco 1947) (Synotaxidae). 189, ventral, note patellar spur (arrow), a rather uniform tibia, and a large excavate TTA; 190, ectal, the E is a direct outgrowth of the tegulum, note also a distinct, cup shaped PC; 191, *S. waiwai* paracymbium; 192, *Nesticus silvestrii* Fage 1929, huge and rigid PC; 193, 194, *Euryopis gertschi* Levi 1951. 193, ventral, conductor absent; 194, ectal, note small membrane between T and E. Scale bars: 189, 190, 192–194 = 100 μm; 191 = 20 μm.

In *Theridula* (Fig. 120), *Euryopis* (Figs. 8–11), and perhaps *Carniella* (Fig. 50; see Agnarsson 2004) the C is absent.

The nomenclature of the theridiid C has been remarkably stable, considering its variability and that it does not always function to conduct the E. Saaristo (1978) generally referred to the C as "conductor A" calling the TTA or an appendage of it "conductor B." Only in a few cases, have the TTA and the C been confused, for example Levi (1963, p. 43, fig. 44) in *Selkirkiella*, where the TTA is



Figures 195–200.—195, *Pimoa rupicola* (Simon 1884) (PEP = pimoid embolic process); 196, *Linyphia triangularis* (EM = embolic membrane, LC = lamella characteristica, R = radix, SPT = suprategulum, TA = terminal apophysis); 197, *Synotaxus monoceros*; 198, *Nesticus cellulanus* (Clerck 1757); 199, *Eidmanella pallida*, part of tegulum showing MA and E; 200, *Euryopis flavomaculata* (redrawn from Levi & Levi 1962). 195–199 reproduced from Agnarsson (2004) with permission from Blackwell Publishing.

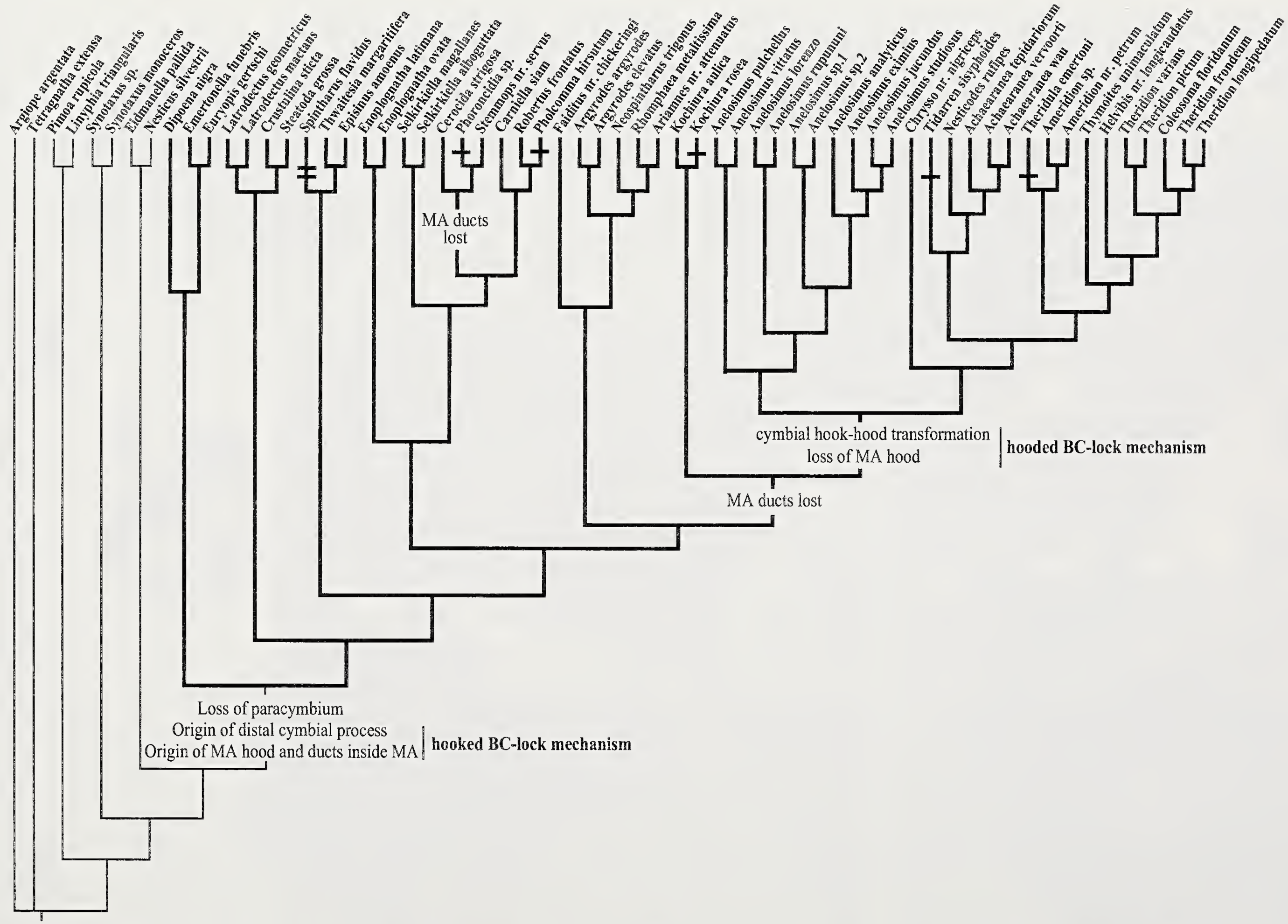


Figure 201.—Evolutionary changes in the unique theridiid BC-lock system. The cladogram is taken from Agnarsson (2004), see Figure 2 for clade names. Theridiidae is indicated with bold lines. Black horizontal bars each indicate one instance of homoplasy: *Spintharus* independently evolved a hooded lock system and lacks the hood on the MA; *Phoroncidia* lacks the MA hood; the MA of *Pholcomma hirsutum* Emerton 1882 does not contain a loop of the sperm duct (but in *P. gibbum* does, see Figs. 91–95); *Kochiura rosea* (Nicolet 1849) lacks the MA hood, *Tidarren* has a hook BC lock system (uniquely among Theridiinae); the lock system of *Theridula* is unique (see text).

strongly modified and forms a long sheath around the E.

Subconductor: In *Anelosimus* (Fig. 63) an outgrowth of the C base overhangs the E. We here name this structure "subconductor" following Agnarsson (2004) and Agnarsson & Kuntner (2005). The only clear reference to the subconductor we are aware of is in Levi (1956, p. 411, fig. 17), and following him, Coddington (1990, p. 42, fig. 94) where in *Anelosimus eximius* (Keyserling 1884), it is labeled as the C. The tiny membranous "true" C, arising from the back of the subconductor (Fig. 63), is missing from their drawings.

Theridiid (theridioid) tegular apophysis: Most theridiids have a tegular apophysis in addition to the ones already accounted for. This apophysis is always a "free" sclerite, connected to the tegulum via a membrane (or sometimes partially imbedded within the tegulum, although never fused to it). Levi generally used the term "radix" for this tegular apophysis but it now seems not to be homologous to any sclerites present in araneids, linyphiids, pimoids, or symphytognathids. Hence Coddington (1990) introduced the term theridiid tegular apophysis (TTA) for this structure. However, because the TTA seems to be present in nesticids (which Coddington (1990) acknowledged) and perhaps in synotaxids, it should be henceforth named the theridioid tegular apophysis (with the same abbreviation, TTA).

Based on the present results, Coddington (1990) did not apply the term TTA consistently in his treatment of theridiid palps. He applied it to the MA whenever the MA had sperm ducts going through it (e.g., figs. 76, 77, 79, 81, p. 41), but to the "true" TTA when the MA was without ducts (e.g., figs. 90, 92, 94, p. 42).

The TTA is a tegular apophysis normally lying in between the E, C, and MA somewhat centrally in the palp (Figs. 4, 5, 8, 9, 11, 17, 18, 23, 24, 27, 30, 32, 33, 36, 39-43, 45, 46, 48, 49, 51, 52, 61, 62, 66, 67, 73, 74, 75–77, 95, 102, 106, 117, 132, 134, 135, 139, 145–148, 151, 152, 155, 156, 163, 164, 171, 172). It is connected to the tegulum via a membrane, usually the same membrane that connects the MA and the E to the tegulum. The TTA commonly terminates in a hook (Figs. 5, 8, 9, 17, 18, 20, 21, 24, 26, 27, 30, 32, 33, 34, 36, 39–42, 46, 48, 52, 54, 95, 102, 145–148) and frequently functions to support the embolus. Based on detailed studies of nesticids (Huber 1993), it is likely that the TTA in theridiids interacts closely with the epigynum during copulation. Usually the TTA has a rugose surface at or near its tip, which may help to stabilize its interaction with the epigynum.

Many names have been applied to the TTA in theridiids; "radix" (e.g., Levi & Levi 1962, figs. 185, 197, 303), "conductor" (Levi 1963d, p. 43, fig. 44), "tegular apophysis" and "conductor B" (Saaristo 1978, p. 119, fig. 194), "median apophysis" (e.g., Coddington 1990, p. 41, fig. 76), "tegular apophysis II" (e.g., Knoflach 1996a, p. 143, fig. 13), and "accessory apophysis" (Levy 1998, p. 33, fig. 48) to name a few.

Despite this confusing nomenclature, in some cases reflecting mistaken homologies, some previous authors have arrived at the same concept that we present here as the TTA. Levi applied the term "radix" very consistently to this sclerite (with exceptions mentioned above) in his many treatments on theridiids, and Knoflach (1991–2002; Knoflach & van Harten 2000, 2001; Knoflach & Thaler 2000; Knoflach & Pfaller 2004) and coauthors have consistently used the term "tegular apophysis II" for it.

Extra tegular apophysis: The spintharines *Episinus* and *Thwaitesia* have palps that are considerably more complex than those of most other theridiids. The C in these taxa is a huge and complex sclerite (note in *Spintharus* the C is also huge and of similar shape; both resemble the C in *Selkirkiella*), and near its distal tip there is an additional tegular apophysis (Figs. 22–25), absent in most other theridiids (but see below). This small, but strongly sclerotized, pointed sclerite, connects to the tegulum via a membrane, and is Knoflach's (1993b, p. 362, fig. 10) "TA3" or tegular apophysis III. This sclerite appears not to be labeled in any of Levi's treatments of these genera.

Similarly, some species of the genera *Enoplognatha* and many other pholcommatines, and of *Neottiura*, have a tegular apophysis, in addition to the MA, TTA, C and E. This apophysis is closely associated with the TTA and is connected to the tegulum in the same manner (Figs. 74, 77, 134–137). Although topologically similar, it seems that the additional tegular apophysis has arisen more than once

across theridiid taxa, and is not homologous. However, this optimization may change as further taxa are added; meanwhile we here label it neutrally as the "extra tegular apophysis."

Embolus: The E is simply "the organ through which the ejaculatory duct opens" (Comstock 1910, p. 173). The E of different groups of spiders can be quite different; for example, it is an outgrowth of the tegulum in *Nesticus* (Fig. 198) and *Synotaxus* (Figs. 189–190, 197), but a free sclerite connected to the tegulum via a membrane in theridiids. (In this case, the sperm duct travels through the membrane between the tegulum and the embolus). Even within theridiids, the E is extremely variable (Figs. 4, 5, 9–12, 15, 17, 20, 22, 25, 26, 29, 30, 32, 33, 34, 39–41, 44–46, 49, 50, 52, 54, 58, 59, 61, 63, 66–69, 76, 77, 82, 83, 85, 89–92, 95, 96, 98, 100, 101, 103–110, 114–123, 125–128, 130, 131, 137, 143, 148–150, 153, 154, 156, 158–160, 165, 166, 169, 193, 194, 200). The E may be split along some or most of its length, as is the case in many *Anelosimus*, (Fig. 54), or it may be split transversally (or fuse to the MA) as in *Achaearanea tepidariorum* (Figs. 109, 119, 121–123, 125–128). In *Achaearanea* spp., the actual extent of the E is problematic. Two alternative interpretations are possible: 1) MA is absent in some species, and the E contains a suture, or 2), the MA has fused to the E. The former is an attractive interpretation in some closely related species, such as *A. wau*, where there is no trace of a MA and the E (which is not split in any way) interacts in the CB-lock. The alternative interpretation would be fusion of the MA to the E in those taxa; both hypotheses could be tested with ontogenetic and phylogenetic data. *Theonoe* is somewhat similar (Figs. 96–98), although apparently the embolus and median apophysis are simply closely associated because the MA clearly contains a loop of the sperm duct, as in related taxa. A remarkable type of E is found in *Stemmops* sp. where the extremely long coiling tip does not contain the sperm duct. Rather it exits through an apophysis that shares a membranous base with the more typical embolus (Agnarsson 2004).

Embolic division b: The E in some *Anelosimus*, is distinctly bipartite and divided into the E spiral and embolic division b (Fig. 54), or Eb (terminology from Levi 1956). The Eb often closely follows and may support, the E. The embolic division b is variable in size, degree of sclerotization, orientation, and rugosity. It is here not considered a potential homolog of other embolic apophyses (following Agnarsson 2006b, and Agnarsson & Kuntner 2005), because it is dissimilar and distinct in topology (branching off the embolus spiral, rather than off the embolus base), and presumably in function.

Embolic sclerite: In several species of *Steatoda*, a unique sclerite is attached by membrane to the E base (Fig. 17). We have not seen this sclerite in any other theridiids, but suggest the name embolic sclerite for it.

Embolic apophysis: The E of several theridiids bears a small apophysis (Figs. 28, 29, 59, 60, 105, 116, 117) here labeled embolic apophysis (see also Agnarsson 2004).

