

Implications of the tympanal hearing organ and ultrastructure of chaetotaxy for the higher classification of Embioptera

CLAUDIA SZUMIK,¹ MARÍA LAURA JUÁREZ,¹ MARTÍN J. RAMÍREZ,²
PABLO GOLOBOFF,¹ AND VERÓNICA V. PEREYRA¹

ABSTRACT

Several slowly evolving characters are evaluated with the main objective of reinforcing the higher classification of Embioptera. An embiopteran femoral auditory organ, described here for the first time, exhibits differences in shape and position that provide diagnostic criteria for higher taxonomic groups in the order. New characters on silk ejectors, bladders, and various types of leg setae are also discussed within a taxonomic framework. The utility of these new traits and their different conditions, for identifying monophyletic groups, was tested by a preliminary phylogenetic analysis.

INTRODUCTION

Until recently, the classification of the Embioptera (webspinners) had been based on a few characters, and we now recognize that many of the genera and families described by E.S. Ross during the past 70 years are clearly not monophyletic (Szumik et al., 2008); in fact, most webspinner genera and families have been defined and characterized almost exclusively on the basis of the male genitalia. This situation has started to change with recent cladistic studies on Embioptera at different taxonomic levels (Szumik, 1994, 1996, 1997, 2004; Szumik et al., 2008, 2017; Miller et al., 2012). Thanks to those quantitative analyses, a general cladogram of the order is becoming more

¹ Unidad Ejecutora Lillo, Consejo Nacional de Investigaciones Científicas y Técnicas, Tucumán, Argentina.

² División Aracnología, Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina.

congruent and stable. In addition, the different groups obtained in the phylogenetic study with the largest number of characters (Szumik et al., 2008) are supported not only by male genitalic characters, but also by characters in other structures such as head, wings, female terminalia, and leg chaetotaxy. Although some groups have remained stable from the first (Szumik, 1996) to the latest cladistic analyses (Szumik et al., 2008; Miller et al., 2012), other sections of the cladogram, particularly in intra- and interfamilial relationships, continue to be unresolved or unstable, such as the family Embiidae. This lack of resolution could arise from both data ambiguity and conflict. Furthermore, the lack of resolution could have been the result of missing entries. For example, in some groups the females are either still unknown or the available specimens are in poor condition or are too old; as a result, the morphological data matrix of Szumik et al. (2008) had missing entries for female characters in 43% of the terminals (out of 186 scored species).

In the context of the higher classification of the order, a more accurate and stable classification would be obtained by including as much new evidence as possible. Specialists trying to resolve the phylogeny of a group often focus on highly variable structures. Slowly evolving characters are sometimes ignored because they are too uniform, and differences between groups can be detected only with comprehensive studies. However, these slow-evolving characters are the ones most likely to give reliable evidence on monophyly for higher groups, providing critical support for the general structure of the cladogram. In previous morphological studies (Szumik, 1996; Szumik et al., 2008, 2017) some rarely modified structures, such as the basal wing union or female terminalia, were included, although the predominant source of characters remained the highly variable characters in male terminalia.

Given that situation, we undertook the present study, focusing our search on slowly evolving characters, with the aim of reinforcing the higher classification of Embioptera. Most of the new findings reported here are morphological traits of male and female legs, never before analyzed (or analyzed only superficially) within a systematic framework. An auditory organ (i.e., tympanal organ) is described for the first time in the order Embioptera; the potential of this new trait can be gauged by the fact that the structure is present, with some variation in shape, in all webspinners.

MATERIALS AND METHODS

TAXON SAMPLING: New characters were observed with a scanning electron microscope (SEM) and stereoscopic microscope. The leg ultrastructure for 18 species (see table 1) of the major groups of Embioptera was analyzed with SEM. The material was dehydrated through an ethanol series, critical-point dried, sputter-coated with Au-Pd, and observed in a FEI XL30-TMP SEM under high vacuum. The study focused on the tarsi and femora of the first legs, which contain the silk glands used to build galleries, and the third legs, which are used to clutch the silk walls. These SEM observations were complemented with stereoscopic microscopy, with a survey of all three leg pairs. The material used in this study belongs to the following institutions: Fundación Miguel Lillo, American Museum of Natural History, Cornell University, and Instituto Nacional de Pesquisas da Amazônia.

TABLE 1. List of taxa analyzed with SEM. Family, species name, sex, total length in mm, number of silk ejectors, number of rows of ejectors ($n = 1$).

Family	Species	Sex	Distribution	Length	Ejectors	Rows
Anisembiidae	<i>Mesembia chamulae</i> Ross, 1984	F	Mexico	9.50	131	8
Anisembiidae	<i>Suassurembia calypso</i> Szumik, 2007	F	Trinidad	9.85	46	6
Anisembiidae	<i>Chelicerca barbara</i> Szumik, 1998	M	Argentina	9.10	69	5
Archembiidae	<i>Archembia dilata</i> Ross, 2001	F	Brazil	17.00	158	14
Archembiidae	<i>Gibocercus chaco</i> Szumik, 1997	F	Argentina	20.35	247	12
Archembiidae	<i>Pachylembia uncinata</i> Ross, 1984	F	Mexico	16.00	139	10
Archembiidae	<i>Parahagadochir trachelia</i> (Navás, 1915)	M	Argentina	16.20	221	12
Clothodidae	<i>Antipaluria caribbeana</i> Ross, 1987	F	Venezuela	21.80	185	10
Clothodidae	<i>Antipaluria urichi</i> (De Saussure, 1896)	F	Trinidad	18.00	155	14
Clothodidae	<i>Clothoda longicauda</i> Ross, 1987	M	Peru	16.70	147	10
Embiidae	<i>Dihybocercus femorata</i> (Navás, 1915)	F	Zaire	19.00	89	8
Embiidae	<i>Embia ramburi</i> Rimsky-Korsakow, 1905	F	Spain	10.00	129	8
Austrolembiidae	<i>Austrolembia incompta</i> Ross, 1963	M	Australia	14.20	117	10
Notoligotomidae	<i>Notoligotoma hardyi</i> (Friederichs, 1914)	F	Australia	12.00	129	8
Oligotomidae	<i>Eosembia</i> sp.	F	SE Asia	—	—	—
Oligotomidae	<i>Haploembia solieri</i> (Rambur, 1842)	F	USA	13.00	234	16
Oligotomidae	<i>Oligotoma saundersii</i> Westwood, 1837	F	USA	11.00	120	8
Teratembiidae	<i>Teratembia geniculata</i> Krauss, 1911	M	Argentina	7.20	80	8

PHYLOGENY: To gain an understanding of the influence of the new traits (13 characters; see appendix 1) on the phylogenetic analyses, they were incorporated into the data matrix of Szumik et al. (2008), and evaluated again. We stress that this is not intended as a full analysis of the order, but instead a preliminary approximation to effect of adding the new characters, and as a means to map the new characters on a phylogeny. The data matrix is available online (<http://www.lillo.org.ar/phylogeny/published/>). The program used for the cladistic analyses was TNT ver. 1.5 (Goloboff et al., 2003, 2008; Goloboff and Catalano, 2016), using the new technology algorithms to search for the optimal trees (Goloboff, 1999) and stopping the analysis when the optimal scores were hit 20 times, following the same procedures described in Szumik et al. (2008).

RESULTS

THE AUDITORY ORGAN

Arthropods are sensitive to acoustic signals; the sound and vibrations allow them not only to identify predators or preys, but also to communicate with other individuals (Cocroft, 2001; Yack, 2004; Cocroft and Rodríguez, 2005). These diverse forms of signals are differentiated by the pattern of wave dispersion in the environment (Greenfield, 2016). Vibrations are transmitted through solids (or substrate), whereas sounds are transmitted through air or water (Cocroft

and Rodríguez, 2005; Greenfield, 2016). In addition, auditory communication is characterized by the use of perceptual organs (Greenfield, 2016). Insects have a great diversity of hearing organs, ranging from hair sensilla to complex tympanal ears, able to receive and process different types of signals and eventually generate a response (Keil, 1997; Yack, 2004). The tympanal ear is characterized by three structures: a tympanal membrane, an air tracheal chamber, and an associated mechanoreceptor, the chordotonal organ (Hoy and Robert, 1996; Yack, 2004). Tympanal organs have evolved convergently several times in insects and have been studied extensively in orders such as Orthoptera, Hemiptera, and Lepidoptera (Hoy and Robert, 1996; Yack, 2004; Strauß and Lakes-Harlan, 2014).

Since the early 20th century, several studies on the behavior of Embioptera (e.g., Melander, 1902; Kershaw, 1914; Mills, 1932) have shown the relevance of the way of life of these insects. Webspinners are gregarious and construct their silk nets and live their entire life inside the silk tubes. More recently, there is evidence of vibration-sensitive embiids detecting parasitoid wasps as well as communicating with conspecifics, especially in connection with the maternal care exhibited in this group (e.g., Edgerly, 1987; Choe, 1994; Proaño et al., 2012). Through lunges, push-ups, and jittering, webspinners generate vibrations that travel throughout their silken tubular domain (Proaño et al., 2012). Concomitant with their gregarious behavior, a hearing organ on the femora is reported here for the first time. This organ, visible with a stereomicroscope, is present in both sexes as well as in all instars.

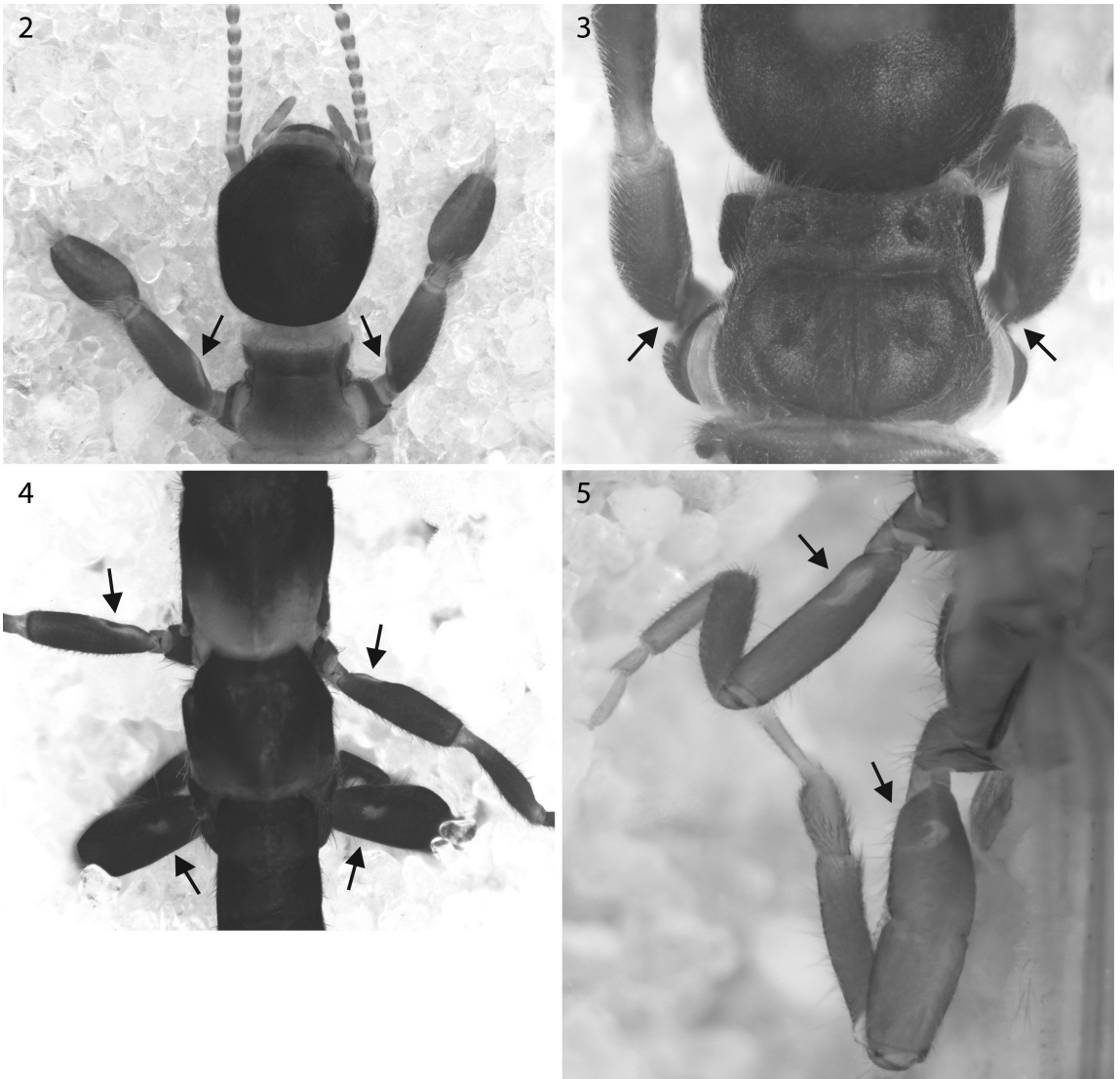
Embioptera have a tubelike shape (fig. 1), with the three pairs of legs positioned to best facilitate spinning as well as movement within the silky tubes. The hearing organ has a tympanal membranous area, indented and poorly sclerotized, clearly differentiated from the rest of the femur (figs. 2–5).

We observed small differences in the position of the tympanal organ in the different legs, but in general on the fore- (figs. 2, 3) and middle (figs. 4, 5) femora it is on the proximal anterior face; on the hind femur it appears on the dorsal face and it is conspicuous in dorsal view (figs. 4–5). These differences are due at least in part to the function and normal position of the legs. Normally, the forelegs, used for spinning, are forward facing; the middle legs (which exert pressure on the opposing walls of the gallery) have a lateral position; and the hind legs are used to clutch to the silk wall, which helps in easily moving forward or backward (fig. 1). Given the position of the legs, the tympanal organs are inconspicuous in the dorsal view of the specimen, which may explain the previous lack of reports on this structure.

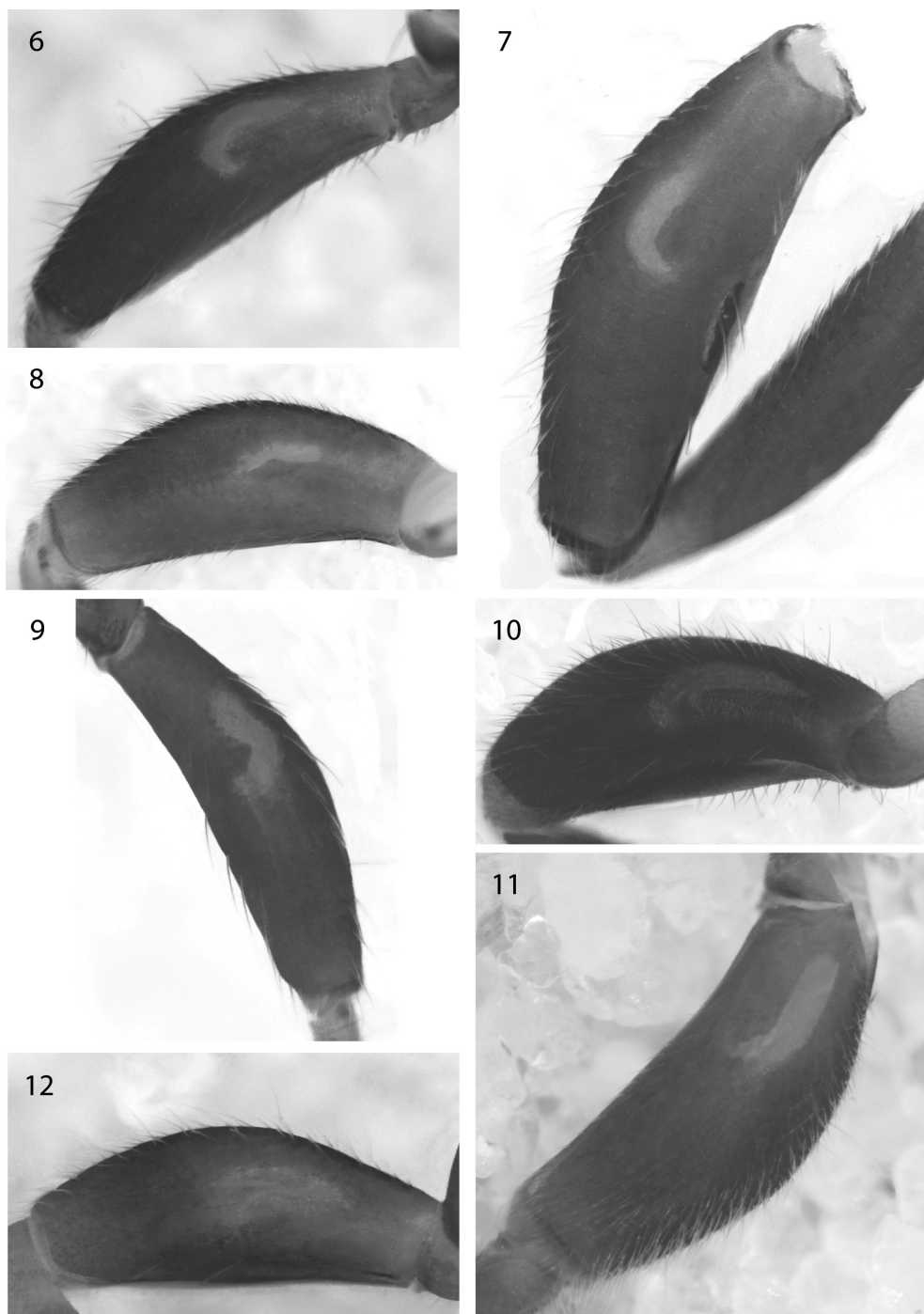
FOREFEMUR: Given the position of foreleg, the auditory organ appears inward and parallel to the body (figs. 2, 3, 6–12). Depending on the taxonomic group, some variation was observed, but the general appearance is a relatively slender and elongate semicircle (e.g., fig. 6). We scored the auditory organ in three states (see appendix 1) based on shape and location: state 0, semicircular with well-defined edges (figs. 6–9); state 1, a curved, slender band, elongated along the femoral axis (figs. 10, 11); state 2, a tiny triangle, a condition observed mostly in species of the genus *Aposthonia* (fig. 12). The most common condition of the auditory organ for the forefemora (fig. 13) is state 0, semicircular (figs. 6–9). Some variations of this general condition are clearly connected to certain taxonomic groups, typically a genus or a family. For example, the



FIG. 1. General view of *Chelicerca barbara* female.



FIGS. 2–5. General view of the auditory organ on femora indicated by black arrows: 2, *Clothoda longicauda* female (Clothodidae) forefemora dorsal view; 3, *Chelicerca wheeleri* male (Anisembiidae) forefemora dorsal view; 4, *Chelicerca barbara* female, middle and hind femora dorsal view; 5, *Chelicerca barbara* male, middle and hind femora anterolateral view.



FIGS. 6–12. Shapes of auditory organ on forefemur: **6**, *Chelicerca barbara* (Anisembiidae) right leg; **7**, *C. wheeleri* right leg; **8**, *Machadoembia angolica* (Embiidae) right leg; **9**, *Teratembia geniculata* (Teratembidae) left leg; **10**, *Australembia nodosa* (Australembidae) right leg; **11**, *Clothoda longicauda* (Clotodidae) left leg; **12**, *Aposthonia indica* (Oligotomidae) right leg.

triangular shape (state 2: fig. 12) characterizes *Aposthonia* (Oligotomidae); the curved slender band, extending longitudinally (state 1: figs. 10, 11) appears several times, separately supporting the genus *Chromatoclothoda*, the genera of Anisembiidae excluding *Chelicerca*, the family Australembiidae, and the family Archembiidae (fig. 13).

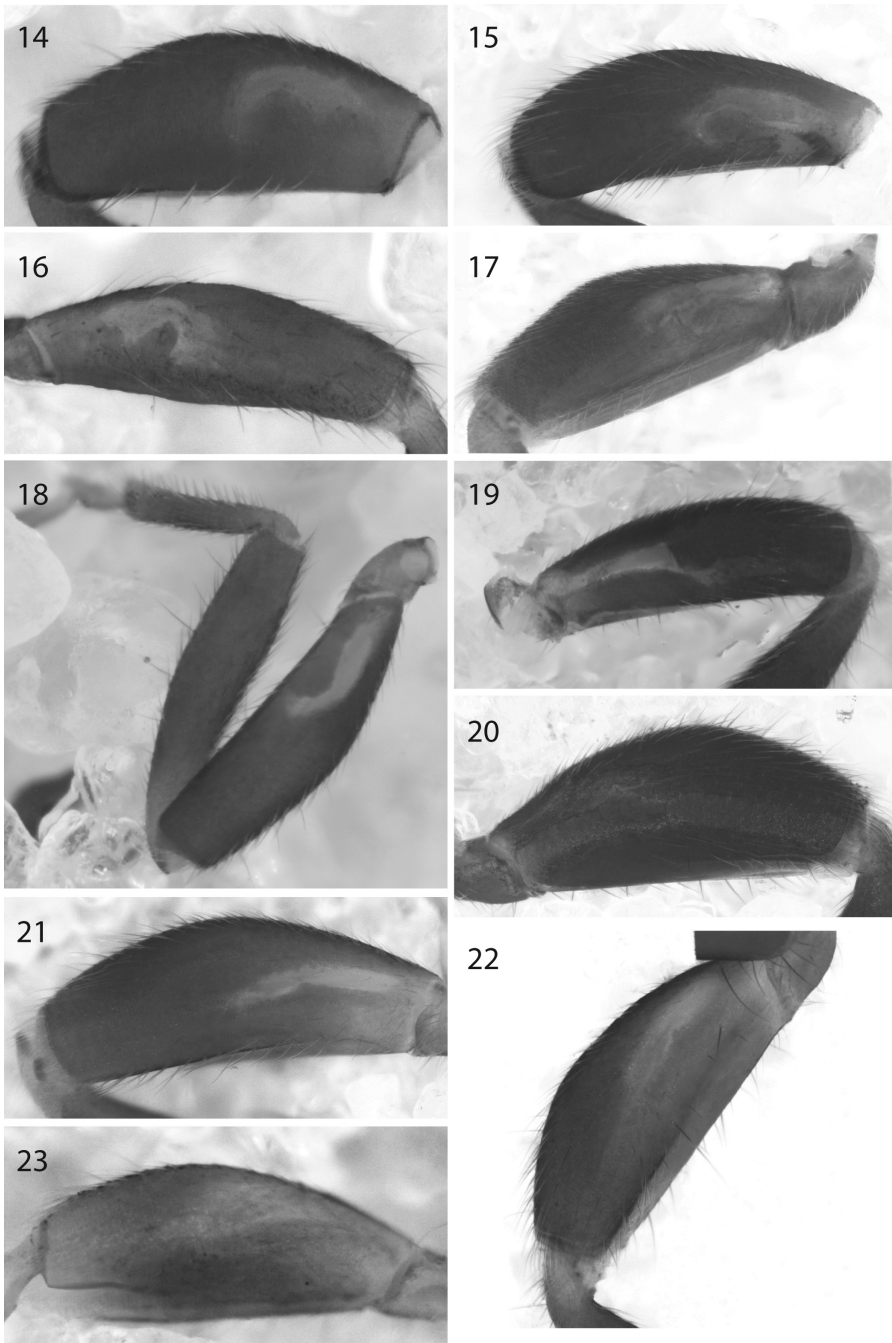
MIDDLE FEMUR: The auditory organ on the second femur has an anterior position (figs. 4, 5). We describe three conditions (appendix 1): state 0, semicircular but with the basal end diffuse and expanded (figs. 14–16); state 1, a long band extended almost to the basal half of the femur (figs. 17–22), sometimes pigmented as strongly as the rest of the femur (e.g., fig. 20); state 2, same condition as state 2 of forefemur (fig. 23). The auditory organ on the middle femur appears to be more homoplastic than that on the forefemur.