CONCLUSIONS

We have reviewed the morphology of the male palpal organ in theridiid spiders and relatives through extensive illustrations and literature review. Using a recently proposed method to evaluate primary homology hypotheses we arrive at a scheme of palpal homology hypotheses for theridiid spiders that is more coherent and congruent than prior attempts. In theridiids, topology—that is to say the relative position of sclerites—seems to be the most reliable criterion to recognize homologous sclerites that differ in various ways such as function, shape, texture, etc., across taxa.

Under this homology scheme, the three most problematic sclerites in the theridiid palp, the median apophysis, the conductor and the theridioid tegular apophysis, can be characterized as follows (left palp, ventral view). The median apophysis is positioned retrolaterally on the mesal side of the tegulum, to which it attaches via a membrane. When present, the MA interacts with the cymbium in the cymbial lock system, and, remarkably, it contains a loop of the sperm duct in basal theridiids. The conductor is positioned close to, but slightly ectal to, the embolus. It is a direct and immovable outgrowth of the tegulum, but can be either membranous or sclerotized and may or may not function to conduct the embolus. The theridiid tegular apophysis is positioned in between the embolus and the median apophysis, caudal to both the embolus and

conductor. It is connected to the tegulum by a membrane, and may or may not conduct the embolus.

All the tegular sclerites provide a number of important characters for phylogenetic analyses, as do various other palpal features such as alveolus position, the cymbial lock system, cymbial and tibial shapes, the sperm duct trajectory, and tibial trichobothrial number and distributions (Fig. 3; see also Agnarsson 2004).

We test a number of hypotheses regarding both homology and evolution of theridiid palpal elements. Two of the more detailed hypotheses are refuted. First, both phylogenetic evidence and the novel homology method refute homology of the basal araneoid paracymbium with the distal theridiid cymbial process (hook or hood). Phylogenetically a hypothesized "transformation" between the two structures is contradicted by the placement of supposedly "intermediate" state taxa (e.g., *Robertus* and *Carniella*) well within Theridiidae, leaving the condition in basal theridiids very dissimilar to that of the outgroups. Putative homology is also refuted by every similarity criterion as the theridiid cymbial process differs from the paracymbium in topology, detailed similarity, and function. Second, Saaristo's (1978) hypothesis that theridiids comprise two main "evolutionary lines" defined by the type of cymbial lock present (his non-homologous locking systems A or B) is also refuted. The two locking mechanisms are instead homologs because the hooked cymbium is primitive, and the hooded cymbium is derived. Thus at least one (and perhaps both) of Saaristo's lineages must be paraphyletic; phylogenetically it is more parsimonious to presume that locking system B is locking system A transformed. Homology criteria also support this transformation view as the cymbial hook and hood share topological and functional similarities.

Our results also show that broad homology hypotheses are especially problematic in the absence of a phylogeny. On the other hand, phylogenetic analysis requires primary homologies, which, if incorrect, cannot be corrected by phylogenetic analysis. We address this "chicken and egg" problem by proposing a procedure that critically compares primary homology hypotheses prior to analysis in order to minimize conflict between classical homology criteria such as topology, function, and special similarity. The method, of course, is not completely independent of phylogeny but rather embedded in the larger context of phylogenetically-based comparative morphology, but in this case it did clarify errors in homology and homoplasy at the "local" phylogenetic level that conventional analysis would have missed. Homology can be effectively tested, not only during phylogenetic analysis, but also prior to it. Both kinds of tests may be helpful whenever character identity (i.e., primary homology) is in doubt.

ACKNOWLEDGMENTS

The manuscript was improved by comments from two anonymous reviewers. Thanks also to Matjaž Kuntner, Martin Ramírez, Maureen Kearney, Philippe Grandcolas, Gustavo Hormiga, and Jeremy Miller who all provided comments on various earlier versions of this manuscript. SEM facilities were provided by the Department of Biological Sciences at the George Washington University. Support for this research was provided by a National Science Foundation PEET grant to Gustavo Hormiga and Jonathan Coddington (DOEB 9712353), The Smithsonian Neotropical Lowland grant, a NMNH "Biodiversity of the Guianas Program" grant, and NSF grant EAR-0228699, all to J. A. Coddington, and a Killam Postdoctoral Fellowship and the USIA Fulbright program to I. Agnarsson

LITERATURE CITED

Agnarsson, I. 2000. *Adansonia* is a baobab tree, not a theridiid spider. Journal of Arachnology 28: 351–352.

Agnarsson, I. 2003. The phylogenetic placement and circumscription of the genus *Synotaxus* (Araneae: Synotaxidae) with a description of a new species from Guyana, and notes on theridioid phylogeny. Invertebrate Systematics 17:719–734.

Agnarsson, I. 2004. Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). Zoological Journal of the Linnean Society 141:447–626.

Agnarsson, I. 2005. A revision and phylogenetic analysis of the American *ethicus* and *rupununi* groups of *Anelosimus* (Araneae, Theridiidae). Zoologica Scripta 34:389–413.

Agnarsson, I. 2006a. A revision of the New World *eximius* lineage of *Anelosimus* (Araneae, Theridiidae) and a phylogenetic analysis using world-

wide exemplars. Zoological Journal of the Linnean Society 146:453–593.

Agnarsson, I. 2006b. Asymmetric female genitalia and other remarkable morphology in a new genus of cobweb spiders (Theridiidae, Araneae) from Madagascar. Biological Journal of the Linnean Society 87:211–232.

Agnarsson I. 2006c. Phylogenetic placement of *Echinotheridion* (Araneae: Theridiidae) – do male sexual organ removal, emasculation, and sexual cannibalism in *Echinotheridion* and *Tidarren* represent evolutionary replicas? Invertebrate Systematics 20:415–429.

Agnarsson, I., L. Avilés, J.A. Coddington & W.P. Maddison. 2006. Social theridiid spider – repeated origins of an evolutionary dead-end. Evolution 60:2342–2351.

Agnarsson, I., W.P. Maddison & L. Avilés. 2007. The phylogeny of the social *Anelosimus* spiders (Araneae: Theridiidae) inferred from six molecular loci and morphology. Molecular Phylogenetics and Evolution. 43:833–851.

Agnarsson, I. & M. Kuntner. 2005. Madagascar: an unexpected hotspot of social *Anelosimus* spider diversity (Araneae: Theridiidae). Systematic Entomology 30:575–592.

Agnarsson, I. & J.X. Zhang. 2006. New species of *Anelosimus* (Theridiidae, Araneae) from Africa and Southeast Asia, with notes on sociality and color polymorphism. Zootaxa 1147:1–34.

Arnedo, M.A., J. Coddington, I. Agnarsson & R.G. Gillespie. 2004. From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. Molecular Phylogenetics and Evolution 31:225–245.

Avilés, L., W.P. Maddison & I. Agnarsson. 2006. A new independently derived social spider with explosive colony proliferation and a female size dimorphism. Biotropica 38:743–753.

Bhatnagar, R.D.S. & J.G. Rempel. 1962. The structure, function, and postembryonic development of the male and female copulatory organs of the black widow spider *Latrodectus curacaviensis* (Müller). Canadian Journal of Zoology 40:465–510.

Brigandt, I. 2003. Homology in comparative, molecular, and evolutionary developmental biology: the radiation of a concept. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 299:9–17.

Coddington, J.A. 1983. A temporary slide-mount allowing precise manipulation of small structures. Pp. 291–292. *In* Taxonomy, Biology and Ecology of Araneae and Myriapoda. (O. Kraus, ed.). Verhandlungen das Naturwisschaften Verhandlung, Hamburg, Germany.

Coddington, J.A. 1986. The monophyletic origin of the orb web. Pp. 319–363. *In* Spiders. Webs, Behavior, and Evolution. (W.A. Shear, ed.). Stanford University Press, Stanford, California.

Coddington, J.A. 1990. Ontogeny and homology in the male palpus of orb weaving spiders and their relatives, with comments on phylogeny (Araneoclada: Araneoidea, Deinopoidea). Smithsonian Contributions to Zoology 496:1–52.

Comstock, J.H. 1910. The palpi of male spiders. Annals of the Entomological Society of America 3:161–186.

de Beer, G. 1971. Homology: An Unsolved Problem. Oxford University Press, Oxford, UK. 16 pp.

de Pinna, M.C.C. 1991. Concepts and tests of homology in the cladistic paradigm. Cladistics 7: 367–394.

Eberhard, W.G. 1985. Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge, Massachusetts. 244 pp.

Farris, J.S. 1983. The logical basis of phylogenetic analysis. Pp. 7–36. *In* Advances in Cladistics, Volume 2. (N.I. Platnick & V.A. Funk, eds.). Columbia University Press, New York.

Forster, R.R., N.I. Platnick & J.A. Coddington. 1990. A proposal and review of the spider family Synotaxidae (Araneae, Araneoidea), with notes on theridiid interrelationships. Bulletin of the American Museum of Natural History 193:1–116.

Gerhardt, U. 1921. Vergleichende Studien über die Morphologie des männlichen Tasters und die Biologie der Kopulation der Spinnen. Versuch einer zusammenfassenden Darstellung auf Grund eigener Beobachtungen. Archiv für Naturgeschichte 87:78–247.

Gerhardt, U. 1923. Weitere sexual-biologische Untersuchungen an Spinnen. Archiv für Naturgeschichte 89:1–225.

Grasshoff, M. 1968. Morphologische Kriterien als Ausdruck von Artgrenzen bei Radnetzpinnen der Subfamilie Araneinae. Abhandlungen der senckenbergischen naturforschenden Gesellschaft 516: 1–100.

Grasshoff, M. 1973. Bau und Mechanik der Kopulationsorgane der Radnetzspinne *Mangora acalypha* (Arachnida, Araneae). Zeitschrift für Morphologie der Tiere 74:241–252.

Griswold, C.E. 1993. Investigations into the phylogeny of the lycosoid spiders and their kin (Arachnida: Araneae: Lycosoidea). Smithsonian Contributions to Zoology 539:1–39.

Griswold, C.E. 2001. A monograph of the living world genera and Afrotropical species of cyatholipid spiders (Araneae, Orbiculariae, Araneoidea, Cyatholipidae). Memoirs of the California Academy of Sciences 26:1–251.

Griswold, C.E., J.A. Coddington, G. Hormiga & N. Scharff. 1998. Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea,

Araneoidea). Zoological Journal of the Linnean Society 123:1–99.

Griswold, C.E., J.A. Coddington, N.I. Platnick & R.R. Forster. 1999. Towards a phylogeny of entelegyne spiders (Araneae, Araneomorphae, Entelegynae). Journal of Arachnology 27:53–63.

Griswold, C.E., M.J. Ramírez, J.A. Coddington & N.I. Platnick. 2005. Atlas of phylogenetic data for entelegyne spiders (Araneae: Araneomorphae: Entelegynae) with comments on their phylogeny. Proceedings of the California Academy of Sciences 56:1–324.

Hall, B.K. 1995. Homology and embryonic development. Evolutionary Biology 28:1–37.

Heimer, S. 1982. Interne Arretierungsmechanismen an den Kopulationsorganen männlicher Spinnen (Arachnida, Araneae). Ein Beitrag zur Phylogenie der Araneoidea. Entomologische Abhandlungen (Dresden) 45:35–64.

Heimer, S. 1986. From where are the Linyphiidae derived? Problems of Araneoidea phylogeny (Arachnida: Araneae). Pp. 117–120. *In* Proceedings of the Ninth International Congress of Arachnology (W.G. Eberhard, Y.D. Lubin & B.C. Robinson, eds.). Smithsonian Institution Press, Washington, DC.

Heimer, S. & W. Nentwig 1982. Thoughts on the phylogeny of the Araneoidea Latreille, 1806 (Arachnida, Araneae). Zeitschrift für Zoologische Systematik und Evolutionsforschung 20: 284–295.

Hormiga, G. 1994a. A revision and cladistic analysis of the spider family Pimoidae (Araneoidea, Araneae). Smithsonian Contributions to Zoology 549:1–104.

Hormiga, G. 1994b. Cladistics and the comparative morphology of linyphiid spider and their relatives (Araneae, Araneoidea, Linyphiidae). Zoological Journal of the Linnean Society 111:1–71.

Hormiga G. 2000. Higher level phylogenetics of erigonine spiders (Araneae, Linyphiidae, Erigoninae). Smithsonian Contributions to Zoology 609: 1–160.

Hormiga, G., W.G. Eberhard & J.A. Coddington. 1995. Web construction behavior in Australian *Phonognatha* and the phylogeny of nephiline and tetragnathid spiders (Araneae, Tetragnathidae). Australian Journal of Zoology 43:313–364.

Huber, B.A. 1993. Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae). Canadian Journal of Zoology 71:2437–2447.

Huber, B.A. 1994. Genital morphology, copulatory mechanism and reproductive biology in *Psilochorus simoni* (Berland, 1911) (Pholcidae; Araneae). Netherlands Journal of Zoology 44:85–99.

Huber, B.A. 1995. Copulatory mechanism in *Holocnemus pluchei* and *Pholcus opilionoides*, with notes on male cheliceral apophyses and stridulatory organs in Pholcidae (Araneae). Acta Zoologica, Stockholm 76:291–300.

Huber, B.A. 1996. Genitalia, fluctuating asymmetry, and patterns of sexual selection in *Physocyclus globosus* (Araneae: Pholcidae). Revue Suisse de Zoologie, supplement 1996:289–294.

Huber, B.A. 2003. Rapid evolution and species-specificity of arthropod genitalia: fact or artifact? Organisms Diversity & Evolution 3:63–71.

Knoflach, B. 1991. *Achaearanea tabulata* Levi, eine für Österreich neue Kugelspinne (Arachnida, Aranei: Theridiidae). Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck 78:59–64.