HIND FEMUR: The tympanal organ on the hind femora is absent in all Embioptera, with the exception of Anisembiidae and Australembiidae (figs. 4, 5). There are two general shapes; one is quite similar to that on the other femora, small and semicircular (figs. 24, 25), and is present only in some species of Anisembiidae. The second shape is in the form of a small, rounded and depressed area, occurring in Australembiidae (fig. 26) and in some species of Anisembiidae (figs. 27, 28). Sometimes, this rounded area is clearly different from the rest of the femur surface because it is membranous and depigmented, although, in some cases, it appears as a very small indentation of the surface (e.g., fig. 26). We coded these conditions as: state 0, tympanal organ semicircular (figs. 24, 25); state 1, small, disklike depressed area (figs. 26–28); state 2, tympanal organ absent. The condition observed in *Anisembia pifical* (fig. 27) was coded as 1, but it seems intermediate between states 0 and 1.

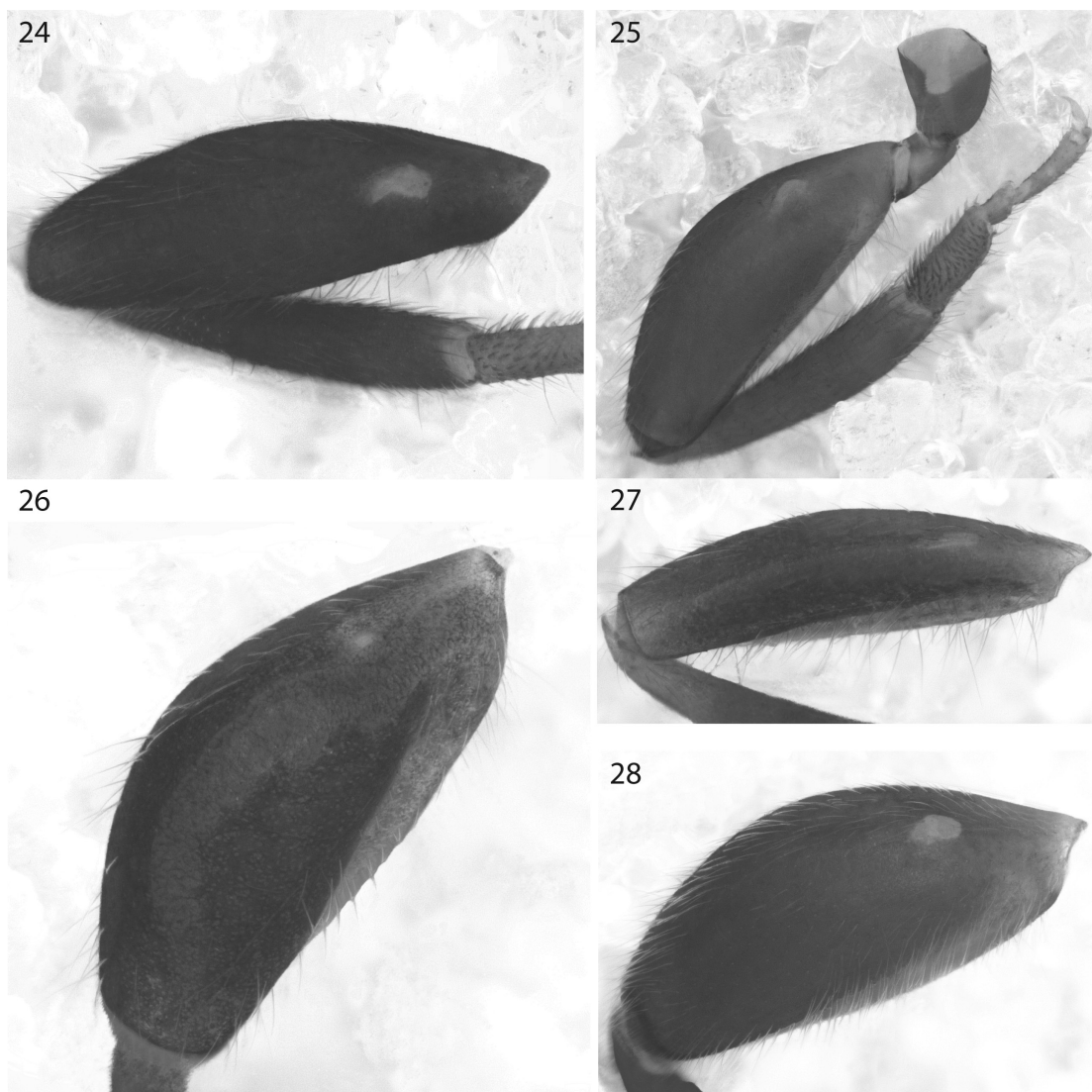
Anisembiidae and Australembiidae are not sister groups; thus, the tympanal organ must have appeared twice independently (fig. 29). According to our results, Australembiidae is supported by a disk-shaped depressed area (state 1, fig. 26). In the case of Anisembiidae, the specimens examined for the genus *Microembia* had poorly preserved hind legs, and for that reason the character for this genus is coded as a missing entry. According to the optimization of the character in the most parsimonious trees, the condition of the small depressed area (state 1: fig. 28) develops first, for the genera that form the sister group of *Chelicerca* (*Anisembia*, *Mesembia*, *Saussurembia*, *Stenembia*; fig. 29), and then turns into the semicircular form (state 0) for most of the species of the genus *Chelicerca*, with three reversals (fig. 29). With the inclusion of more taxa, as well as fresher specimens of *Microembia*, this sequence of transformation may need to be reassessed.

THE SPINNING APPARATUS

The most striking apomorphy of the order—the ability to produce silk from the basal segment of the foretarsus—has been documented since the earliest studies on Embioptera, starting with Grassi and Sandias (1894), who discovered that the silk glands in Embioptera are in the first legs and not in the mouth, as had been maintained for years by other entomologists. Rimsky-Korsakow (1905) first made a detailed description of the silk glands, ducts, and setae from which the silk is extruded (i.e., spinning apparatus). Since the pioneering works of Rim-



FIGS. 14–23. Shapes of auditory organ on middle femur: **14**, *Chelicerca wheeleri* (Anisembiidae) right leg; **15**, *Mesembia chamulae* (Anisembiidae) right leg; **16**, *Teratembia geniculata* (Teratembidae) left leg; **17**, *Clothoda longicauda* (Clothodidae) right leg; **18**, *Chelicerca barbara* (Anisembiidae) left leg; **19**, *Australembia nodosa* (Australembidae) left leg; **20**, *Biguembia copo* (Archembiidae) left leg; **21**, *Machadoembia angolica* (Embiidae) right leg; **22**, *Dihybocercus femorata* (Embiidae) right leg; **23**, *Aposthonia indica* (Oligotomidae) right leg.



FIGS. 24–28. Shapes of auditory organ on right hind femur: **24**, *Chelicerca barbara* (Anisembiidae); **25**, *C. wheeleri* (Anisembiidae); **26**, *Australembia incompta* (Australembiidae); **27**, *Anisembia piticial* (Anisembiidae); **28**, *Mesembia chamulae* (Anisembiidae).

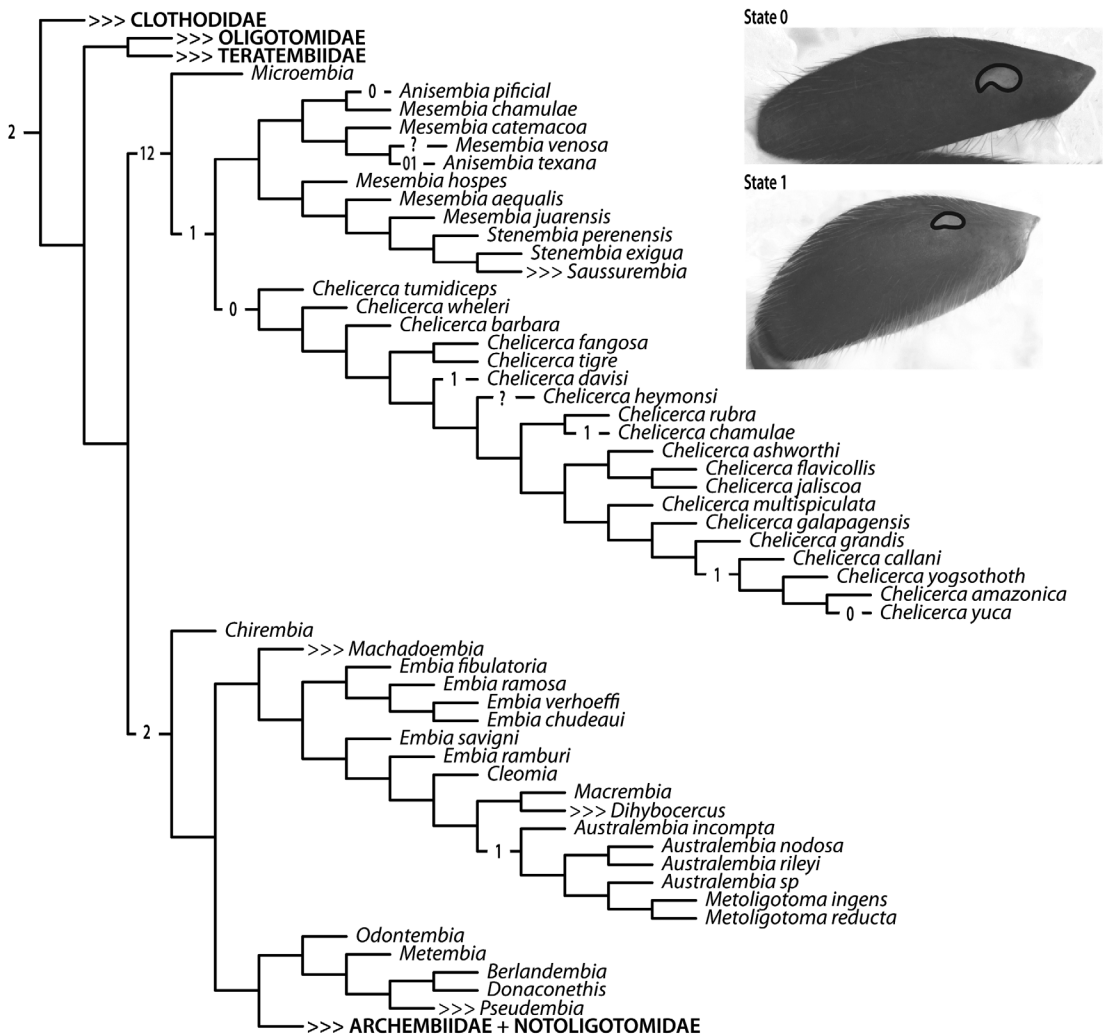


FIG. 29. Optimization of the shape of tympanal organ in the hind femur: state 0, small and semicircular; state 1, small disk depressed area; state 2, absent.

sky-Korsakow (1905, 1910, 1914), several interesting studies of the spinning apparatus in Embi-
optera have been made (Mukerji, 1927; Barth, 1954; Alberti and Storch, 1976; Nagashima et
al., 1991; Dubitzky and Melzer, 1999), using different approaches (morphological, physiologi-
cal, and histological). The spinning behavior and properties of silk were also assessed and
described by Edgerly et al. (2002, 2012); Okada et al. (2008); Collin et al. (2009); Büsse et al.
(2015) and McMillan et al. (2016).

The spinning apparatus is composed of glands in the basal segment of the foretarsus, each
gland consisting of a reservoir surrounded by gland tissue that secretes the silk into the reser-
voir. The secretion is passed through a single duct connected to a silk ejector (modified hollow
seta), through which it is released (Nagashima et al., 1991). The ventral face of the basal seg-

ment of the foretarsus is always depigmented, poorly sclerotized, quite soft, and normally whitish, yellowish, or orangish. On this soft surface there is a great diversity of setae and modified microtrichia (i.e., silk ejectors and combs); many of these had already been observed by Rimsky-Korsakow (1905).

SILK EJECTORS: The silk ejectors (modified microtrichia) are more or less curved filaments (figs. 30–32), with a lumen and an apical opening. In this study we describe for the first time two types of silk ejectors:

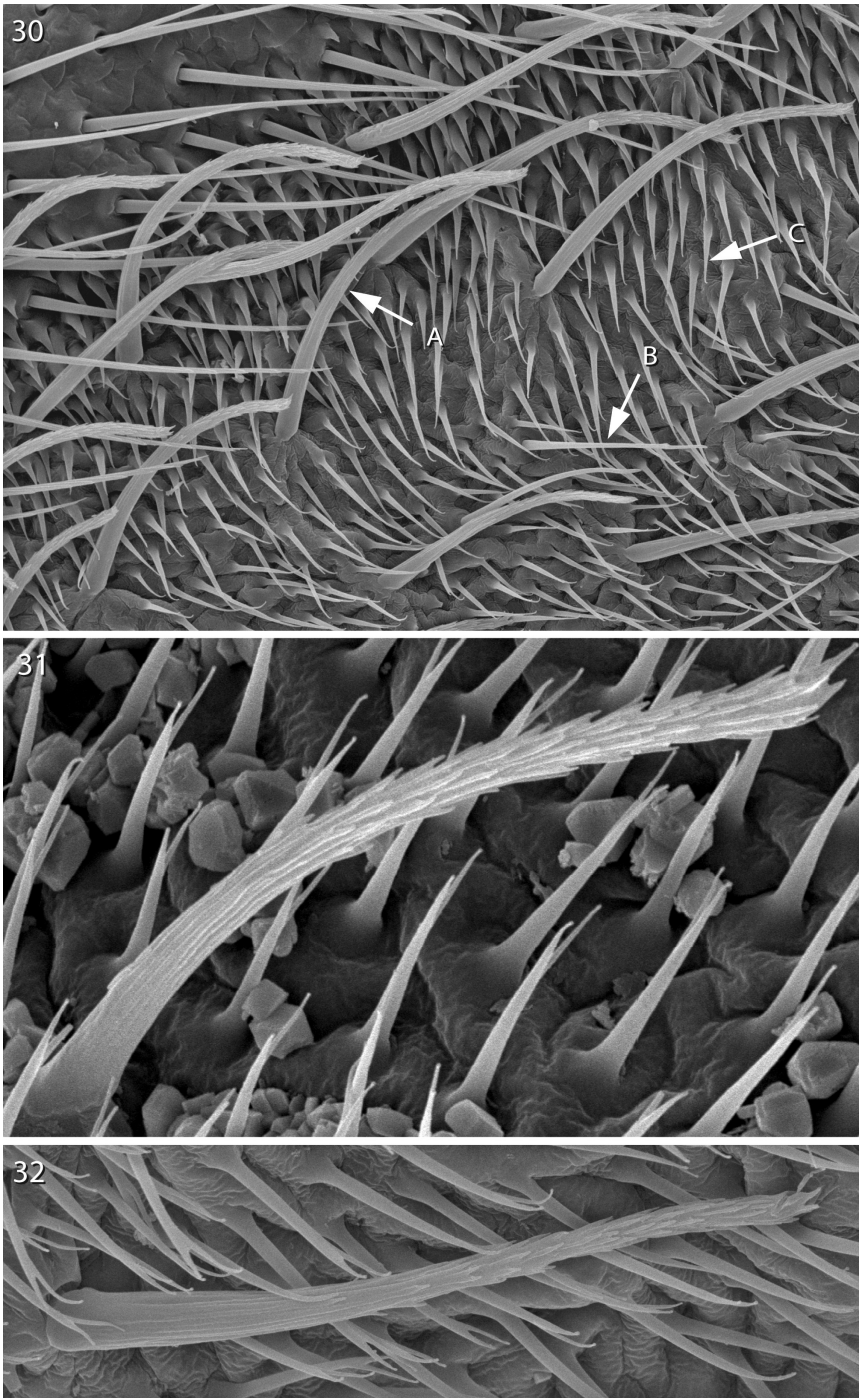
Silk ejectors of Type I are the most abundant type, long, robust and longitudinally striated (figs. 30A–31). Striations or serrate ridges along the ejector end in acute tips (fig. 31). The serrate ridges can start on the basal, medial, or apical section of the ejector, depending on the position of the ejector (with ridges starting basally on the ejectors closer to the edge of the tarsus) and the species observed (figs. 31, 32). The silk ejector ends in a spiraled cone with two or three acute tips (figs. 31, 32); again, differences in the tip exist, depending on the position of the silk ejector and the species observed. All webspinners have this type of ejector.

Silk ejectors of Type II are few and sparsely distributed, never close to the edge of the tarsus, slender and nearly straight (fig. 30B). Their surface is smooth, ending in a rounded tip where the lumen opens.

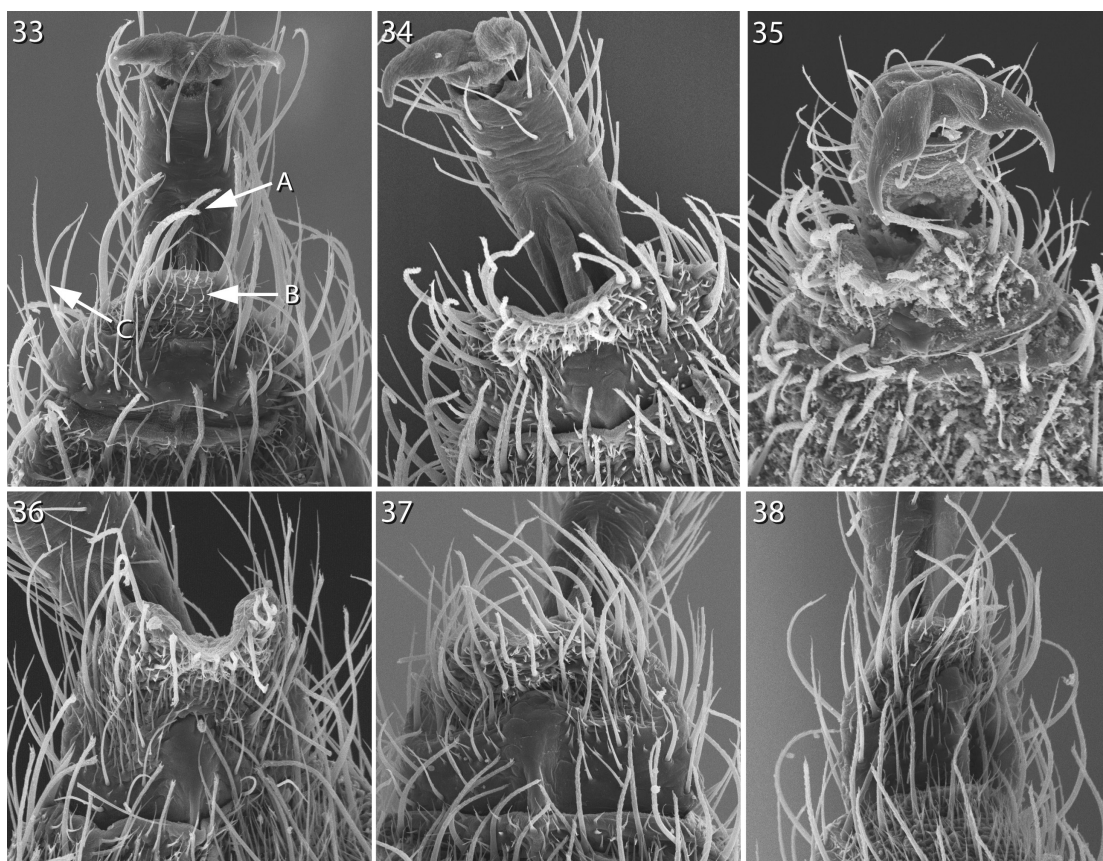
This study demonstrates that silk ejectors of Type I are also present on the ventral face of the foretarsus midsegment (see figs. 33–38). Previously, only Nagashima et al. (1991) had reported ejectors, and no glands, on the midsegment, which meant that silk glands were thought to be present only in the basal segment. No other study on the silk apparatus reported observations on any segment other than the basal one.

Both types of silk ejectors are arranged nearly longitudinally in rows (figs. 39–50) and are denser distally on both segments of the tarsus. The ventral view of the silk apparatus of 12 species belonging to eight families of Embioptera (figs. 39–50) shows clear variations in the number and rows of ejectors. Variation in the number of ejectors among the different species is to be expected from the fact that the thickness and consistency of nest silk also varies widely (e.g., Okadaa et al., 2008; Collin et al., 2009). In addition, a direct proportional relationship between individual size and number of ejectors could be expected, as larger species tend to be hairier. This correlation was also suggested by Collin et al. (2009) comparing two species (*Antipaluria urichi* and *Oligotma nigra*). However, the number of rows and setae are apparently not related to the size of the specimen; for example, the oligotomid *Haploembia solieri* (13 mm long) has approximately 234 ejectors distributed in 16 rows of ejectors (fig. 39), but the much larger embiid *Dihybocercus femorata* (19 mm long) has only 89 ejectors distributed in eight rows (fig. 43) (see table 1 for additional examples). The same lack of correlation between body length and the number of glands or reservoirs (i.e., number of ejectors) also was observed by Büsse et al. (2015).

Ejector shape may differ within the same specimen. Ejectors on the edge are clearly curved, whereas the others, in general, are less curved (e.g., figs. 40, 45–46). Aside from these differences in the same specimen—curved or less curved, robust or slender—we coded the number of silk ejector types as a character, with two states (see appendix 1): state 0, presence



FIGS. 30–32. Silk ejectors and combs on basal segment of foretarsus: **30**, detail of ventral left margin view of *Gibocercus chaco* (Archembiidae): A, silk ejector Type I, B, silk ejector Type II, C, comb setae; **31**, silk ejector Type I of *Oligotoma saundersii* (Oligotomidae), slightly curved; **32**, silk ejector Type I of *Gibocercus chaco*, erect and slender.

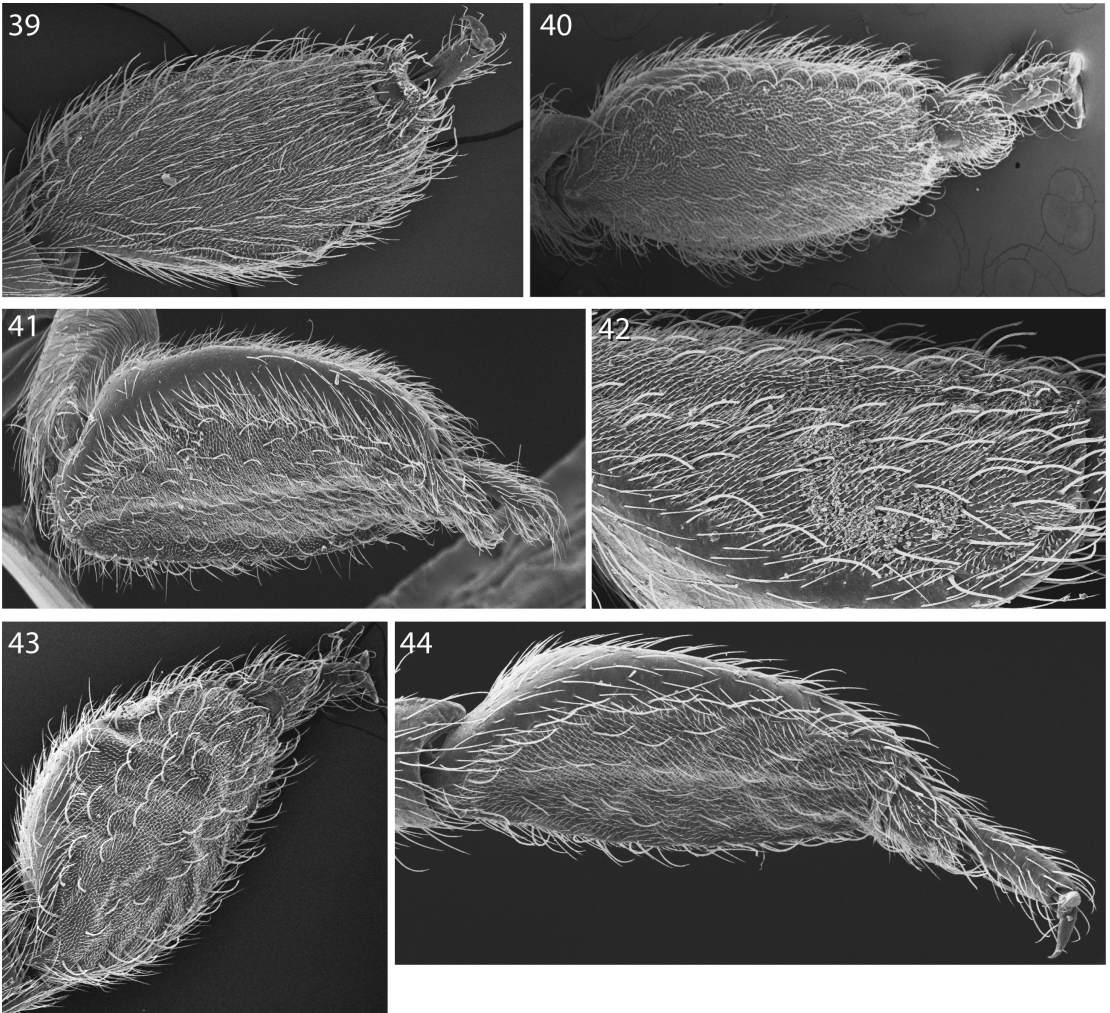


FIGS. 33–38. Ventral view of midsegment of foretarsus: **33**, *Saussurembia calypso* (Anisembiidae) left leg: A, silk ejector Type I, B, comb setae, C, serrate hairs; **34**, *Haploembia solieri* (Oligotomidae); **35**, *Notoligotoma nitens* (Notoligotomidae); **36**, *Australembia incompta* (Australembiidae) left leg; **37**, *Pararhagadochir trachelia* (Archembiidae); **38**, *Teratembia geniculata* (Teratembidae).

of a single type of ejector (Type I); state 1, presence of both types of ejectors (types I and II). The majority of Embioptera have two types of ejectors; the loss of the Type II silk ejector is apparently a synapomorphy of Australembiidae (fig. 51), with parallelisms in some species of *Teratembia* (Teratembidae) and *Chelicerca* (Anisembiidae). We found that the silk produced by *Chelicerca* and *Teratembia*, which generally have a low number of silk ejectors, is extremely delicate and translucent.