Knoflach, B. 1992. Neue Robertus-Funde in den Alpen: *R. mediterraneus* Eskov und *Robertus* sp. (Arachnida, Aranei: Theridiidae). Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck 79:161–171.

Knoflach, B. 1993a. *Theridion conigerum* Simon—rediscovered in Austria (Araneida: Theridiidae). Bulletin of the British Arachnological Society 9: 205–208.

Knoflach, B. 1993b. Das Mannchen von *Episinus theridioides* Simon (Arachnida: Araneae, Theridiidae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 66:359–366.

Knoflach, B. 1994. Zur Genitalmorphologie und Biologie der *Crustulina*-Arten Europas (Arachnida: Araneae, Theridiidae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 67:327–346.

Knoflach, B. 1995. Two remarkable afromontane Theridiidae: *Proboscidula milleri* n. sp. and *Robertus calidus* n. sp. (Arachnida, Araneae). Revue suisse de Zoologie 102:979–988.

Knoflach, B. 1996a. *Steatoda incomposita* (Denis) from southern Europe, a close relative of *Steatoda albomaculata* (Degeer) (Araneae: Theridiidae). Bulletin of the British Arachnological Society 10:141–145.

Knoflach, B. 1996b. Three new species of *Carniella* from Thailand (Araneae, Theridiidae). Revue suisse de Zoologie 103:567–579.

Knoflach, B. 1996c. Das Männchen von *Simitidion agaricographum* (Levy & Amitai) (Arachnida: Araneae, Theridiidae). Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck 83:149–156.

Knoflach, B. 1996d. Die Arten der *Steatoda phalerata*-Gruppe in Europa (Arachnida: Araneae, Theridiidae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 69:377–404.

Knoflach, B. 1997. Zur Taxonomie, Verbreitung und Sexualbiologie von *Theridion adrianopoli* Drensky (Arachnida: Araneae, Theridiidae). Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck 84:133–148.

Knoflach, B. 1998. Mating in *Theridion varians*

Hahn and related species (Araneae: Theridiidae). Journal of Natural History 32:545–604.

Knoflach, B. 1999. The comb-footed spider genera *Neottiura* and *Coleosoma* in Europe (Araneae, Theridiidae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 72:341–371.

Knoflach, B. 2002. Copulation and emasculation in *Echinotheridion gibberosum* (Kulczynski 1899), Araneae, Theridiidae. Pp. 139–144. *In* European Arachnology 2000. Proceedings of the 19th European Colloquium of Arachnolgy (S. Toft & N. Scharff, eds.). Arhus University Press, Arhus, Denmark.

Knoflach, B. 2004. Diversity in the copulatory behaviour of comb-footed spiders (Araneae, Theridiidae). Denisia, Kataloge der Oberösterreichischen Landesmuseen, Neue Serie 12:161–256.

Knoflach, B. & K. Pfaller. 2004. Kugelspinnen – eine Einführung (Arachnida, Araneae). Denisia, Kataloge der Oberösterreichischen Landesmuseen, Neue Serie 12:111–160.

Knoflach, B. & A. van Harten. 2000. Palpal loss, single palp copulation and obligatory mate consumption in *Tidarren cuneolatum* (Tullgren, 1910) (Araneae, Theridiidae). Journal of Natural History 34:1639–1659.

Knoflach, B. & A. van Harten. 2001. *Tidarren argo* sp. nov. (Araneae: Theridiidae) and its exceptional copulatory behaviour: emasculation, male palpal organ as a mating plug and sexual cannibalism. Journal of Zoology, London 254:449–459.

Knoflach, B. & K. Thaler. 2000. Notes on Mediterranean Theridiidae (Araneae) – I. Memorie della Societa Entomologica Italiana 78:411–442.

Kuntner, M. 2005. A revision of *Herennia* (Araneae, Nephilidae, Nephilinae), the Australasian 'coin spiders'. Invertebrate Systematics 19:391–436.

Kuntner, M. 2006. Phylogenetic systematics of the Gondwanan nephilid spider lineage Clitaetrinae (Araneae, Nephilidae). Zoologica Scripta 35:19–62.

Kuntner, M. 2007. A monograph of *Nephilengys*, the pantropical 'hermit spiders' (Araneae, Nephilidae, Nephilinae). Systematic Entomology 32:95–135.

Ledoux, J.-C. & A. Canard. 1981. Initiation à létude Systematique des Araignée. J.-C. Ledoux, Domazan, Aramon, France. 56 pp.

Lehtinen, P.T. 1967. Classification of the cribellate spiders and some allied families, with notes on the evolution of the suborder Araneomorpha. Annales Zoologici Fennici 4:199–468.

Levi, H.W. 1953a. New and rare *Dipoena* from Mexico and Central America. American Museum Novitates 1639:1–11.

Levi, H.W. 1953b. Spiders of the genus *Dipoena* from America North of Mexico (Araneae, Theridiidae). American Museum Novitates 1647:1–39.

Levi, H.W. 1954a. The spider genera *Episinus* and *Spintharus* from North America, Central America and the West Indies (Araneae: Theridiidae). Journal of the New York Entomological Society 52:65–90.

Levi, H.W. 1954b. The spider genus *Theridula* in North and Central America and the West Indies (Araneae: Theridiidae). Transactions of the American Microscopical Society 73:331–343.

Levi, H.W. 1954c. Spiders of the genus *Euryopis* from North and Central America (Araneae, Theridiidae). American Museum Novitates 1666:1–48.

Levi, H.W. 1954d. Spiders of the new genus *Theridiotis* (Araneae: Theridiidae). Transactions of the American Microscopical Society 73:177–189.

Levi, H.W. 1955a. The spider genera *Chrysso* and *Tidarren* in America (Araneae: Theridiidae). Journal of the New York Entomological Society 63:59–81.

Levi, H.W. 1955b. The spider genera *Coressa* and *Achaearanea* in America north of Mexico (Araneae, Theridiidae). American Museum Novitates 1718:1–33.

Levi, H.W. 1955c. The spider genera *Oronota* and *Stemmops* in North America, Central America and the West Indies (Araneae: Theridiidae). Annals of the Entomological Society of America 48:333–342.

Levi, H.W. 1956. The spider genera *Neottiura* and *Anelosimus* in America (Araneae: Theridiidae). Transactions of the American Microscopical Society 74:407–422.

Levi, H.W. 1957a. The North American spider genera *Paratheridula*, *Tekellina*, *Pholcomma*, and *Archerius* (Araneae: Theridiidae). Transactions of the American Microscopical Society 76:105–115.

Levi, H.W. 1957b. The spider genera *Crustulina* and *Steatoda* in North America, Central America,and the West Indies (Araneae, Theridiidae). Bulletin of the Museum of Comparative Zoology 117:367–424.

Levi, H.W. 1957c. The spider genera *Enoplognatha*, *Theridion*, and *Paidisca* in America north of Mexico (Araneae, Theridiidae). Bulletin of the American Museum of Natural History 112:5–123.

Levi, H.W. 1958. Numbers of species of black widow spiders (Theridiidae: *Latrodectus*). Science 127:1055.

Levi, H.W. 1959a. The spider genera *Achaearanea*, *Theridion* and *Sphyrotinus* from Mexico, Central America and the West Indies (Araneae, Theridi-

idae). Bulletin of the Museum of Comparative Zoology 121:57–163.

Levi, H.W. 1959b. The spider genus *Coleosoma* (Araneae, Theridiidae). Breviora of the Museum of Comparative Zoology 110:1–8.

Levi, H.W. 1959c. The spider genus *Latrodectus* (Araneae: Theridiidae). Transactions of the American Microscopical Society 78:7–43.

Levi, H.W. 1960. The spider genus *Styposis* (Araneae, Theridiidae). Psyche 66:13–19.

Levi, H.W. 1961. Evolutionary trends in the development of palpal sclerites in the spider family Theridiidae. Journal of Morphology 108:1–9.

Levi, H.W. 1962a. More American spiders of the genus *Chrysso* (Araneae, Theridiidae). Psyche 69:209–237.

Levi, H.W. 1962b. The spider genera *Steatoda* and *Enoplognatha* in America (Araneae, Theridiidae). Psyche 69:11–36.

Levi, H.W. 1963a. The American spider genera *Spintharus* and *Thwaitesia*. Psyche 70:223–234.

Levi, H.W. 1963b. American spiders of the genera *Audifia*, *Euryopis* and *Dipoena* (Araneae: Theridiidae). Bulletin of the Museum of Comparative Zoology 129:121–185.

Levi, H.W. 1963c. American spiders of the genus *Achaearanea* and the new genus *Echinotheridion* (Araneae, Theridiidae). Bulletin of the Museum of Comparative Zoology 129:187–240.

Levi, H.W. 1963d. The American spiders of the genus *Anelosimus* (Araneae, Theridiidae). Transactions of the American Microscopical Society 82:30–48.

Levi, H.W. 1963e. American spiders of the genus *Theridion* (Araneae, Theridiidae). Bulletin of the Museum of Comparative Zoology 129:481–589.

Levi, H.W. 1963f. The spider genera *Cerocida*, *Hetschkia*, *Wirada* and *Craspedisia* (Araneae: Theridiidae). Psyche 70:170–179.

Levi, H.W. 1964a. The American spiders of the genera *Styposis* and *Pholcomma* (Araneae, Theridiidae). Psyche 71:32–39.

Levi, H.W. 1964b. American spiders of the genus *Episinus* (Araneae: Theridiidae). Bulletin of the Museum of Comparative Zoology 131:1–25.

Levi, H.W. 1964c. American spiders of the genus *Phoroncidia* (Araneae: Theridiidae). Bulletin of the Museum of Comparative Zoology 131:65–86.

Levi, H.W. 1964d. The spider genera *Stemmops*, *Chrosiothes*, and the new genus *Cabello* from America. Psyche 71:73–92.

Levi, H.W. 1964e. The spider genus *Helvibis* (Araneae, Theridiidae). Transactions of the American Microscopical Society 83:133–142.

Levi, H.W. 1964f. The spider genus *Thymoites* in America (Araneae: Theridiidae). Bulletin of the Museum of Comparative Zoology 130:445–471.

Levi, H.W. 1966. The three species of *Latrodectus* (Araneae), found in Israel. Journal of Zoology, London 150:427–432.

Levi, H.W. 1967a. Cosmopolitan and pantropical species of theridiid spiders (Araneae: Theridiidae). Pacific Insects 9:175–186.

Levi, H.W. 1967b. Habitat observations records and new South American theridiid spiders (Araneae, Theridiidae). Bulletin of the Museum of Comparative Zoology 136:21–37.

Levi, H.W. 1967c. The theridiid spider fauna of Chile. Bulletin of the Museum of Comparative Zoology 136:1–20.

Levi, H.W. 1968. The spider family Hadrotarsidae and the genus *Hadrotarsus*. Transactions of the American Microscopical Society 87:141–145.

Levi, H.W. 1969. Notes on American theridiid spiders. Psyche 76:68–73.

Levi, H.W. 1972. Taxonomic-nomenclatorial notes on misplaced theridiid spiders (Araneae: Theridiidae) with observations on *Anelosimus*. Transactions of the American Microscopical Society 91:533–538.

Levi, H.W. & L.R. Levi. 1962. The genera of the spider family Theridiidae. Bulletin of the Museum of Comparative Zoology 127:1–71.

Levy, G. 1998. Fauna Palaestina. Arachnida III. Araneae: Theridiidae. Israel Academy of Sciences and Humanities, Jerusalem. 226 pp.

Menge, A. 1866. Preußische Spinnen. Erste Abtheilung. Schriften der Naturforschenden Gesellschaft in Danzig (N.F.) 1:1–152

Menge, A. 1868. Preußische Spinnen. II. Abtheilung. Schriften der Naturforschenden Gesellschaft in Danzig (N.F.) 2:153–218.

Menge, A. 1869. Preußische Spinnen. III. Abtheilung. Schriften der Naturforschenden Gesellschaft in Danzig (N.F.) 2:219–264.

Merrett, P. 1963. The palpus of male spiders of the family Linyphiidae. Proceedings of the Zoological Society of London 140:347–467.

Miller, J.A. 2007. Review of erigonine spider genera in the Neotropics (Araneae: Linyphiidae, Erigoninae). Zoological Journal of the Linnean Society 149. Supplement 1. Pp. 1–263.

Miller, J.A. & I. Agnarsson. 2005. A redescription of *Chrysso nigriceps* (Araneae, Theridiidae) with evidence for maternal care. Journal of Arachnology 33:711–717.

Miller, J.A. & G. Hormiga. 2004. Clade stability and the addition of data: A case study from erigonine spiders (Araneae: Linyphiidae, Erigoninae). Cladistics 20:385–442.

Nelson, G.J. 1978. Ontogeny, phylogeny, paleontology and the biogenetic law. Systematic Zoology 27:324–345.

Osterloh, A. 1922. Beiträge zur Kenntnis des Kopulationsapparates einiger Spinnen. Zeitschrift für Wissenschaftliche Zoologie 119:29–421.

Patterson, C. 1982. Morphological characters and

homology. Pp. 21–74. *In* Problems of Phylogenetic Reconstruction. (K.A. Joysey & A.E. Friday, eds.). Academic Press, London.

Platnick, N.I. 2006. The World Spider Catalog, version 6.5. American Museum of Natural History, New York. Online at http://research.amnh.org/entomology/spiders/catalog/index.html

Rieppel, O. 1994. Homology, topology, and typology: the history of modern debates. Pp. 63–100. *In* Homology: The Hierarchical Basis of Comparative Biology. (B.K. Hall, ed.). Academic Press, San Diego, California.

Rieppel, O. 2001. Preformationist and epigenetic biases in the history of the morphological character concept. Pp. 55–75. *In* The Character Concept in Evolutionary Biology. (G.P. Wagner, ed.). Academic Press, San Diego, California.

Rieppel, O. & M. Kearney. 2002. Similarity. Biological Journal of the Linnean Society 75:59–82.

Saaristo, M.I. 1978. Spiders (Arachnida, Araneae) from Seychelles Islands, with notes on taxonomy. Annales Zoologici Fennici 15:99–126.

Saaristo, M.I. 2006. Theridiid or cobweb spiders of the granitic Seychelles islands (Araneae, Theridiidae). Phelsuma 14:49–89.

Scharff, N. & J.A. Coddington. 1997. A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). Zoological Journal of the Linnean Society 120:355–434.

Shear, W.A. 1967. Expanding the palpi of male spiders. Breviora of the Museum of Comparative Zoology 259:1–27.

Sierwald, P. 1990. Morphology and homologous features in the male palpal organ in Pisauridae and other spider families, with notes on the taxonomy of Pisauridae (Arachnida: Araneae). Nemouria, Occasional Papers of the Delaware Museum of Natural History 35:1–59.

Uhl, G., B.A. Huber & W. Rose. 1995. Male pedipalp morphology and copulatory mechanism in *Pholcus phalangioides* (Fuesslin, 1775) (Araneae, Pholcidae). Bulletin of the British Arachnological Society 10:1–9.

Wagner, G.P. 2005. The developmental evolution of avian digit homology: an update. Theoretical Biosciences 124:165–183.

Wunderlich, J. 1978. Zur Taxonomie und Synonymie der Taxa Hadrotarsidae, *Lucarachne* Bryant 1940 und *Flegia* C.L. Koch & Berendt 1854, (Arachnida: Araneida: Theridiidae). Zoologische Beiträge 24:25–31.

Appendix A – Abbreviations

BC	bulb-cymbium lock mechanism
BH	basal haematodocha
C	conductor
CA	cymbial apophysis
Cb	conductor base
CHd	theridiid cymbial hood
CHk	theridiid cymbial hook
Cy	cymbium
E	embolus
EA	embolic apophysis
Eb	embolic division b
ES	embolic sclerite
ETS	extra tegular sclerite
MA	median apophysis
MH	median haematodocha
PC	paracymbium
SC	subconductor
ST	subtegulum
T	tegulum
Tp	tegular pit
TTA	theridiid tegular apophysis

Appendix B – Material Examined (deposited in the CTh Collection Thaler & Knoflach)

For additional material examined, see Agnarsson (2004).

Achaearanea lunata (Clerck 1757). Austria, Northern Tyrol, Innsbruck, Hötting, 15 May 1992, leg. Knoflach.

Achaearanea riparia (Blackwall 1834). Italy, Treviso, Quartier del Piave, Palu, pitfall trap, 1989/1990, leg. Targa.

Crustulina guttata (Wider 1834). Austria, Northern Tyrol, Ötztal, Längenfeld, 14 April 1992, leg. Knoflach.

Dipoena melanogaster (C.L. Koch 1837). Austria, Northern Tyrol, Innsbruck, Hötting, 15 May 1992, leg. Knoflach.

Enoplognatha latimana Hippa and Oksala 1982. Austria, Burgenland, Parndorf, 1988, leg. Thaler, Meyer, Steinberger.

Enoplognatha ovata (Clerck 1757). Austria, Northern Tyrol, Innsbruck, Martinswand, 3 August 1991, leg. Knoflach. Telfs, Zimmerberg, 17 July 1991, leg. Bertrandi. Ötztal Bahnhof, Forchet, 16 May 1992, leg. Knoflach. Kufstein, Langkampfen, tree eclector, 28 June–23 July 1988, leg. Thaler, Meyer, Steinberger.

Enoplognatha thoracica (Hahn 1833). Italy, Veneto, Treviso, pitfall trap, 1990–1991, leg. Schiroto, Paletti.

Episinus angulatus (Blackwall 1836). Austria, Northern Tyrol, Innsbruck, Kranebitten, 12 July 1991, leg. Knoflach.

Episinus theridioides Simon 1873. France, Corsica, Col de Vizzavona, 1100–1400 m, 1 October 1974, leg. Thaler.

Episinus truncatus Latreille 1809. Austria, Northern Tyrol, Innsbruck, Kranebitten, 20 July 1991, leg. Knoflach.

Euryopis flavomaculata (C.L. Koch 1836). Austria, Vienna, Lobau, 19 May–2 June 1972, leg. Steiner.

Keijia tincta (Walckenaer 1802). Austria, Northern Tyrol, Innsbruck, Kranebitten, 10 May 1991, leg. Knoflach.

Kochiura aulica (C.L. Koch 1838). Croatia, Rovinj, 29 July–26 August 1965, leg. Thaler.

Lasaeola tristis (Hahn 1833). Austria, Northern Tyrol, Ötztal, Sautener Forchet, 26 June 1992, leg. Bertrandi.

Neottiura bimaculata (Linné 1767). Austria, Northern Tyrol, Innsbruck, Kranebitten, 23 June 1991, leg. Knoflach.

Pholcomma gibbum (Westring 1851). Austria, Northern Tyrol, Innsbruck surroundings, Halltal, 13 June 1992, leg. Thaler.

Robertus neglectus (O. Pickard-Cambridge 1871). Italy, Veneto, Treviso, Riese, pitfall trap 1990–1991, leg. Schiroto, Paoletti. Germany, near Immendingen, Zimmern, leg. Wunderlich 1973.

Robertus scoticus Jackson 1914. Austria, Carinthia, Großglockner 1700 m, pitfall trap, 1978, leg. Thaler.

Robertus ungulatus Vogelsanger 1944. Austria, Northern Tyrol, Innsbruck surroundings, Lanser Moor, fen, pitfall trap, 14 May–18 September 1963, leg. Thaler.

Rugathodes bellicosus (Simon 1873). Austria, Northern Tyrol, Obergurgl, 2600 m, 26 June 1992, leg. Thaler.

Simitidion simile (C.L. Koch 1836). Italy, Trentino, Civezzano, 30 April 1990, leg. Foddai.

Steatoda bipunctata (Linné 1758). Austria, Northern Tyrol, Innsbruck, Hötting, November 1992, leg. Knoflach.

Steatoda triangulosa (Walckenaer 1802). Italy, Toscana, Grosseto, Castiglione, 8 June 1987, leg. Thaler.

Theonoe minutissima (O. Pickard-Cambridge 1879). Germany, Kempten, Schorenmoos, 15 December 1974–17 May 1975, leg. Mendl.

Theridion conigerum Simon 1914. Germany, Oberharz, Ilsenhütte, June 1972, Staatliches Museum für Tierkunde Dresden, leg. Heimer.

Theridion nigrovariegatum Simon 1873. Switzerland, Unterengadin, Ramosch, 12 July 1987, leg. Thaler.

Theridion ohlerti (Thorell 1870). Austria, Northern Tyrol, Kühtai, 2200 m, 18 June 1992, leg. Bertrandi.

Theridion petraeum L. Koch 1872. Austria, Northern Tyrol, Innsbruck surroundings, Patscher-kofel, 2200 m, 7 July 1991, leg. Knoflach.

Theridion pictum (Walckenaer 1802). Austria, Northern Tyrol, Innsbruck West, university surroundings, 14 May 1992, leg. Knoflach.

Theridion pinastri L. Koch 1872. Austria, Northern Tyrol, Ötztal, Sautener Forchet, 26 June 1992, leg. Bertrandi.

Theridion sisyphium (Clerck 1757). Austria, Northern Tyrol, Innsbruck surroundings, Gnadenwald, 11 June 1991, leg. Bertrandi.

Manuscript received 14 June 2006, revised 22 May 2007.

2007. The Journal of Arachnology 35:396–406

SUBLETHAL EXPOSURE TO A NEUROTOXIC PESTICIDE AFFECTS ACTIVITY RHYTHMS AND PATTERNS OF FOUR SPIDER SPECIES

William J. Tietjen[1] and **Alan B. Cady**[2,3]: [1]Department of Biology, Bellarmine University, Louisville, Kentucky 40205 USA; [2]Department of Zoology, Miami University, Oxford, Ohio 45056 USA

ABSTRACT. Four species from three families of spiders were exposed to sublethal concentrations of the neurotoxic pesticide malathion: *Schizocosa ocreata* (Hentz 1844), *Rabidosa rabida* (Walckenaer 1837), *Frontinella communis* (Hentz 1850), and *Salticus scenicus* (Clerck 1757). Spider activity was recorded using a proprietary computer vision system equipped with artificial intelligence routines. Exposure to malathion changed the spiders' propensity to move, levels and patterns of activity, and distance moved. Dosed spiders increased their activity between 12 and 40%, depending on the species. Continuous recordings for ≥ 24 h revealed the peak activity for dosed *R. rabida* and *S. scenicus* was shifted ~ 1 h earlier than controls. Spiders exposed to malathion also significantly increased the distance they moved per locomotory bout. This is consistent with the action of an organophosphate neurotoxin acting as an acetylcholinesterase inhibitor. Thus, exposure to sublethal doses of malathion appears to affect the neural basis for these spider's normal diel periodicities, time budgets, and patterns of locomotion, probably reducing their efficiency as agents of biological control.

Keywords: *Rabidosa*, *Salticus*, *Schizocosa*, *Frontinella*, malathion, behavior

Many insecticides kill arthropods by affecting the nervous or endocrine systems. Since an animal's behavior is governed by interactions among nerve and/or endocrine cells, it is not surprising that low and sublethal doses of pesticides influence behavior. Insecticides used to control insect pests can also affect spider populations either directly (through death) or indirectly (via changes in behavior or physiology). Given the likely importance of spiders in insect control (Mansour et al. 1980; Riechert 1998, 1999; Maloney et al. 2003), pesticide toxicity has been evaluated for these important predators. Where the susceptibility of several spider species to 30 pesticides was tested, toxicity ranged from no mortality (biological compounds, herbicides, fungicides), to medium mortality (pyrethrins, organophosphates, carbamates), and high toxicity cyclocompounds (Mansour & Nentwig 1988).

Field trials investigating the impact of some toxins on spiders have shown varying responses. The application of broad-spectrum insecticides in apple orchards significantly decreased spider and harvestman populations (Epstein et al. 2000). Populations of lycosid, linyphiid, and other non-insect arthropod predators (harvestmen and centipedes) in fields sprayed with organophosphates (at standard application rates) versus water did not change their densities as sampled by pitfall trapping (Hodge & Vink 2000). Other tests and field surveys have suggested that many insecticides have little effect on spider population densities (Riechert & Lockley 1984; Hilburn & Jennings 1988; VanDenBerg et al. 1990) leading Integrated Pest Management (IPM) researchers to rate the risk of these toxins to beneficial arthropods as low (Higley & Wintersteen 1992). A recent review, however, cautions that some pesticides, even in concentrations below recommended field application rates, can result in high mortality (Maloney et al. 2003).

Sublethal doses of insecticides may affect the physiology and behavior of insects (Haynes 1988; Longley & Jepson 1996; Delpuech et al. 1998; Venkateswara et al. 2005) and arachnids (Chu et al.1976, 1977; Samu & Vollrath 1992; Amalin et al. 2000; James &

[3] Corresponding author. E-mail: cadyab@muohio.edu

Price 2002; Tietjen 2006). Low doses of malathion (1,2-di(ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate) increased walking speed of wasps, while other compounds caused continuous wing-fanning and/or flight (Haynes 1988). For arachnids, exposure to formamindine compounds affected the activity and dispersal of some mites and ticks (Hollingworth 1976).

Most research on sublethal doses of pesticides has concentrated on the economically-important insects (Haynes 1988) with few studies assessing their effects on spider biology even though spiders are important generalist predators. More investigations have focused on measuring direct mortality to spiders (Amalin et al. 2000; Epstein et al. 2000; Van Erp et al. 2002) rather than observing behavioral changes resulting from pesticide exposure (Samu & Vollrath 1992; Shaw et al. 2006). Hodge & Vink (2000) indicated that although lycosid and linyphiid spiders may not be good bioindicator candidates, they should be investigated to elucidate physiological responses from sublethal exposure. Individuals of *Lycosa hilaris* surviving a single exposure to diazinon in the field showed 87% inhibition of cholinesterase activity (Van Erp et al. 2002), making this enzyme in wolf spiders a possible biomarker for organophosphate contamination.

Preliminary observations in the laboratory suggested that spiders dosed with sublethal concentrations of malathion were more active than control animals (Tietjen unpubl. data). To further explore this observation, methods were developed to determine if altered spider activity is measurable and if the underlying mechanisms producing these changes in activity could be identified. Because different species of insects have varying responses to insecticides (Haynes 1988), several spider species from differing families and with different foraging strategies were tested. Results show that exposure to malathion changed the spiders' propensity to move, their levels and patterns of activity, and the distance moved during locomotor activity.

METHODS

Test animals.—Data were collected for the salticid *Salticus scenicus* (Clerck 1757), the linyphiid *Frontinella communis* (Hentz 1850), and the lycosids *Schizocosa ocreata* (Hentz 1844) and *Rabidosa rabida* (Walckenaer 1837). All spiders (except *S. scenicus*) were collected from an old field on the Kentucky State University Research Farm (38.2° N, 84.9° W) that had been pesticide-free for ≥ 10 yr. *Salticus scenicus* were gathered from the surface of old grain bins adjacent to the field. Spiders were collected during the 1996–2000 field seasons.

All species (except *Frontinella*) were housed in 14-cm diam × 2.5-cm high plastic Petri dishes positioned upside-down so water could be delivered in the gap between the top and bottom of the dish. A 2-cm diam access hole was cut in the top of the dish to permit delivery of food items (the hole was corked when not in use). *Frontinella* were housed in 8.5-cm × 5-cm dishes and provided water through a moistened cotton wad in the container lid. Spiders were fed a natural diet of appropriately-sized insects (obtained via sweep-netting) on alternate days. Filter paper was the substratum for all species.