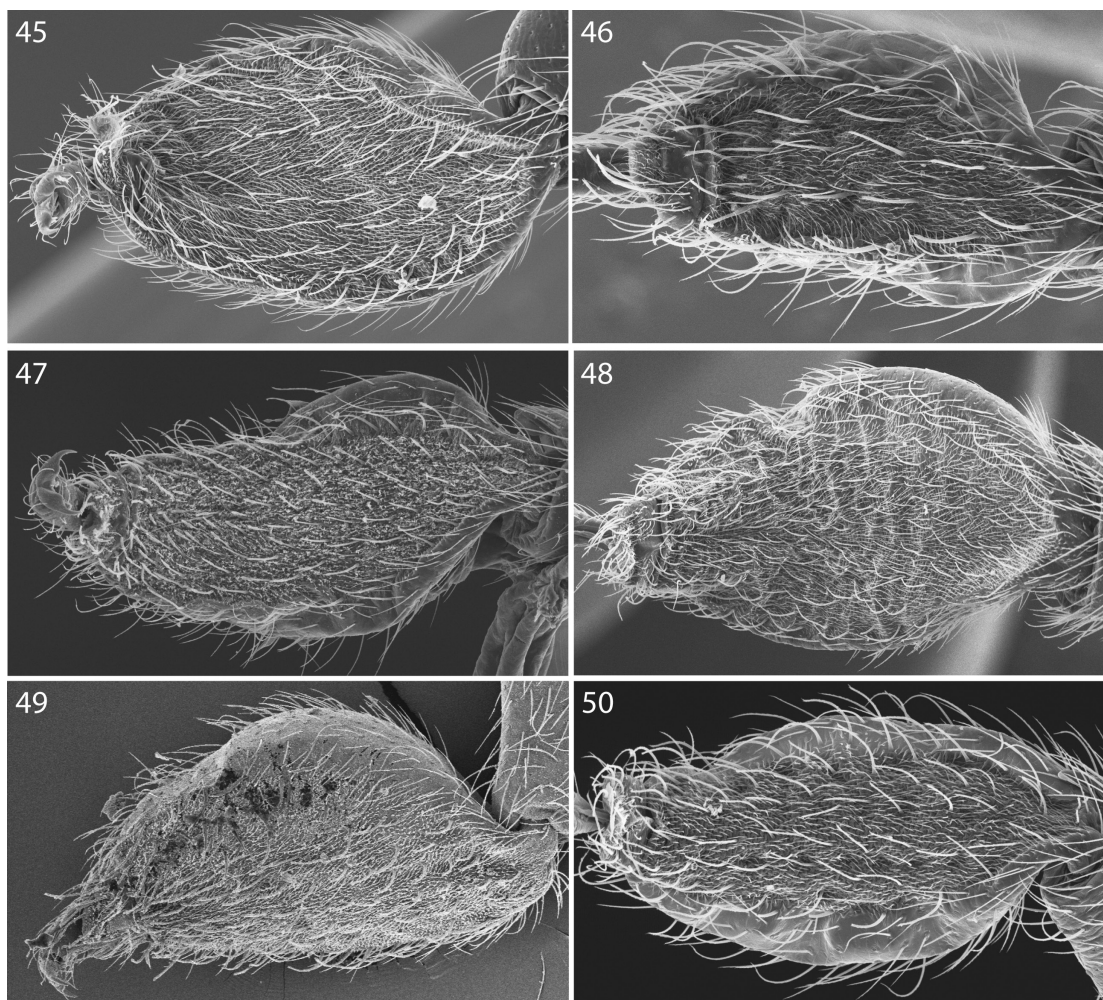
We coded a second ejector character, the length of Type I ejectors: state 0, ejectors (less than 80 μm); and state 1, long ejectors (more than 100 μm). Long ejectors are a synapomorphy of a subgroup of Archembiidae, but they also appear in parallel in some species of Clothodidae (fig. 52). In some species of *Antipaluria* this character could not be observed; those species are scored in the matrix as missing entries.

COMB SETAE: Comb setae are modified microtrichia that assist in building the silk wall (see Ross, 2000: 23) and are more or less densely distributed among the ejectors (figs. 30C, 53–62). They are mainly present on the basal segment of the hind tarsus but can



FIGS. 39-44. Ventral view of basal segment of foretarsus: **39**, *Haploembia solieri* (Oligotomidae), left leg; **40**, *Clothoda longicauda* (Clothodidae), right leg; **41**, *Antipaluria caribbeana* (Clothodidae), left leg; **42**, *Oligotoma saundersii* (Oligotomidae), left leg; **43**, *Dihybocercus femorata* (Embiidae), right leg; **44**, *Chelicerca barbara* (Anisembiidae), left leg.

also be found (in lower numbers) on the midsegment of the hind tarsus (e.g., figs. 33B, 34). The shape of the combs on the edge of the basal segment of the tarsus is nearly triangular, either basally broad and recumbent (figs. 53, 54) or slender and erect (figs. 55, 56). On the ventral surface of the foretarsal basal segment the combs are tiny, tubular, and with bifid apices (figs. 57–60). Depending on the taxonomic group, the comb setae can be either scarce or dense distributed. Apically bifid comb setae were not noted in detail in previous studies, but we observed here that one of the tips is normally shorter and more slender than the other, both gently tapering toward the tip and ending in a minuscule sphere (figs. 61, 62).



FIGS. 45–50. Ventral view of basal segment of foretarsus: 45, *Australembia incompta* (Australembiidae) left leg; 46, *Saussurembia calypso* (Anisembiiidae) left leg; 47, *Notoligotoma hardy* (Notoligotomidae) right leg; 48, *Pararhagadochir trachelia* (Archembiiidae) right leg; 49, *Embia ramburi* (Embiidae) right leg; 50, *Teratembia geniculata* (Teratembiiidae) right leg.

CHAETOTAXY OF HIND TARSI

The hind tarsi in Embioptera are used to clutch to the silk wall, helping the insect to easily move forward or backward. These two actions are possible because of the highly developed hind femora and the large size of the depressor muscle (see Ross, 2000), which is another synapomorphy of the order (Szumik, 1996). Details of the bladders (euplantulae) and setae on the hind tarsus appeared for the first time in the taxonomic descriptions of Silvestri (1912, 1921). Later, the presence or absence of a middle bladder (a modified euplantula) on the basal segment of the hind tarsus was often mentioned in taxonomic works (e.g., Davis, 1936; Ross, 2000). Variation in shape of the basal segment of the hind tarsus, position and size of the

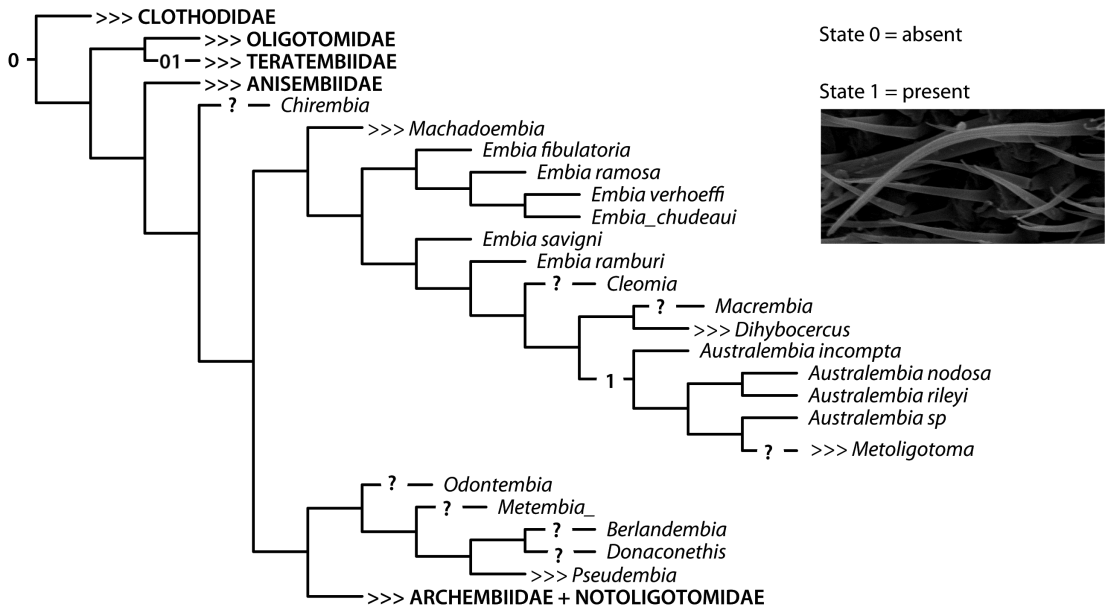


FIG. 51. Optimization of the silk ejectors Type II: state 0, absent; state 1, present.

middle bladder, and the number of lines of setae on anterolateral and retrolateral faces of the basal segment of the hind tarsus have also been included in taxonomic treatments (e.g., Szumik, 1994) and in cladistic studies (e.g., Szumik et al., 2008).

The number, arrangement, and direction of the setae on lateral and ventral surfaces, as well as other types of setae and microtrichia on the basal segment of the hind tarsus, are described here (see general view of basal segment of hind tarsus: figs. 63–70). In this study we describe a diversity of setae and microtrichiae, as well some details of the bladders.

BROAD SETAE: Broad setae are strong, short, conoid, and longitudinally striate (figs. 71, 72). These setae are present only on the lateral and ventral surfaces of the basal segment of the hind tarsus (figs. 63, 64, 66–69); Ross (2000: 24) called these setae “peg-like.” On the anterolateral half of the basal segment, these setae are directed transversally to the segment (see figs. 63, 68, 69). The number and distribution of broad setae are useful in alpha taxonomy studies (e.g., Szumik, 1997) as well as in higher classification (e.g., Szumik, 2004).

JAGGED SETAE: Jagged setae occur in Anisembiidae and Teratembidae. They are equivalent to the broad setae but have three or four serrations (figs. 65, 70, 73, 74). We coded these two types of setae as state 0, broad setae (figs. 63, 64, 66–69, 71, 72); and state 1, jagged setae (figs. 65, 70, 73–74) (see appendix 1). State 1 is present only in Anisembiidae and Teratembidae, where it is best interpreted as a synapomorphy for each family (fig. 91).