For all species (except *S. ocreata*), penultimate or antepenultimate spiders were captured and raised to adulthood in the laboratory, and only adult females were used to reduce variance due to gender. *Schizocosa ocreata* spiderlings tested were from the same egg sac when < 1 wk old. Data were collected during their early communal behavior before dispersal of the spiderlings, commencing on day-three post-eclosion.

Dosing procedures.—Adult female spiders were exposed for 24 h to 10 µl of 10^{-5} malathion (1,2-di(ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate) (w/w distilled water) applied to the center of a filter paper substrate covering the bottom of a standard glass Petri dish. Control animals were exposed to 10 µl of distilled water under the identical protocol. The 24-h exposure was chosen based on preliminary tests of mortality and to allow potential variation in individual activity to be summed over a day, thus minimizing variations in pesticide exposure among animals. Spiders were not exposed to pesticides by spraying because of potential problems delivering a replicable dose to the animals. Spiders were tested 24 h after exposure to allow for potential recovery and to ensure that no animals died as a result of pesticide application. *Frontinella* females were returned to their webs after exposure to the pesticide.

Schizocosa ocreata spiderlings could not be

Table 1.—Mean distance (± SD) moved by four spider species. "Dosed" spiders were exposed to malathion, while controls were not. Treatment group sample sizes are indicated in parentheses. The length of recording time for each experiment also is indicated. Statistical analyses depicted are from a one-way ANOVA across spider species.

Species	Time	Distance moved		ANOVA
		Control spiders	Dosed spiders	
Schizocosa ocreata, Lycosidae	15 min	1.1 mm ± 1.97 (20)	1.3 mm ± 1.94 (27)	$F = 8.81$, $P < 0.003$
Frontinella communis, Linyphiidae	24 h	1.1 cm ± 9.6 (20)	1.5 cm ± 10.08 (20)	$F = 84.66$, $P < 0.0001$
Rabidosa rabida, Lycosidae	24 h	3.5 cm ± 8.14 (40)	5.8 cm ± 9.72 (40)	$F = 272.72$, $P < 0.0001$
Salticus scenicus, Salticidae	7 da	1.5 cm ± 1.39 (22)	1.7 cm ± 1.22 (17)	$F = 4.79$, $P < 0.02$

dosed using this technique because they produced a silk platform separating them from contact with the pesticide. Therefore, a vacuum-dosing technique modeled after Pluthero & Threlkeld (1980) was used. Spiderlings were introduced into a chamber in groups of five. The atmosphere was partially evacuated from the chamber and then the chamber was pierced with a syringe needle containing 10 µl of the dosing compound (10^{-5} malathion or distilled water). The sudden change in atmospheric pressure caused the solution to enter the chamber and vaporize. Spiderlings were covered with a fine mist and the compound was forced into the booklungs. Spiderlings remained in the chamber for 1 min and were tested 24 h later. Adult spiders were not dosed using this technique because preliminary work indicated that some would become active and contact the condensate on the interior of the dosing chamber. Others would remain inactive and had little excess exposure to the condensate. These differential behavioral responses to vacuum and the sudden return to normal pressure would increase dosage variability for the adults compared to the filter paper technique.

An initial examination of dosed animals found they were not overtly adversely affected by pesticide exposure. The coordination of their locomotion, grooming, and other general behaviors appeared to be normal when compared to control animals. A light tap on the cages of dosed spiders elicited a startle response that was not seen for control animals, but otherwise the general responses of the two groups were similar. None of the dosed animals nor the control animals died during the experiment (except a few *S. scenicus* during the long-term observations; see below).

Recording spider activities.—Spider activity was recorded using a computer-controlled digital camera (EDC 1000, Electrim Corporation, Princeton, NJ, USA). Methods were similar to those described by Tietjen (1980, 1981, 1982). During recording, spiders (except *Frontinella*) were housed in plastic Petri dish arenas with a filter paper substratum. The diameter of the arena was selected relative to the animal size (5 cm for *S. ocreata* spiderlings, 8.5 cm for *F. communis*, and *S. scenicus*, and 13.5 cm for *R. rabida*). Female *F. communis* were observed while on their own webs built in their container 2 wk before dosing. The camera was suspended from the ceiling by a tripod head that allowed the apparatus to focus on spiders of varying size. For each run a cm ruler was placed in the field of view to permit metric conversion.

Spider recordings with single *S. ocreata*, *F. communis*, & *R. rabida*.—One version of the camera software permitted the logging of a single spider at a time. This was used to record the activity of *S. ocreata* spiderlings and *F. communis* and *R. rabida* females. Recording intervals were set at one frame per 10 s (except for *F. communis*, which recorded at one frame per min). For each interval, the current time and X/Y coordinates of the spider were logged to a file. These coordinates were serially accumulated to enable frequency and distance measurements over time. For *S. ocreata*, movements were recorded between 20:00 and 21:30 (10-s intervals, Table 1) in

an effort to minimize circadian effects. Illumination for this species was provided by a 40 W incandescent lamp positioned at a 30° angle to the recording arena, approximately 2 m away from the test animals, and was not varied over the recording period for either species. Recordings of *F. communis* and *R. rabida* females ran for 24 h (1-min intervals, Table 1), and ambient illumination was provided by a skylight. Data collection alternated between recording dosed and control spiders. New arenas were used for each run.

Long-term recordings with multiple *S. scenicus*.—Improvements to the software allowed simultaneous recording of up to 10 animals individually-housed in Petri dishes for up to 7 d. For these observations, *S. scenicus* were tested in groups of 5 dosed and 5 control animals. The computer was programmed to continuously sample and adjust the camera's exposure, allowing automated recording of spiders under natural photoperiod provided by a skylight in the recording room. The system adjusted to passing clouds, bright sunlight, and inclement weather. During the evening hours infrared LEDs illuminated the recording area since the camera was sensitive to these wavelengths while spiders are not (Crain 1949; DeVoe et al. 1969). The temperature and humidity in the recording room tracked outdoor conditions. The arenas were surrounded with black paper rings to visually isolate the test animals. In addition, arenas were set in a sand basin placed on a granite base supported by partially-evacuated tennis balls. This apparatus dampened vibrations caused either by animal activities in adjacent arenas or human activities in a nearby room. Vibrations also were minimized by the first-floor location of the recording room and a concrete-slab floor separated from the rest of the building structure. (Vibration-reduction techniques were not employed when recording the activities of *S. ocreata*, *R. rabida*, and *F. communis*. This is not considered to be problematic for these species since their activities were recorded individually and not in groups of ten.) Sampling rates for groups of *S. scenicus* were one image/10 s. During the recording period, spiders were unattended to avoid external stimuli.

Salticus scenicus was chosen for the long-term observations since they were available as adults from late spring to early fall, whereas *R. rabida* were available for collection for 3 wk and would survive as adults in the lab for only ~ 6 wk. Therefore, testing 80 *Rabidosa* for a week in groups of 10 would not have been possible. *Salticus scenicus* were undisturbed for each 7-da recording period. Water was supplied continuously to the edges of the arenas by a wicking system. Prey were not introduced while recording because previous observations indicated that dosed animals often did not attempt prey-capture, and the artificial intelligence routines of the machine-vision program were not sufficiently robust to reliably differentiate between spiders and prey located in the same container. Thus, feeding during the recording period would add an uncontrollable variable. Initiation of the experimental runs was timed with the feeding schedule so *S. scenicus* received food the day before the test. Of the 25 spiders in each treatment, one control and two of the dosed animals died during the long-term recordings. Two control and six of the dosed animals were removed from analysis because they appeared to suffer from dehydration caused by failure of the water-delivery system.

Analyses.—The activity levels, expressed as distance-moved, were analyzed using conventional statistics (Stata, Stata Corporation, College Station, TX, USA). For all but *S. ocreata*, the time that individuals moved were also analyzed with circular statistics (Batschelet 1965). In addition, *R. rabida* and *S. scenicus* data were teased-apart through a Fourier analysis (Tietjen 1982) to inspect frequency and duration information. Because the data were collected at 10 s intervals, over 2.3 million data points were generated from the long-term observations of *S. scenicus*, making data sets too large for the available commercial statistical packages. Therefore, data were pooled into 1-min intervals, reducing the data set to less than 400,000 points. Those one-minute intervals having no movement also were discarded, further reducing the data set to 43,000 points without affecting the analyses, and allowing the use of standard statistics packages. Although the original 10 s interval data sets for spiders (except *S. scenicus*) were considerably smaller, only the *F. communis* data could have been directly analyzed. Therefore, all 24-h data (*S. ocreata*, *F. communis*, *R. rabida*) were analyzed at a 1-min resolution.

Voucher specimens of spider species used

Table 2.—Circular statistical analyses for control and "dosed" (= exposed to malathion) spiders (*Frontinella pyramitella*, Linyphiidae; *Rabidosa rabida*, Lycosidae; *Salticus scenicus*, Salticidae). The mean vector (μ) is the mean peak activity "strength" expressed as the length of μ (r) and the circular standard deviation. Significance in the "Raleigh Test" column indicates that the spiders had a preferred time for peak activity. Significance for Watson's F-test indicates that control and dosed spiders had a shift in the time of peak activity. Sample sizes are in parentheses.

Species and treatment	Mean vector (μ)	Length of μ (*r*)	Circular standard deviation	Raleigh test of uniformity	Watson's F-test for two circular means
R. rabida control (40)	08:16	0.232	94.80°	$P < 0.0001$	$F = 84.91$, $P < 0.0001$
R. rabida dosed (40)	07:25	0.254	97.99°	$P < 0.0001$	
S. scenicus control (22)	14:02	0.470	70.31°	$P < 0.0001$	$F = 462.92$, $P < 0.0001$
S. scenicus dosed (17)	12:45	0.654	52.74°	$P < 0.0001$	
F. communis control (20)	14:46	0.01	171.00°	$P > 0.05$	not applicable
F. pyramitella dosed (20)	07:08	0.08	129.75°	$P > 0.01$	

here are deposited in the arthropod collection of Miami University, Oxford, Ohio.

RESULTS

Distance moved and circadian rhythms.—The distances moved per time period for all spider species significantly increased when dosed with malathion (Table 1). A circular analysis of the daily time data for *R. rabida* and *S. scenicus* indicated that their activity over 24-h periods was not uniform (Raleigh Test, $P < 0.0001$; Table 2). In addition, there was a change in the mean peak activity time for these two species. Control *R. rabida* females had a peak activity at 08:16 while the peak activity for dosed animals was at 07:25, a progression of nearly 1 h (Fig. 1). Dosed *S. scenicus* females also shifted their peak activity to over an hour earlier in the day from 14:02 for the controls to 12:45 for the dosed spiders (Fig. 1). These activity shifts were significant for both species (Watson's F-Test, $P < 0.0001$; Table 2). Dosed female *S. scenicus* also showed several bursts of increased activity during evening hours between 22:00 and 05:00, while spurious activity was not apparent for *R. rabida* (Fig. 1).

Female *F. communis* movements also were analyzed using circular statistics. Control *F. communis* had a non-significant activity peak at 14:46 (Watson's F-Test, $P > 0.05$; Table 2). Dosed *F. communis* had a significant peak activity at 07:08 (Watson's F-Test, $P < 0.01$; Table 2). Because only one of the treatments had a significant activity peak, Watson's F-Test for two circular means was not performed. The large circular standard deviation (almost 180 degrees short of a full circle for the control group) may be an artifact caused by constant lighting conditions during the recording period. It is important to note, however, that this does not affect the analyses related to distance-moved since those data are independent of and not coupled to timing of activity bouts. Since both control and experimental spiders were tested under constant-light conditions, the differences in distance-moved can only be explained by the experimental protocol.

Patterns of activity.—Fourier analyses were performed on data recorded from *R. rabida* and *S. scenicus* to investigate the small-scale patterning of activity that summate to produce a circadian rhythm (Table 3; Figs. 2, 3). The power spectra for *R. rabida* indicate that dosed spiders showed higher frequencies at higher power compared to control animals (Table 3). Spectral components were generally more well-defined for dosed animals, and there were changes in the low-frequency portions of the spectra (Fig. 2). The shift by dosed animals to shorter time intervals with higher power reflects the earlier activity peaks observed in dosed *R. rabida* (Fig. 1).