SERRATE HAIRS: Long, slender setae, serrated on the outer curvature are present either along the entire seta or only apically (on foretarsus: figs. 33C, 53–54; on hind tarsus: fig. 75). We confirm here that they are present on every tarsus and that they occur in low numbers on the other leg segments. These setae are most numerous on the dorsal face but are also present

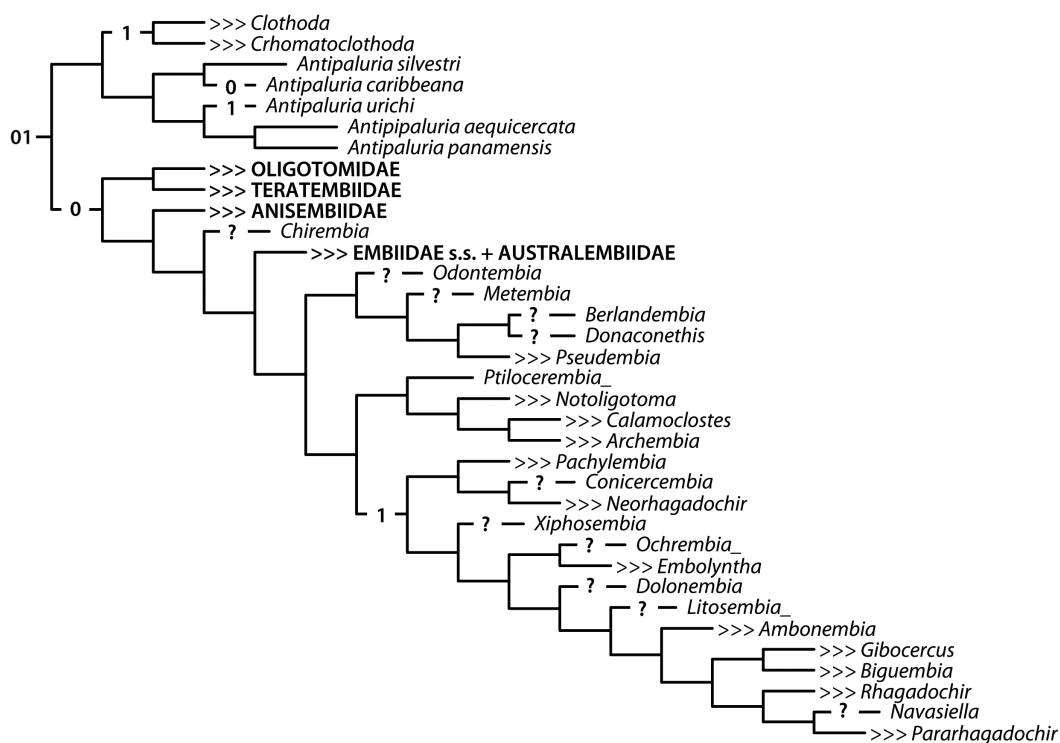


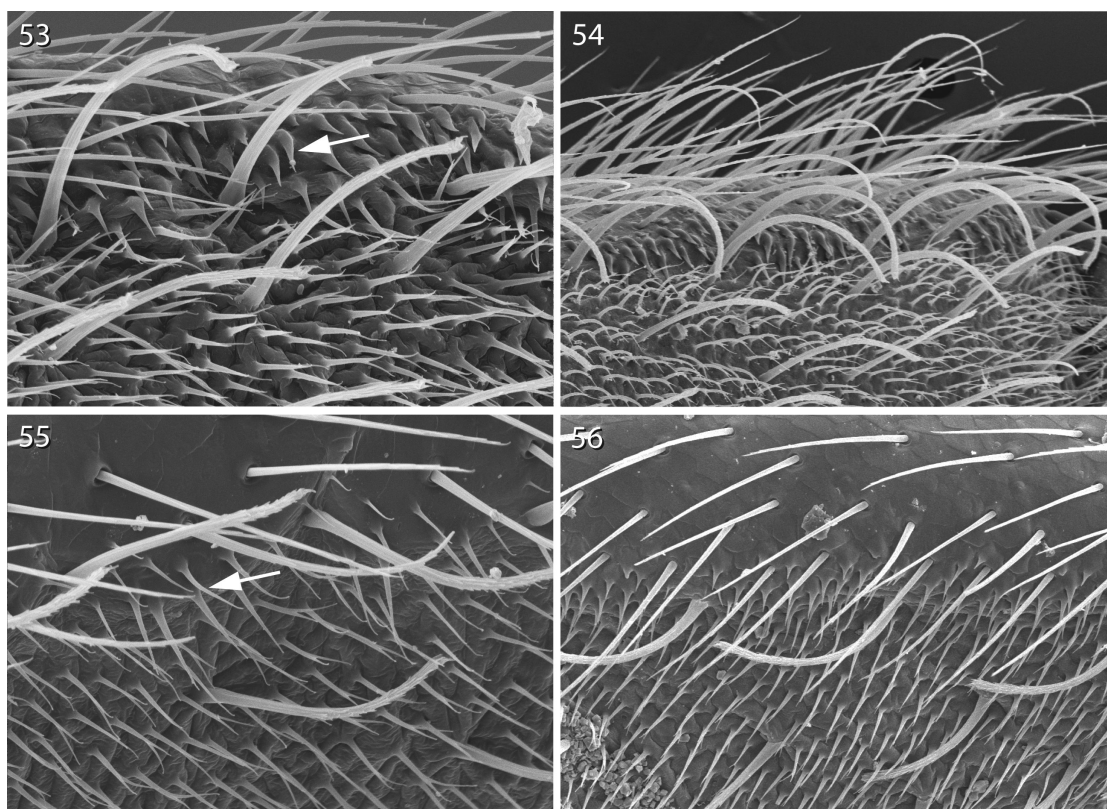
FIG. 52. Optimization of the silk ejectors length: state 0, short as in Embiidae (less than 80 μm); state 1, long (more than 100 μm).

in low numbers on the lateral and ventrobasal faces of the basal segment of the hind tarsus of all Embioptera. A similar seta was described by Slifer and Sekhon (1973) as tactile hairs on the antennae of two species of Embioptera. These setae were also observed by Ross (2000) on the hind tarsus only.

THREE-PRONGED SETAE: Close to or extending over the bladders of the basal segment of hind tarsus are three-pronged setae. The most striking characteristic of this setal type is that it has a pair of subdistal forks and a central straight apical filament (fig. 76). Slifer and Sekhon (1973: 213) described a similar seta as a thick-walled chemoreceptor on the antenna but also observed them on several parts of the body.

SLENDER HOOK SETAE: The slender hook seta is quite thin and distally arcuate (figs. 77, 78); this type is found in low numbers on the ventral face of the basal segment of the hind tarsus. We coded this as state 0, present (figs. 77, 78); and state 1, absent. Almost all webspinners have these setae (see appendix 1). Their absence appears as a synapomorphy of Embiidae sensu stricto + Australembiidae and also for the genus *Notoligotoma* (fig. 91).

BLADDERS OF HIND TARSUS: A character often used in Embioptera taxonomy is the presence (figs. 63, 66–68) or absence (figs. 64, 65, 69, 70) of a middle bladder on the ventral face of the basal segment of the hind tarsus. An apical bladder (i.e., euplantula, always present in the basal and midsegment of the hind tarsus) is more or less developed according to the taxonomic group (e.g., figs. 63–64, 70, 85–90).

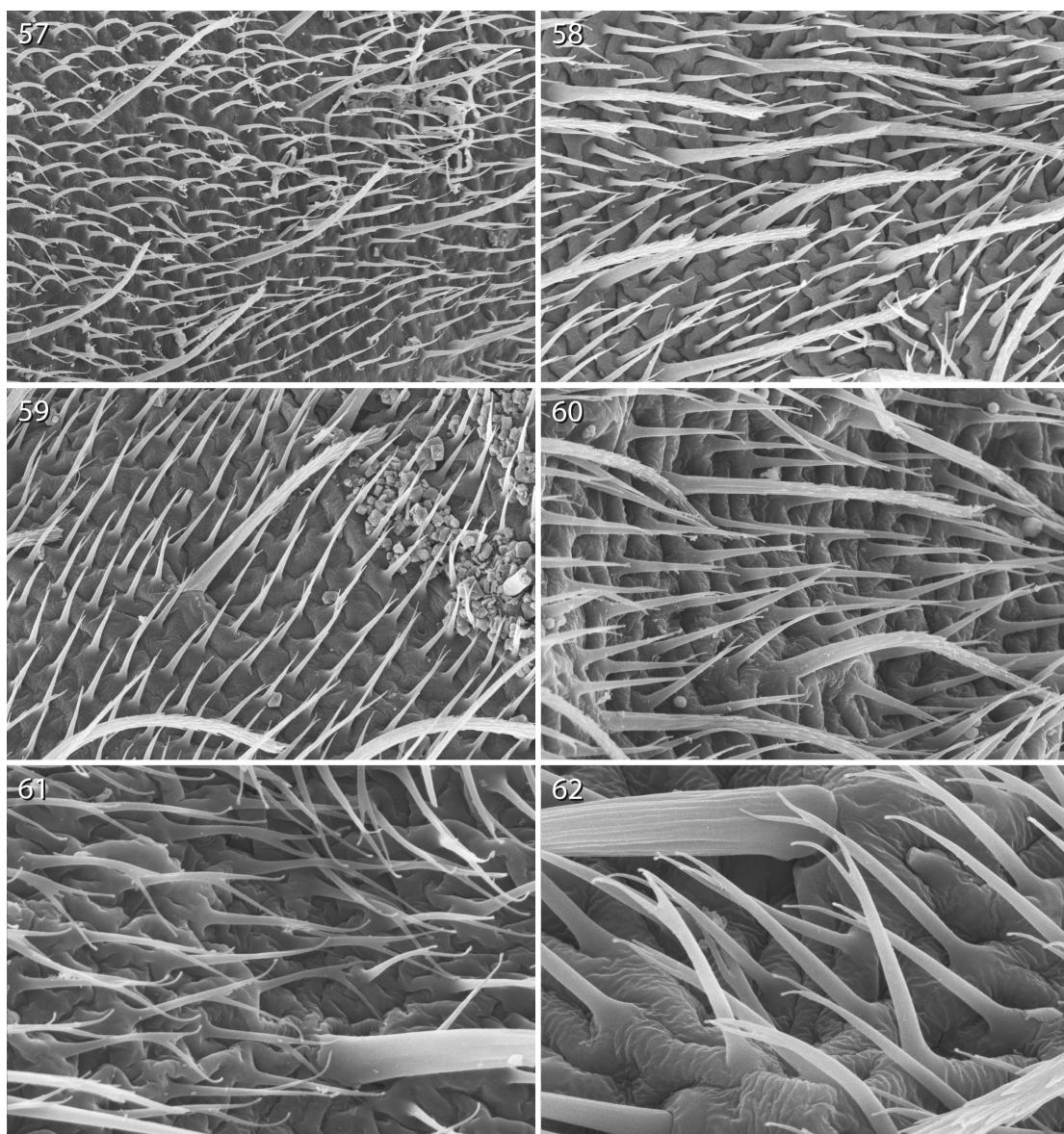


FIGS. 53–56. Comb setae on the edge and ventral surface of foretarsus basal segment. **53**, *Australembia incompta* (Australembiidae): arrow indicating broad and recumbent comb setae; **54**, *Antipaluria urichi* (Clothodidae); **55**, *Chelicerca barbara* (Anisembiidae): arrow indicating slender comb setae; **56**, *Oligotoma saundersii* (Oligotomidae).

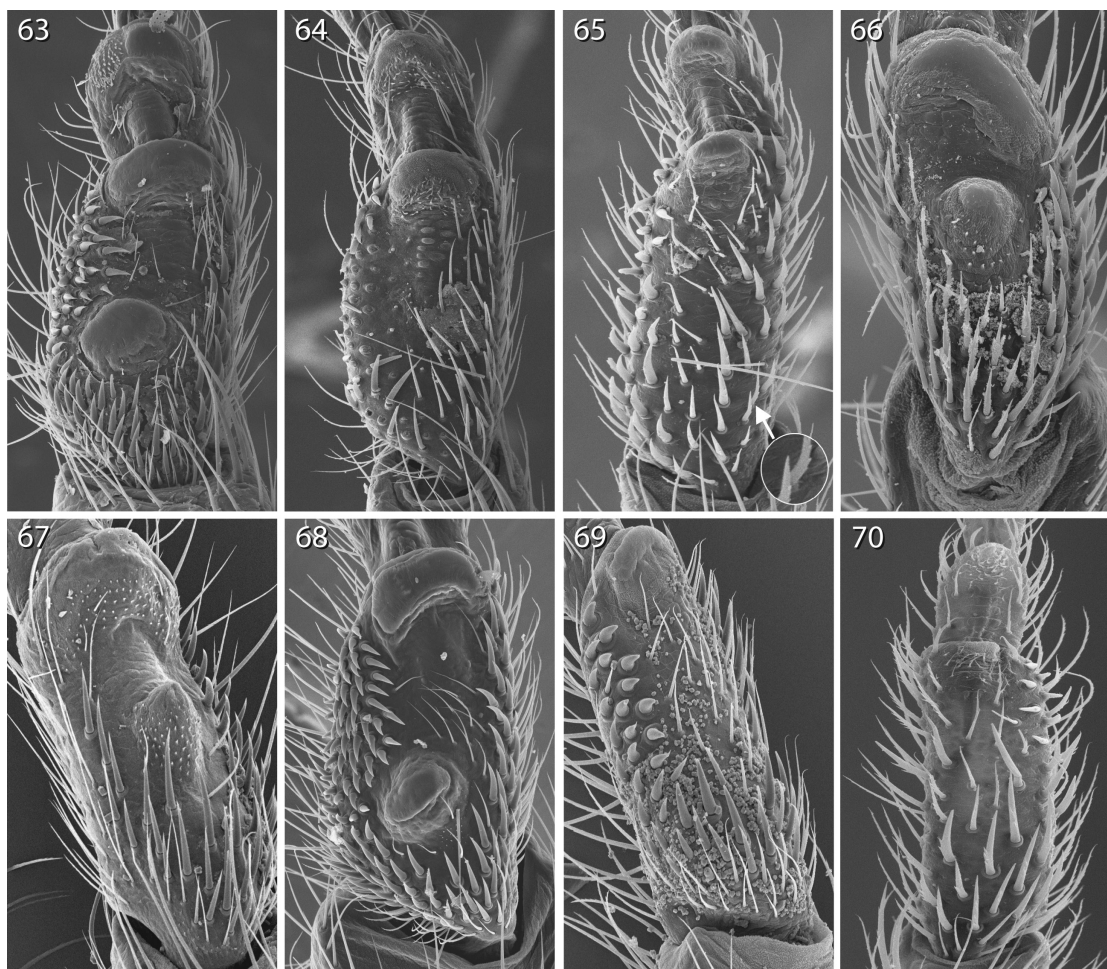
Bladders are unsclerotized, whitish, and globose. In some groups these bladders can be asymmetrical (e.g., figs. 63, 68), with the bladder more developed on the anterolateral face of the segment (the side in contact with the silk wall). In other groups the bladders are almost perfectly symmetrical (figs. 66, 67). The surface of the bladders is softly sculptured (figs. 79) or smooth (fig. 80). We coded this condition as state 0, sculpture present; and state 1, sculpture absent (see appendix 1). Sculptured bladders appear three times, in the family Anisembiidae and the genera *Teratembia* (Teratembidae) and *Archembia* (Archembidae).

We observed that some taxa may have a small curved seta, probably a mechanoreceptor, on the basal edge of the middle bladder or apical bladder (figs. 83, 84). According to our results the presence of mechanoreceptor setae on the medial bladder is a synapomorphy of both Clothodidae and the clade *Notoligotoma* + *Calamoclostes* + *Archembia* (appendix 1). This curved seta occurs on the basal edge of the apical bladder in the genus *Notoligotoma* and the species *Antipaluria urichi*.

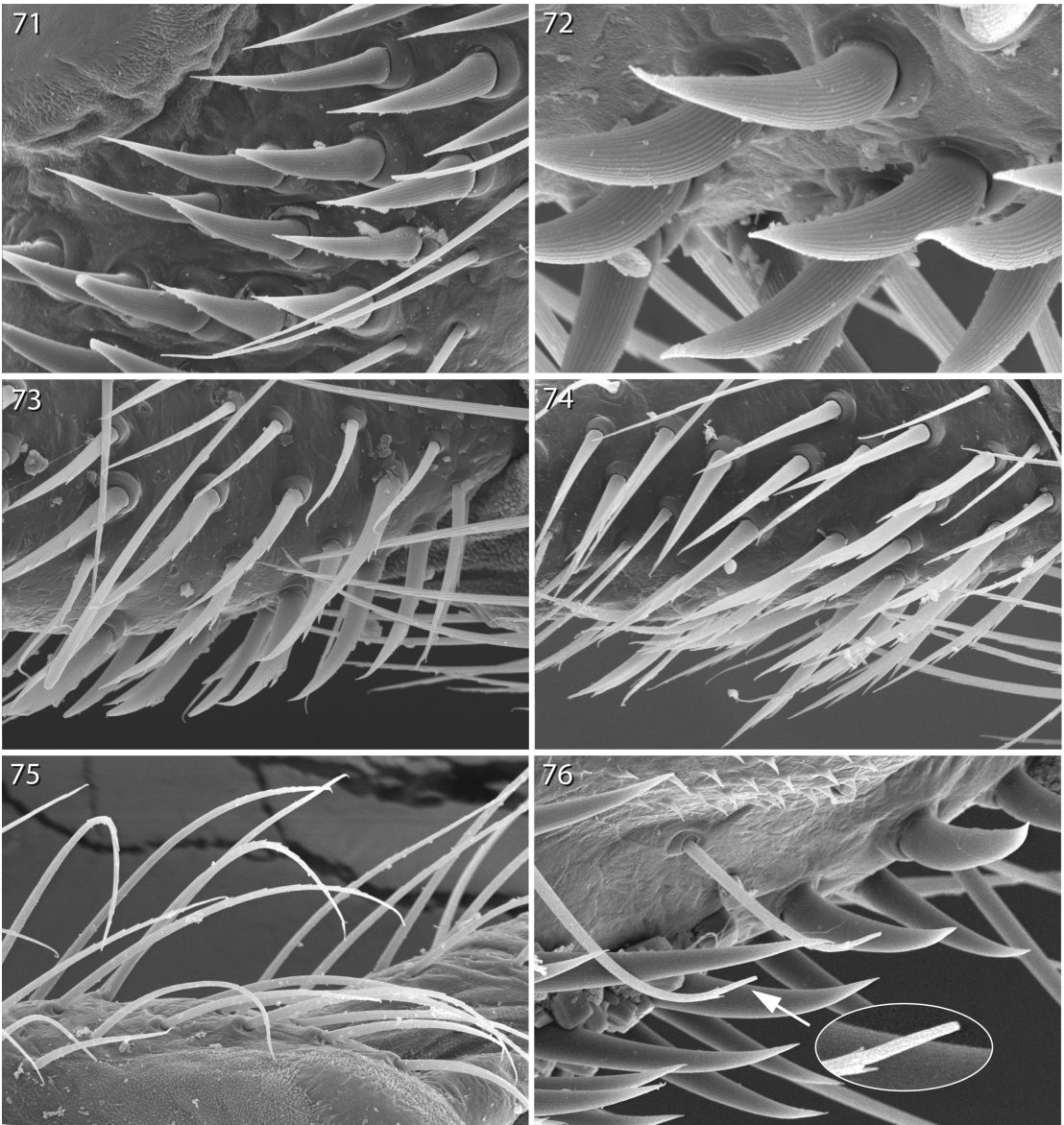
Likewise, a small area covered by microtrichia can be present on or near the middle and apical bladders. These microtrichia show variations in shape as well as position (e.g., figs. 67, 82, 86–90). Three characters (see appendix 1) were scored describing the microtrichia on the medial and apical



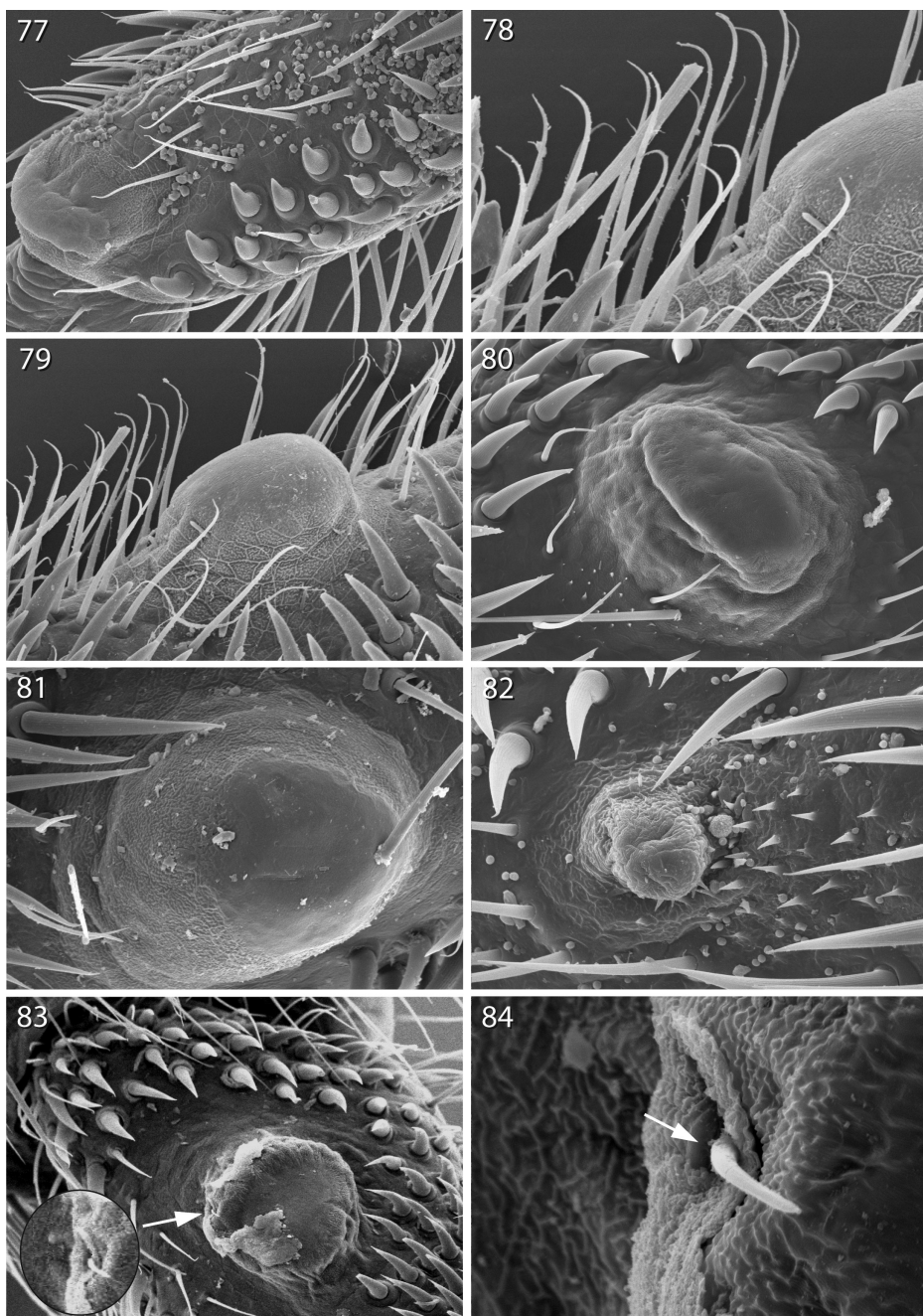
FIGS. 57–62. Comb setae on ventral surface of foretarsus basal segment: **57**, *Clothoda longicauda* (Clothodidae); **58**, *Haploembia solieri* (Oligotomidae); **59**, *Oligotoma saundersii* (Oligotomidae); **60**, *Pararhagadochir trachelia* (Archembiidae); **61**, *Saussurembia calypso* (Anisembiidae); **62**, *Gibocercus chaco* (Archembiidae).