An examination of the power spectra for *S. scenicus*, on the other hand, indicates a longer periodicity and lower power for dosed animals (Table 3). Also note that the power for each time period for dosed animals is less than half that seen in the controls (Fig. 3), which is a greater difference than observed for *R. rabida*. In addition, the power spectra for *S. scenicus* have a lower noise component when com-

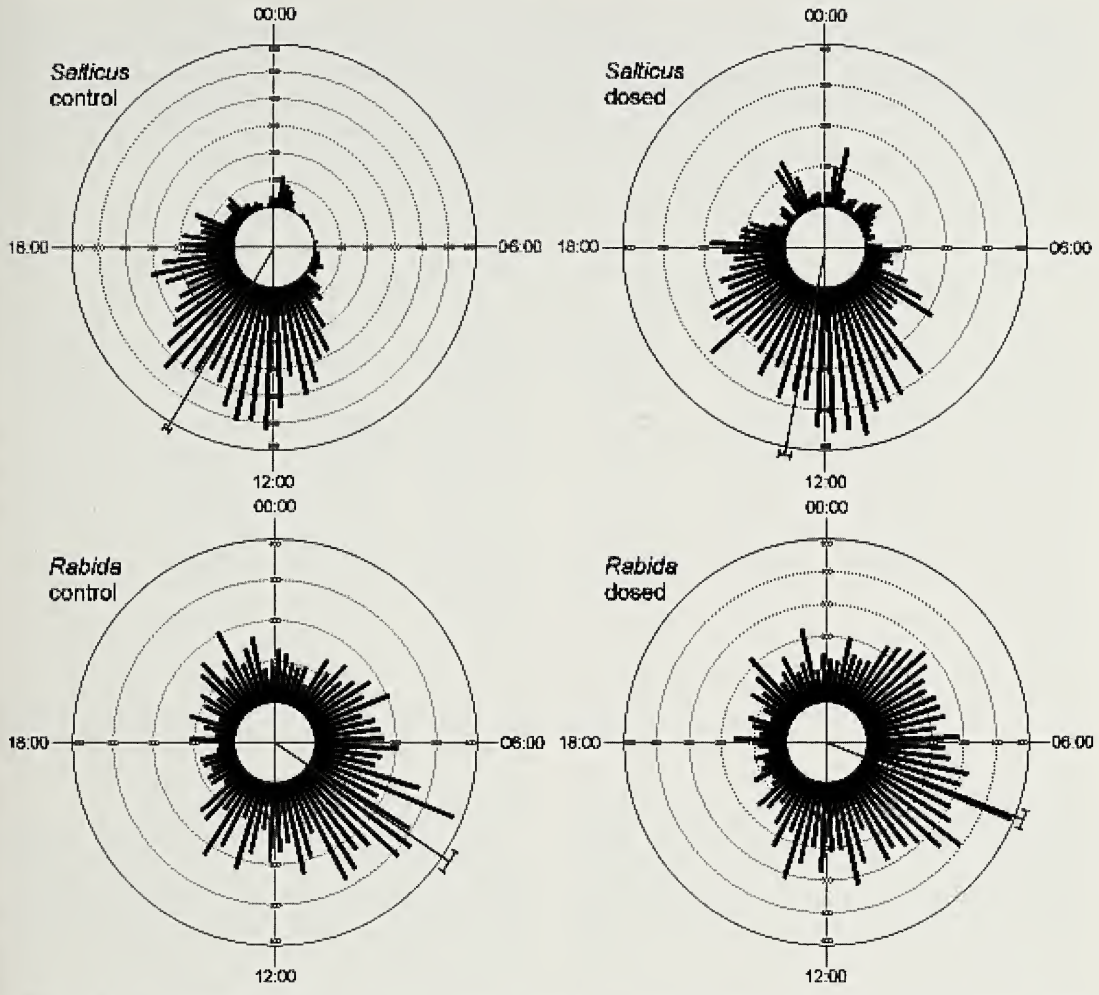


Figure 1.—Circadian responses of two spider species to malathion. Circular graphs depict a 24-h period to show changes in the circadian activity of control *Salticus scenicus* (Salticidae) and *Rabidosa rabida* (Lycosidae) compared to animals dosed with malathion. The indicated variations of the mean vectors are the 95% confidence limits. Sample sizes: *R. rabida* control (c) and dosed (d) = 40; *S. scenicus* c = 22, d = 17.

pared to that of *R. rabida* (compare Figs. 2, 3). Interestingly, many of the spectral components over most of the spectral range for dosed and control animals align with one another (Figs. 2, 3). Thus, a different mechanism must be responsible for the observed shifts in mean activity rhythms of *S. scenicus* versus *R. rabida*, suggesting different behavioral/physiological mechanisms controlling circadian rhythms in these two species.

An independent measure of the time-pattern elucidated by the Fourier analysis can be obtained by comparing the number of bouts of movement among the species and treatments. The number of movement-bouts for control *R. rabida* was 11,304 while dosed animals significantly decreased their movement-bouts to 10,721 ($\chi^2 = 15.43$, $df = 1$, $P > 0.001$). Thus, since dosed *R. rabida* moved longer distances than control spiders (Table 1), they must have a propensity to move farther once a movement-bout begins. Coupled with the higher frequency and power spectra illustrated from the Fourier analysis, these results explain the greater distance moved by dosed *R. rabida* compared to controls (Table 1).

The number of movement-bouts for dosed *S. scenicus* (28,957.18; adjusted) showed a significant increase of 29.4% compared to control animals (20,772; $\chi^2 = 1347.24$, $df =$

Table 3.—Power spectra of activity for control and "dosed" (= exposed to malathion) spiders *Rabidosa rabida* (Lycosidae) and *Salticus scenicus* (Salticidae). The ten strongest spectral components and their frequencies (expressed as time) are presented. Data are sorted in descending order based on power. Dosed *R. rabida* had higher frequencies at higher power compared to control animals, indicating earlier activity peaks for those spiders exposed to malathion. Dosed *S. scenicus* had longer periodicity and lower power than controls, showing dosed spiders had a shift of activity rhythm similar to *R. rabida*, but with a different pattern of activity from that of *R. rabida*.

Rabidosa rabida				*Salticus scenicus*			
Control		Dosed		Control		Dosed	
Time	Power	Time	Power	Time	Power	Time	Power
00:00:16	28,473.08	00:00:50	37,775.29	00:00:16	937,233.87	00:00:16	350,529.26
00:00:50	19,021.45	00:00:16	25,704.70	00:08:11	76,580.08	00:08:11	21,683.22
00:01:24	6,756.55	00:01:24	9,986.70	00:17:14	38,015.42	00:01:24	14,351.12
18:30:14	5,642.32	08:01:47	8,359.91	00:35:19	33,560.74	01:11:29	13,916.73
07:05:33	5,575.28	06:56:13	7,755.35	00:03:40	32,688.73	02:23:49	11,719.87
04:48:30	5,457.57	00:16:40	7,566.96	01:11:29	26,941.13	04:48:30	11,612.50
13:45:24	5,110.31	22:34:56	6,913.85	02:23:49	25,173.79	00:35:19	11,025.64
05:54:03	4,838.85	19:06:24	6,885.65	04:48:30	24,732.63	00:17:10	10,440.23
07:51:37	4,638.15	04:58:40	6,463.89	00:05:56	13,925.17	00:03:40	5,131.22
08:35:41	4,564.55	02:43:02	6,415.13	00:07:37	13,127.38	00:53:24	3,729.32

1, $P < 0.0001$). Since the mean distance-moved increased only slightly from 1.5 cm for control animals to 1.7 cm for the dosed spiders (Table 1), the distance covered during a movement-bout must, on average, be shorter for dosed than control spiders, otherwise a greater disparity in the average distance moved would be expected. Since the data were pooled into 1-min intervals, the more frequent short-distance movements would sum within a minute and could only be inferred through the above analyses.

The pronounced decrease of the power spectrum strength for dosed *S. scenicus*, coupled with the greater number of movement-bouts, implies that high-frequency compo-

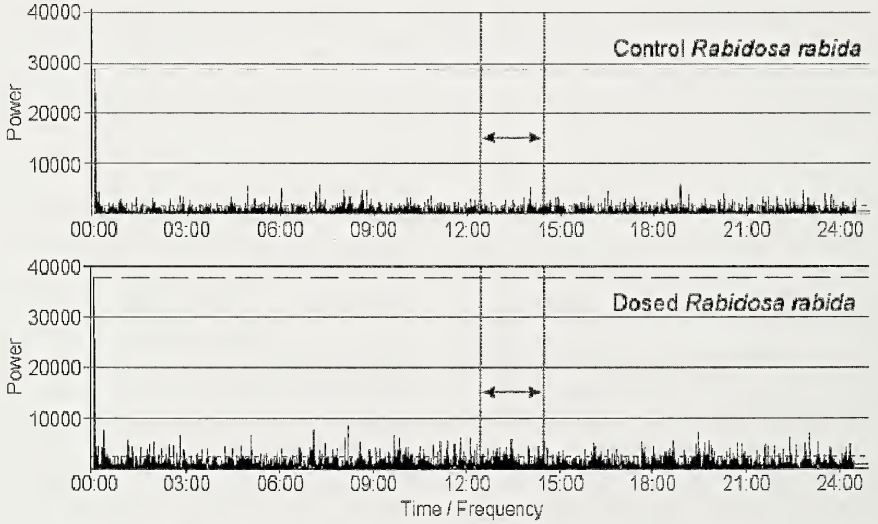


Figure 2.—Power spectra of activity by the spider *Rabidosa rabida* (Lycosidae). Spiders dosed with malathion exhibited a generally stronger spectrum than controls. The most significant spectral changes are with frequencies in the time period highlighted by the vertical lines. Abscissa represents time; ordinate depicts spectral power. Sample sizes: control and dosed = 40.

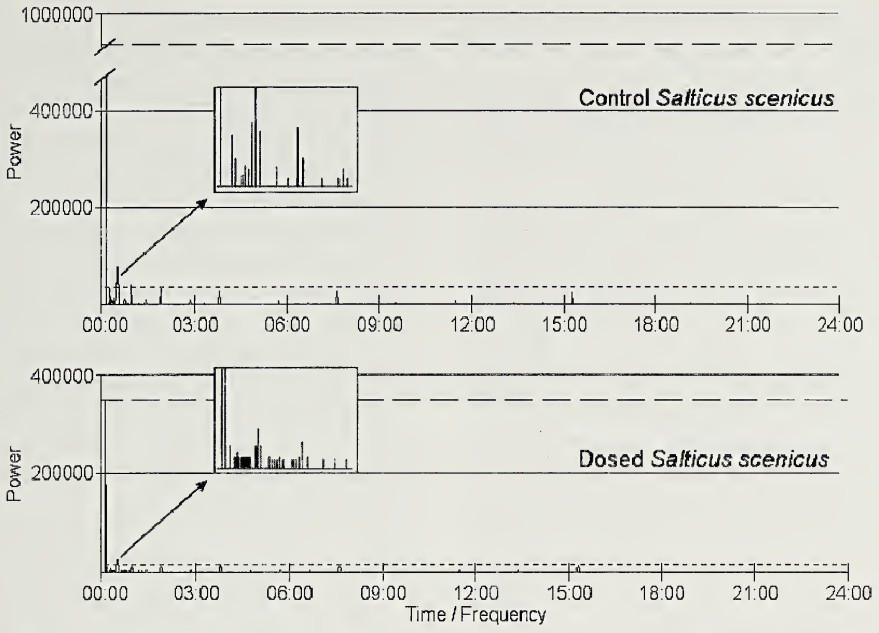


Figure 3.—Power spectra of activity for the spider *Salticus scenicus* (Salticidae). Spiders dosed with malathion showed a longer periodicity and lower power. Note that many of the spectral peaks align over time for the two treatments. Insets are enlargements of the spectra for the first hour. Abscissa represents time; ordinate depicts spectral power. Sample sizes: control = 22, dosed = 17.

nents of low power should be seen in the power spectrum. Indeed, close examination of the spectra reveals a large number of short-term frequencies of low power for dosed spiders not seen in the controls (compare insets in Fig. 3). These components reflect both the progression of more than an hour of the mean vector from circular analysis (Fig. 1) and the increase in movement-bouts by *S. scenicus*.

DISCUSSION

Sublethal exposure of these four spider species to malathion affected changes in the amount, duration, and patterns of their activities. Although there were no overt crippling effects to the spiders from this neurotoxin, the observed alterations of their behavior could have significant consequences to their ultimate survival. Furthermore, the spiders' efficiencies as predators could be impaired, decreasing their value as agents of biological control.

Alterations of circadian rhythms.—Changes in a spider's total and peak activity are likely to affect the time budget of the dosed animals (Cloudsley-Thompson 1960). All spider species tested here that could be fully analyzed using circular statistics became active earlier in the day, possibly forcing them to forage when competitors and/or predators are present, or causing them to be active when their normal prey are unavailable. For example, field data suggest that *S. scenicus*' time budget is adjusted so their peak activity coincides to that of their primary prey on the surface of grain bins, the Angoumois grain moth, *Sitotroga cerealella* (Olivier 1789). In this specialized habitat, grain moths exit bins through the top vents between 13:00 and 14:30, probably in response to the mid-afternoon heat. Timed observations throughout the day indicated that during peak hours, 47% of grain moths on the bin surface were being fed upon by *S. scenicus* (1243 grain moths were scored), with only eight of the moths attacked by other spider species (Tietjen unpubl. data). In the present study, the daily period of greatest activity for control *S. scenicus* brackets the greatest availability of their primary prey at this location (Fig. 1). The shift of over an hour by dosed spiders could desynchronize them with the peak activity of their prey, reducing their predatory efficiency. Furthermore, the spurious activity bursts between 22:00 and 05:00 observed in *S. scenicus* could put them

at risk since these spiders rely heavily on vision for prey-detection and predator-avoidance.

Differences among the taxa tested were readily apparent, thus the effects of malathion on one spider species does not have the same effect on all species. Both *S. scenicus* and *R. rabida* showed an analogous mean daily activity change, but Fourier analyses indicated that different behavioral/physiological mechanisms must be responsible for the observed shifts in mean activity rhythms of *S. scenicus* versus *R. rabida*. Lycosids and salticids have differing levels of acetylcholinesterase activity in their protocerebrum (Meyer & Idel 1977). Perhaps the influence of malathion differentially influences neural integration in *S. scenicus* and *R. rabida*. The lack of spurious activity on the part of *R. rabida* compared to *S. scenicus* may be due to the lycosid's higher general activity throughout the day as evidenced by the smaller mean vector between treatments (r in Table 2; Fig. 1).

The circular analyses of *F. communis* showed a significant mean vector for dosed animals but not for the controls. Although the constant lighting conditions may have contributed to these effects, the mean activity peak for dosed animals suggests that exposure to malathion may affect the interactions between light receptors and the circadian clock. For example, the visual system may override the internal clock for control animals while dosed *F. communis* were not as strongly affected by the constant lighting conditions because of visual aberrations or a change in the coupling between photoreceptors and their base-line circadian rhythms affected by the inhibition of acetylcholinesterase.

Altered locomotor behaviors.—The propensity for dosed *S. scenicus* to move more frequently but for shorter distances may be due to visual distortions experienced by these spiders. While exploring the effects of malathion on prey-capture by *S. scenicus*, abnormalities in visual processing were discovered (Tietjen, unpub. data). Visual errors may cause dosed animals to move at a different rate or pattern. The protocerebral ganglia of salticids and lycosids show very high activities of acetylcholinesterase (Meyer & Idel 1977), which a center of vision for these animals (Meyer 1991). Since malathion is an acetylcholinesterase inhibitor, this pesticide could impair visual processing and subsequent motor activities based on visual sensory input. If the time budget of *S. scenicus* includes a certain level of locomotor behavior, the short-duration spectra (depicted in the inset of Fig. 3) may represent a compensatory mechanism by the spiders to maintain a set amount of activity despite problems with visual processing or other integration systems. Apparently, in the presence of malathion, the underlying decision-making process over-compensates, resulting in a greater distance covered and a precession of the mean time of activity.

Pesticide exposure could cause different behavioral abnormalities at different life stages. Spiderlings, for example, may increase their general activity while adults may show a decrease. The increased activity of *S. ocreata* spiderlings after malathion contact could disrupt the duration of maternal care during a very vulnerable time in their lives. These spiderlings are carried on their mother's dorsal abdomen and cephalothorax for up to 10 days (Rovner et al.1973). This affords them some protection and access to resources such as water and food while gathering strength. By dispersing from the mother too soon, dosed spiderlings would have increased exposure to predation, desiccation, and starvation, severely decreasing the probability of their survival.

General and specific effects.—The current analyses indicate that sublethal doses of malathion have generalized effects on behavior (such as increased activity) and also have specific effects on behavioral components, including the propensity to move, the distance a spider moves during an activity bout, and the small-scale patterning of locomotor behavior. Evidence of high acetylcholinesterase activity in the leg ganglia and connective ring systems of the ventral nerve cord in spiders (Meyer & Pospiech 1977; Meyer 1991) attests to the importance of proper acetylcholine regulation to locomotor coordination and control. Restricted inhibition of the stimulatory action from acetylcholine by malathion would lead to overactivity in these neural tracts central to locomotor activity.

Although one might expect that general behavioral alterations would be common, specific effects on spider behaviors by pesticides have been seen in other contexts. As an example, dosed *R. rabida* males exhibit completely normal courtship behavior in response

to female pheromone or in the presence of females (Tietjen 2006). However, when females indicated a willingness to mate, most dosed males were unable to shift from courtship behavior to copulation (40 control males mated, 2 of 40 dosed males; Tietjen 2006). In fact, all males that could not switch to mating behavior were killed by the normal females (Tietjen 2006). Cholinesterase activity was inhibited for laboratory male *Lycosa hilaris* (Van Erp et al. 2002). Considering the neural integration and sensory feedback necessary to perform complex motor patterns (Milde & Seyfarth 1988; Gronenberg 1990), any impairment of neural transmission or control could significantly affect sexually-oriented behaviors.

The possibility that various neuropesticides could be used to elucidate the fine structure of behavior deserves future investigation. If different classes of pesticides affect different receptors and/or portions of the nervous system (Meyer & Idel 1977; Meyer 1991), it might be possible to use these chemicals as probes to selectively disable portions of a behavioral sequence and gain a greater understanding of the normal behavior of an animal.

Pest management researchers should carefully consider potential sublethal effects on the behavior, physiology, and reproduction of non-target species when planning an IPM scheme. This study suggests that malathion exposure may undermine the efficacy of spiders as biocontrol agents even if surveys find field population densities are not affected. Although live spiders are found to still occupy the treated areas, this study shows that these animals are probably behaviorally impaired. Studies should be performed on a wide variety of spiders from different families, life stages, and varying foraging strategies. There is the possibility that feeding on exposed prey may compromise the behavioral repertoire of otherwise unexposed spiders. In addition, data from the present experiments indicate that different species may have variable underlying responses to the same pesticide, further complicating assumptions in pest management when considering the biological component of IPM.

ACKNOWLEDGMENTS

This study was supported by grants from the United States Department of Agriculture (grant 94-37311-1186), and National Institutes of Health (grant P20RR16481). Drs. P. Cushing, D. Mott, and G. Stratton provided valuable comments that greatly improved an earlier draft of this document.

LITERATURE CITED

Amalin, D.M., J.E. Peña, S.J. Yu & R. McSorley. 2000. Selective toxicity of some pesticides to *Hibana velox* (Araneae: Anyphaenidae), a predator of citrus leafminer. Florida Entomologist 83: 254–262.

Batschelet, E. 1965. Statistical Methods for the Analysis of Problems in Animal Orientation and Certain Biological Rhythms. American Institute of Biological Science, Washington, DC. 57 pp.

Chu, Y.I., C.C. Hor & B.J. Chen. 1976. The effect of BPMC and Unden on the predation of *Lycosa* spider. National Taiwan University Phytopathologist and Entomologist 18:42–57.

Chu, Y.I., K.K. Ho, W.K. Peng, P.W. Lai & I.H. Chen. 1977. Relative toxicity study on six dust insecticides to planthopper, leafhopper and their predator, the wolf spider. National Taiwan University Phytopathologist and Entomologist 5:37–45.

Cloudsley-Thompson, J.L. 1960. Adaptive function of circadian rhythms. Cold Spring Harbor Symposium on Quantitative Biology 25:345–355.

Crane, J. 1949. Comparative biology of salticid spiders at Rancho Grande, Venezuela. IV. An analysis of display. Zoologica 34:159–214.

Delpuech, J.-M., B. Froment, P. Fouillet, F. Pompanon, S. Janillon & M. Boulétreau. 1998. Inhibition of sex pheromone communications of *Trichogramma brassicae* (Hymenoptera) by the insecticide chlorpyrifos. Environmental Toxicology and Chemistry 17:1107–1113.

DeVoe, R.D., R.J.W. Small & J.E. Zvargulis. 1969. Spectral sensitivities of wolf spider eyes. Journal of General Physiology 54:1–32.

Epstein, D.L., R.S. Zack, J.F. Brunner, L. Gut & J.J. Brown. 2000. Effects of broad-spectrum insecticides on epigeal arthropod biodiversity in Pacific Northwest apple orchards. Environmental Entomology 29:340–348.

Gronenberg, W. 1990. The organisation or plurisegmental mechanosensitive interneurons in the central nervous system of the wandering spider *Cupiennius salei*. Cell and Tissue Research 260:49–61.

Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annual Review of Entomology 33:149–168.

Higley, L.G. & W.K. Wintersteen. 1992. A novel approach to environmental risk assessment of pesticides as a basis for incorporating environmental costs into economic injury levels. American Entomologist 38:34–39.

Hilburn, D.J. & D.T. Jennings. 1988. Terricolous spiders (Araneae) of insecticide-treated spruce-fir forests in west-central Maine. Great Lakes Entomologist 21:105–114.

Hodge, S. & C.J. Vink. 2000. An evaluation of *Lycosa hillaris* as a bioindicator of organophosphate pesticide contamination. New Zealand Plant Protection 53:226–229.

Hollingworth, R.M. 1976. Chemistry, biological activity, and uses of formamidine pesticides. Environmental Health Perspectives 14:57–69.

James, D.G. & T.S. Price. 2002. Fecundity in two-spotted mite (Acari: Tetranychidae) is increased by direct and systematic exposure to imidacloprid. Journal of Economic Entomology 95:729–732.

Longley, M. & P.C. Jepson. 1996. The influence of insecticide residues on primary parasitoid and hyperparasitoid foraging behaviour in the laboratory. Entomologia Experimentalis et Applicata 81:259–269.

Malony, D., F.A. Drummond & R. Alford. 2003. Spider predation in agroecosystems: Can spiders effectively control pest populations? Maine Agricultural and Forest Experiment Station Technical Bulletin 190. 32 pp.

Mansour, F. & W. Nentwig. 1988. Effects of agrochemical residues on four spider taxa: laboratory methods for pesticide tests with web-building spiders. Phytoparasitica 16:317–326.

Mansour, F., D. Rosen, A. Shulov & H.N. Plaut. 1980. Evaluation of spiders as biological control agents of *Spodoptera littralis* larvae on apple in Israel. Acta Oecologica et Applicata 1:225–232.

Meyer, W. 1991. The biology of excitatory neurotransmitters in the central nervous system of spiders (Arachnida: Araneida). Neurochemical Life Science Advances 10:85–103.

Meyer, W. & K. Idel. 1977. The distribution of acetylcholinesterase in the central nervous system of jumping spiders and wolf spiders (Arachnida, Araneida: Salticidae et Lycosidae). Journal of Comparative Neurology 173:717–744.

Meyer, W. & B. Pospiech. 1977. The distribution of acetylcholinesterase in the central nervous system of web-building spiders (Arachnida, Araneae). Histochemistry 51:201–208.

Milde, J.J. & E.-A. Seyfarth. 1988. Tactile hairs and leg reflexes in wandering spiders: physiological and anatomical correlates of reflex activity in the leg ganglia. Journal of Comparative Physiology A 162:623–631.

Pluthero, F.G. & S.F.H. Threlkeld. 1980. Vacuum injection measurements of susceptibility to insecticides. Journal of Economic Entomology 73: 424–426.

Riechert, S.E. 1998. The role of spiders and their conservation in the agroecosystem. Pp. 211–237. *In* Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests (C.H. Pickett & R.L. Bugg, eds.). University of California Press, Berkeley, California.

Riechert, S.E. 1999. The hows and whys of successful pest suppression by spiders: insights from case studies. Journal of Arachnology 27:387–396.

Riechert, S.E. & T. Lockley. 1984. Spiders as biological control agents. Annual Review of Entomology 29:299–320.

Rovner, J.S., G.A. Higashi & R.F. Foelix. 1973. Maternal behavior in wolf spiders: the role of abdominal hairs. Science 182:1153–1155.

Samu, F. & F. Vollrath. 1992. Spider orb web as bioassay for pesticide side effects. Entomologia Experimentalis et Applicata 62:117–124.

Shaw, E.M., M. Waddicor & A.M. Langan. 2006. Impact of cypermethrin on feeding behaviour and mortality of the spider *Pardosa amentata* in arenas with artificial 'vegetation'. Pest Management Science 62:64–68.

Tietjen, W.J. 1980. Sanitary behavior by the social spider *Mallos gregalis* (Dictynidae): distribution of excreta as related to web density and animal movements. Psyche 87:59–73.

Tietjen, W.J. 1981. An inexpensive microcomputer-based image digitizer. Behavior Research Methods & Instrumentation 13:347–350.

Tietjen, W.J. 1982 Influence of activity patterns on the social organization of *Mallos gregalis* (Araneae, Dictynidae). Journal of Arachnology 10: 75–84.

Tietjen, W.J. 2006. Pesticides affect the mating behavior of *Rabidosa rabida* (Araneae, Lycosidae). Journal of Arachnology 34:285–288.

Van den Berg, A.M., A.S. Dippenaar-Schoeman & H.J. Schoonbee. 1990. The effect of two pesticides on spiders in South African cotton fields. Phytophylactica 22:435–441.

Van Erp, S., L. Booth, R. Gooneratne & K. O'Halloran. 2002. Sublethal responses of wolf spiders (Lycosidae) to organophosphorous insecticides. Environmental Toxicology 17:449–456.

Venkateswara Rao, J., K. Parvathi, P. Kavitha, N.M. Jakka & R. Pallela. 2005. Effect of chlorpyrifos and monocrotophos on locomotor behaviour and acetylcholinesterase activity of subterranean termites, *Odontotermes obesus*. Pest Management Science 61:417–421.

Manuscript received 31 August 2004, revised 17 May 2007.

2007. The Journal of Arachnology 35:407–408

SHORT COMMUNICATION

REPLACEMENT NAMES FOR *ONCOPUS* AND ONCOPODIDAE (ARACHNIDA, OPILIONES)

Hüseyin Özdikmen: Department of Biology, Faculty of Science and Arts, University of Gazi, Ankara 06500, Turkey. E-mail: ozdikmen@gazi.edu.tr

Adriano Brilhante Kury: Dept. Invertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, São Cristóvão 20.940-040, Rio de Janeiro, RJ, Brazil.

ABSTRACT. A junior homonym was detected amongst the Arachnida and the replacement name *Sandokan* is proposed for *Oncopus* Thorell 1876 (Opiliones, Laniatores). Accordingly, nine new combinations are herein proposed for all nine valid species currently included in *Oncopus* (Opiliones). In addition, we propose the replacement name Sandokanidae new name for Oncopodidae.

Keywords: *Sandokan*, Sandokanidae, homonymy

The genus *Oncopus* was described by Herrich-Schaeffer (1855) with the type species *Paidia citrosa* Geyer 1832 by subsequent designation (Prout 1934) in Lepidoptera (Geometroidea, Geometridae, Sterrhinae). This genus was originally proposed in the Lithosiidae (now Arctiidae), but has since been transferred to the Geometridae; its first published use in this family appears to have been by Prout (1934). It is currently a valid generic name in Lepidoptera (Scoble 1999). The genus *Oncopus* was proposed by Thorell (1876) with the type species *Oncopus doriae* Thorell 1876 by original designation in Arachnida (Opiliones, Laniatores, Grassatores, Phalangodoidea, Oncopodidae). The name is currently used as a valid generic name in Opiliones as the type genus of the family Oncopodidae Thorell 1876 (Schwendinger & Martens 2004). Both usages of the name *Oncopus* are listed by Neave (1940).

However, the name *Oncopus* Thorell 1876 is invalid under the rule of homonymy, being a junior homonym of *Oncopus* Herrich-Schaeffer (1855). Under the International Code of Zoological Nomenclature (International CZN 1999) it must be rejected and replaced. In accordance with article 60 of the International Code of Zoological Nomenclature, fourth edition (1999), we propose to substitute the junior homonym *Oncopus* Thorell 1876 for the nomen novum *Sandokan*.

As a result of this, *Oncopus* Thorell 1876 is replaced with *Sandokan* new name. The following new combination is established: *Sandokan doriae* (Thorell 1876) new combination, along with eight other new combinations for all nine valid species currently included in *Oncopus* (Opiliones).

In addition to this, we herein propose the replacement name Sandokanidae new name for the family name Oncopodidae because its type genus *Oncopus* Thorell 1876 is invalid and the type genus of a family-group name must be valid.

SYSTEMATICS

Order Opiliones

Family **Sandokanidae** new name

Oncopodidae Thorell 1876:134 (as Oncopodinae).

Type genus.—*Sandokan* new name.

Remarks.—The name *Oncopus* has been used in Opiliones as the stem for other family-group names, currently in disuse, and should be automatically replaced with the new name and appropriate ending if they are ever considered to be valid: Oncopodoidea Thorell 1876 (Kratochvíl 1958:380, for a superfamily). The infraordinal name Oncopodomorphi Šilhavý (1961, p. 265) is not ruled by the International Code of Zoological Nomenclature.

Genus **Sandokan** new name

Oncopus Thorell 1876:134–135, junior homonym of *Oncopus* Herrich-Schaeffer 1855.

Type species.—*Oncopus doriae* Thorell 1876 by original designation.

Etymology.—Sandokan is the name of the character of a prince-pirate from Borneo created by the Italian writer Emilio Salgari (1862–1911), appear-

ing in a series of novels starting with "I pirati della Malesia" (1883). The gender is masculine.

Species account and distribution.—Nine species; known from Thailand (extremely doubtful record), peninsular Malaysia, Singapore, Sumatra and its islands to the east and on Borneo, according to Schwendinger & Martens (2004).

The following new combinations are proposed, and all species are removed from *Oncopus*:

Sandokan doriae (Thorell 1876) new combination. This name was given in honor to the marquis Giacomo Doria, a man's name. It is the same case as the specific name "feae" (see below).

Sandokan feae (Thorell 1890) new combination. The species name is based on the man's name [Leonardo] Fea and, according to Latin grammar, being a noun of the first declension it should form the genitive *-ae*. According to the International Commission on Zoological Nomenclature (1999) Article 31.1.1, a species-group name, if a noun in the genitive case formed directly from a latinized personal name, must follow the Latin grammar rules (cf. the example presented therein for Poda). Therefore "feae" is the correct original spelling (Article 32.2) and must be maintained.

Sandokan hosei (Pocock 1897) new combination.

Sandokan lingga (Schwendinger & Martens 2004) new combination.

Sandokan malayanus (Schwendinger & Martens 2004) new combination.

Sandokan megachelis (Schwendinger 1992) new combination.

Sandokan tiomanensis (Schwendinger & Martens 2004) new combination.

Sandokan expatriatus (Schwendinger & Martens 2004) new combination.

Sandokan truncatus (Thorell 1891) new combination.

We thank Dr Miguel Angel Alonso-Zarazaga (Museo Nacional de Ciencias Naturales, Madrid, Spain) who provided comments on a draft of this work. This study was supported by grant #520406/98-2 from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to ABK.

LITERATURE CITED

Herrich-Schäffer, G.A.W. 1855. Systematische Bearbeitung der Schmetterlinge von Europa, zugleich als Text, Revision und Supplement zu Jakob Hübner's Sammlung Europäischer Schmetterlinge. Regensburg 6:1–178.

International Commission on Zoological Nomenclature. 1999. International Code of Zoological Nomenclature. Fourth Edition. International Trust for Zoological Nomenclature, London.

Kratochvíl, J. 1958. Die Höhlenweberknechte Bulgariens (Cyphophthalmi und Laniatores). Práce Brněnské základny Československé akademie věd (= Acta Academiae Scientiarum Čechoslovenicae Basis Brunensis) Brno 30(375):371–396.

Neave, S.A. 1940. Nomenclator Zoologicus. A List of Names of the Genera and Subgenera in Zoology from the Tenth Edition of Linneaus 1758 to the End of 1935. M-P. Volume 3. Zoological Society of London, London. Pp. 1–1065.

Prout, L.B. 1934. Geometridae: Subfamilia Sterrhinae. *In* Lepidopterorum Catalogus, editus a F. Bryk's-Gravenhage (E. Strand, ed.). Volume 61: 1–176. W. Junk, Berlin.

Schwendinger, P.J. & J. Martens. 2004. A taxonomic revision of the family Oncopodidae IV. The genus *Oncopus* Thorell (Opiliones, Laniatores). Revue Suisse de Zoologie 111:139–174.

Scoble, M.J. (ed.) 1999. Geometrid Moths of the World, a Catalogue. Volume 2. CSIRO Publishing, Collingwood, Victoria, Australia & Apollo Books, Stenstrup, Denmark.

Šilhavý, V. 1961 ["1960"]. Die Grundsätze der modernen Weberknechttaxonomie und Revision des bisherigen Systems der Opilioniden. XI. Internationaler Kongress für Entomologie (Vienna, 17–25 August, 1960) Verhandlungen. Volume 1: 262–267.

Thorell, T.T.T. 1876. Descrizione di alcune specie di Opilioni dell'Arcipelago Malese appartenenti al Museo Civico di Genova. Annali del Museo Civico di Storia Naturale, Genoa (series 1) 9: 111–138.

Manuscript received 11 April 2005, revised 10 August 2005.

INSTRUCTIONS TO AUTHORS

(revised December 2006)

General: Manuscripts must be in English and should be prepared in general accordance with the current edition of the *Council of Biological Editors Style Manual* unless instructed otherwise below. Authors should consult a recent issue of the *Journal of Arachnology* for additional points of style. Manuscripts longer than three printed journal pages should be prepared as Feature Articles, shorter papers as Short Communications. One invited Review Article will be published in the third issue of each year at the discretion of the editors. Suggestions for review articles may be sent to the Managing Editor.

Submission: Send one electronic version of the entire manuscript (in PDF or Microsoft Word format) to the Managing Editor of the *Journal of Arachnology*: **Paula E. Cushing, *Managing Editor,* Denver Museum of Nature and Science, Zoology Department, 2001 Colorado Blvd., Denver, CO 80205-5798 USA** [*Telephone:* (303) 370-6442; *FAX:* (303) 331-6492; *E-mail:* PCushing@dmns.org or PECushing@juno.com].

The Managing Editor will acknowledge receipt of the manuscript, assign it a manuscript number and forward it to a Subject Editor for the review process. Correspondence relating to manuscripts should be directed to the appropriate Subject Editor and should include the manuscript number. If the manuscript is accepted, the author will be asked to submit the final copy electronically to the appropriate Subject Editor. Submission of final illustrations is detailed below. Authors are expected to return revisions promptly. Revised manuscripts that are not returned in a reasonable time period will be considered new submissions.

Voucher Specimens: Specimens of species used in your research should be deposited in a recognized scientific institution. All type material must be deposited in a recognized collection/institution.

FEATURE ARTICLES

Title page.—The title page includes the complete name, address, and telephone number of the corresponding author, a FAX number and electronic mail address if available, the title in capital letters (with no more than 40 characters and spaces per line), each author's name and address, and the running head.

Running head.—The author's surname(s) and an abbreviated title should be typed in capital letters and must not exceed 60 characters and spaces. The running head should be placed near the top of the title page.

Abstract.—The heading in capital letters should be placed at the beginning of the first paragraph set off by a period. A second abstract, in a language appropriate to the nationality of the author(s) or geographic region(s) emphasized, may be included.

Keywords.—Give 3–5 appropriate keywords following the abstract. Keywords should not duplicate words in the title.

Text.—Double-space text, tables, legends, etc. throughout. Three levels of heads are used.

- The first level (METHODS, RESULTS, etc.) is typed in capitals and on a separate line.
- The second level head is **bold**, begins a paragraph with an indent and is separated from the text by a period and a dash.
- The third level may or may not begin a paragraph but is *italicized* and separated from the text by a colon.

Use only the metric system unless quoting text or referencing collection data. All decimal fractions are indicated by a period (e.g., −0.123). Include geographic coordinates for collecting locales if possible.

Citation of references in the text: Cite only papers already published or in press. Include within parentheses the surname of the author followed by the date of publication. A comma separates multiple citations by the same author(s) and a semicolon separates citations by different authors, e.g., (Smith 1970), (Jones 1988; Smith 1993), (Smith & Jones 1986, 1987; Jones et al. 1989). Include a letter of permission from any person who is cited as providing unpublished data in the form of a personal communication.

Citation of taxa in the text: Include the complete taxonomic citation for each arachnid taxon when it first appears in the manuscript. For Araneae, this information can be found online at http://research.amnh.org/entomology/spiders/catalog/INTRO2.html. For example, *Araneus diadematus* Clerck 1757. Taxon author does not need to be included in Literature Cited.

Literature Cited section.—Use the following style and include the full unabbreviated journal title. Personal web pages should not be included in Literature Cited. These can be cited within the text as (John Doe, pers. website) without the URL. Institutional websites can be included in Literature Cited. Examples:

Huber, B.A. & W.G. Eberhard. 1997. Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). Canadian Journal of Zoology 74:905–918.

Krafft, B. 1982. The significance and complexity of

communication in spiders. Pp. 15–66. *In* Spider Communications: Mechanisms and Ecological Significance. (P.N. Witt & J.S. Rovner, eds.). Princeton University Press, Princeton, New Jersey.

Platnick, N.I. 2006. The World Spider Catalog, Version 7.0. American Museum of Natural History, New York. Online at http://research.amnh.org/entomology/spiders/catalog/INTRO1.html

Footnotes.—Footnotes are permitted only on the first printed page to indicate current address or other information concerning the author. All footnotes are placed together on a separate manuscript page. Tables and figures may not have footnotes.

Taxonomic articles.—Consult a recent taxonomic article in the *Journal of Arachnology* for style or contact the Subject Editor for Taxonomy and Systematics. Papers containing original descriptions of focal arachnid taxa should be given in the Literature Cited section.

Tables.—Each table, with the legend above, should be placed on a separate manuscript page. Only horizontal lines (no more than three) should be included. Tables may not have footnotes; instead, include all information in the legend. Make notations in the text margins of the final manuscript to indicate the preferred location of tables in the printed text.

Illustrations.—Original illustrations (hard copy) should not be sent until the article is accepted for publication. Electronic submission of final illustrations is acceptable if the author strictly adheres to the Digital Art Spec guidelines posted at the following website: http://www2.allenpress.com/allen_press/apguides/Digital_Art_Spec.pdf. Hard copies of final illustrations must still be submitted to the Editor-in-Chief to ensure that the electronic version matches the printed copy. Address all questions concerning illustrations to the Editor-in-Chief of the *Journal of Arachnology*: **James E. Carrel, *Editor-In-Chief*, Division of Biological Sciences, 209 Tucker Hall, University of Missouri-Columbia, Columbia, MO 65211-7400, USA** [*Telephone:* 573-882-3037; *FAX:* 573-882-0123 *E-mail:* carrelj@missouri.edu]. All hard-copy art work must be camera-ready—i.e., mounted and labeled—for reproduction. Figures should be arranged to fit (vertically and horizontally) the printed journal page, either one column or two columns, with a minimum of wasted space. When reductions are to be made by the printer, pay particular attention to width of lines and size of lettering in line drawings. Multiple photos assembled on a single plate should be mounted with a minimum of space separating them. In the case of multiple illustrations mounted together, each illustration must be numbered sequentially rather than given an alphabetic sequence (e.g., Fig. 1a, 1b, 1c numbering is not allowed). Distribution maps should be considered figures and numbered consecutively with figures. Written on the back should be the name(s) of author(s) and an indication of top edge. The overall dimensions should be no more than 11 inches (28 cm) × 14 inches (36 cm). Larger drawings present greater difficulty in shipping and greater risks of damage for which the *Journal of Arachnology* assumes no responsibility. On the hard copies of illustrations sent by the author (upon acceptance), indicate whether the illustration should be one column or two columns in width and indicate the preferred position of illustrations within the text. Make notations in the text margins of a hard copy of the final manuscript or provide this information in electronic mail posted to the Editor-in-Chief. Color plates can be printed, but the author must assume the full cost, currently about $600 per color plate.

Legends for illustrations should be placed together on the same page(s) and separate from the illustrations. Each plate must have only one legend, as indicated below:

Figures 1–4. *A-us x-us*, male from Timbuktu. 1, Left leg; 2, Right chelicera; 3, Dorsal aspect of genitalia; 4, Ventral aspect of abdomen. Scale = 1.0 mm.

Assemble manuscript.—The manuscript should appear in separate sections or pages in the following sequence; title page, abstract, text, footnotes, tables with legends, figure legends, figures.

Page charges, proofs and reprints.—Page charges are voluntary, but non-members of AAS are strongly encouraged to pay in full or in part for their article ($75/journal page). The author will be charged for changes made in the proof pages. Hard copy or PDF reprints are available only from Allen Press and should be ordered when the author receives the proof pages. Allen Press will not accept reprint orders after the paper is published. The *Journal of Arachnology* also is published by BioOne. Therefore, you can download the PDF version of your article from the BioOne site or the AAS site if you are a member of AAS or if your institution is a member of BioOne. PDFs of articles older than one year will be made freely available from the AAS website.

SHORT COMMUNICATIONS

Short Communications are usually limited to three journal pages, including tables and figures. The format is open, but internal headings (Methods, Results, etc.) are omitted. They are printed in a smaller (10 point) typeface. An Abstract is required.

CONTENTS

The Journal of Arachnology

Volume 35 Featured Articles Number 2