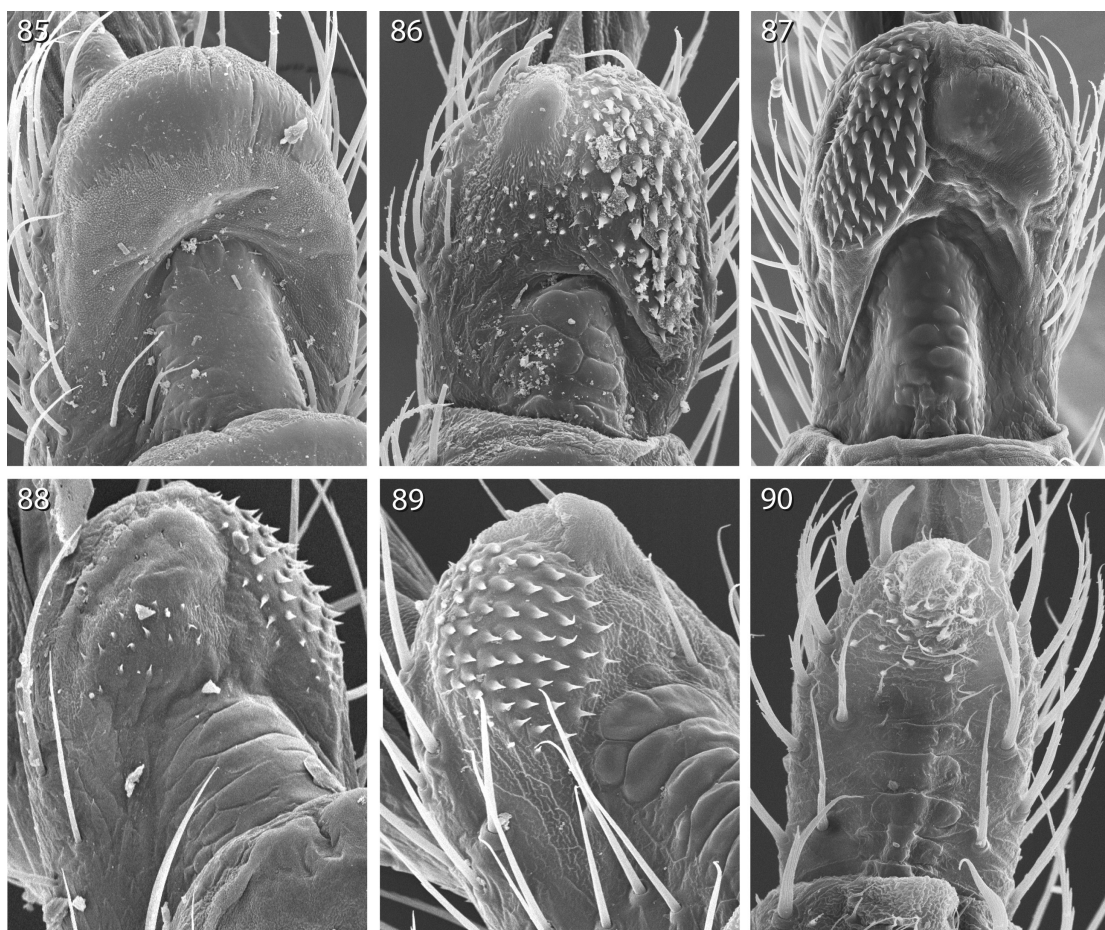
FIGS. 63–70. Hind tarsus, ventral view: **63**, broad setae in *Antipaluria caribbeana* (Clothodidae) left leg; **64**, broad setae in *Auqualembia incompta* (Auqualembiidae) left leg; **65**, jagged setae in *Chelicerca barbara* (Anisembiidae) left leg; an enlarged section of this seta is shown in the adjacent circle; **66**, broad setae in *Notoligotoma hardy* (Notoligotomidae) right leg; **67**, broad setae in *Dihibocercus femorata* (Embiidae) right leg; **68**, broad setae in *Gibocercus chaco* (Archembiidae) left leg; **69**, broad setae in *Oligotoma saundersii* (Oligotomidae) left leg; **70**, jagged setae in *Teratembia geniculata* (Teratembidae) right leg.



FIGS. 71–76. Chaetotaxy of hind basitarsus: 71, broad setae of *Antipaluria urichi* (Clothodidae); 72, broad setae of *Clothoda longicauda* (Clothodidae); 73, jagged setae of *Chelicerca barbara* (Anisembiidae); 74, jagged setae of *Saussurembia calypso* (Anisembiidae); 75, serrate hairs on lateroapical surface of *Clothoda longicauda* (Clothodidae); 76, three-pronged setae on lateroapical surface of *Dihybocercus femorata* (Embiidae); an enlarged section of this type of setae is shown in the adjacent circle.



FIGS. 77–84. Chaetotaxy and middle bladder: 77, slender hook of *Oligotoma saundersi* (Oligotomidae); 78, slender hook of *Archembia lacombea* (Archembiidae); 79, middle bladder with sculpture on *Archembia lacombea* (Archembiidae); 80, middle bladder with few microtrichia of *Gibocercus chaco* (Archembiidae); 81, middle bladder without sculpture or microtrichia *Pachylembia uncinata* (Archembiidae); 82, small middle bladder with microtrichia *Pararhagadochir trachelia* (Archembiidae); 83, mechanoreceptor on middle bladder *Antipaluria urichi* (Clothodidae); 84, detail of mechanoreceptor (circle in fig. 83) on middle bladder of *Antipaluria urichi* (Clothodidae).



FIGS. 85–90. Apical bladder of hind tarsus midsegment: **85**, *Clothoda longicauda* (Clothodidae) left leg; **86**, *Notoligotoma hardyi* (Notoligotomidae) right leg; **87**, *Gibocercus chaco* (Archembiidae) left leg; **88**, *Dihybocercus femorata* (Embiidae) right leg; **89**, *Oligotoma saundersii* (Oligotomidae) left leg; **90**, *Teratembia geniculata* (Teratembidae) right leg.

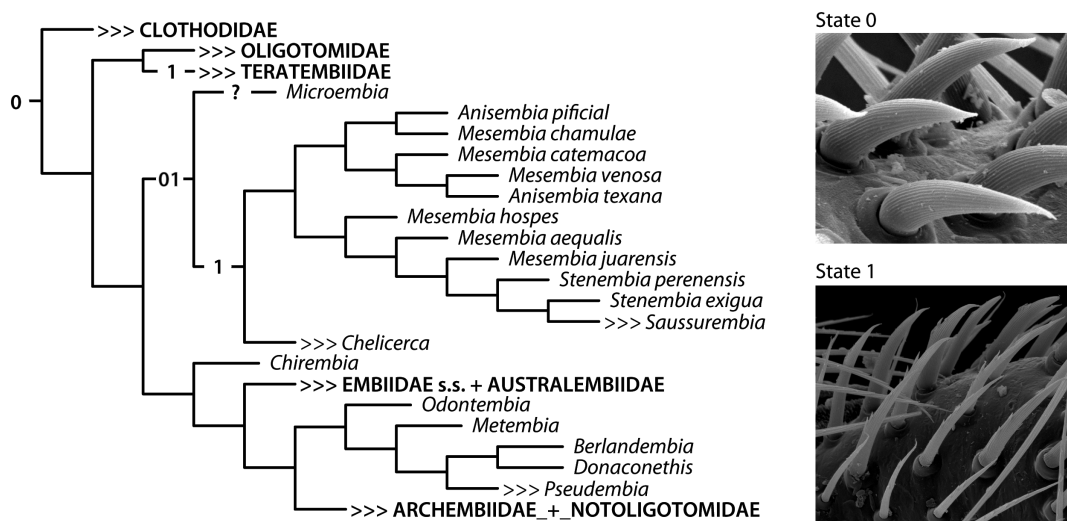


FIG. 91. Optimization of broad/jagged setae: state 0, broad setae; state 1, jagged setae.

bladders of the basal segment of hind tarsus. However, we found more variation in the microtrichia of the apical bladder of the hind tarsus midsegment (figs. 85–90). In some groups the microtrichia are uniformly distributed (figs. 67, 70, 90); in others the microtrichia are concentrated on the external side of the bladder (anterolateral face: figs. 63, 86–89) or are absent (figs. 65, 69, 85). Uniform distribution is a synapomorphy of Australembiidae (figs. 64, 93) and Teratembidae (figs. 70, 90, 93). Absence of microtrichia is a synapomorphy of several genera, such as *Clothoda* (Clothodidae, e.g., fig. 85), *Chelicerca* (Anisembidae, e.g., fig. 65) and *Pachylembia* (Archembiidae, see fig. 93).

FINAL COMMENTS

This study is a first attempt to search for stable characters to reinforce the classification of Embioptera. New traits on legs were observed, described, and analyzed within a cladistic framework. Perhaps the most striking new finding is an auditory tympanal organ, described for the first time in this order. In the past Embioptera were commonly preserved on slides (e.g., see Ross, 1943), and this protocol (always producing dorsal views of the mounted specimens) prevented the tympanal organ from being observed. An additional disadvantage of mounting the specimens on slides is that specimen clearing makes this structure much less evident, to the point of being undetectable. The structure is also inconspicuous under SEM as it is a membranous area, and quite difficult to observe with typical microscopy, especially on poorly pigmented specimens (e.g., Teratembidae, Oligotomidae) or specimens with several patches of different colors (e.g., Australembidae).

The tympanal organ of Embioptera resembles that of other insects, e.g., the suborder Ensifera, although the shape, position, and size of this structure in Embioptera is quite unique. In Ensifera, it is present on only the proximal part of the foretibia, consisting of two oval membranes on the anterior and posterior faces of the tibia (Yack, 2004: 323). Two species of Mantophasmatodea have five scolopidial organs on the legs, one of which is on the femur (Eberhard

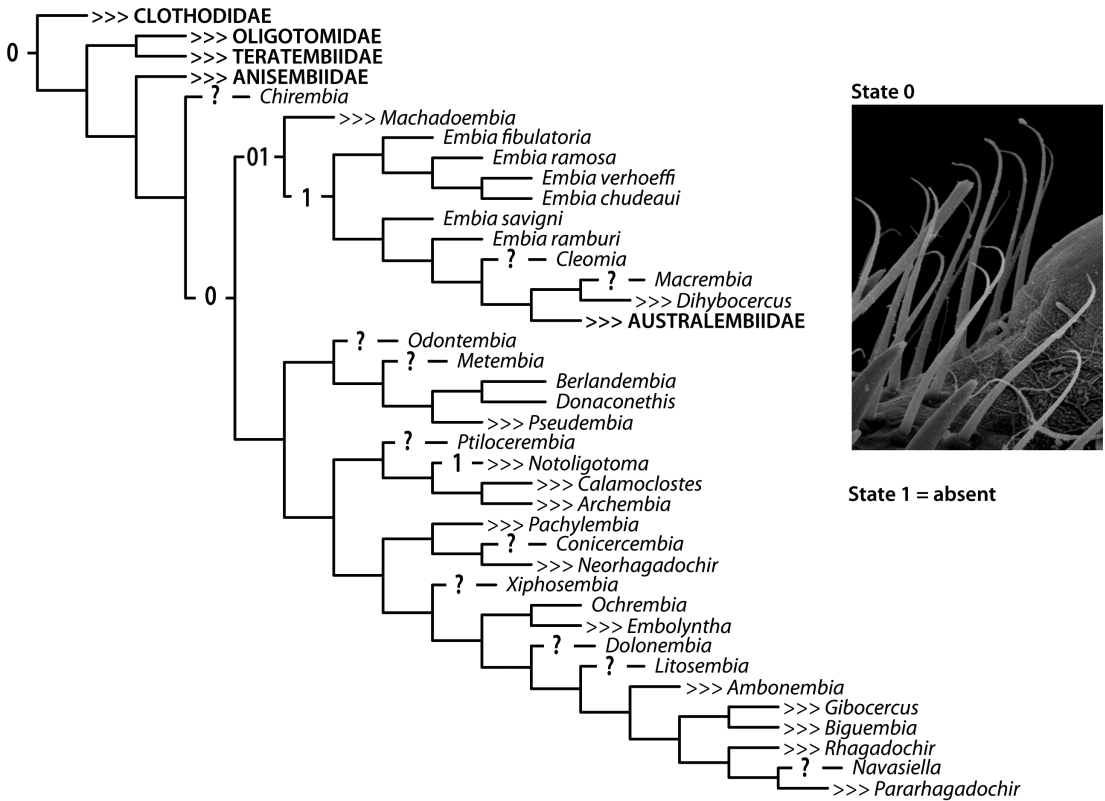


FIG. 92. Optimization of the slender hook setae: state 0, present; state 1, absent.

et al., 2010) as in Embioptera. The question of whether the structure observed here is homologous with that found in other insect orders is beyond the scope of the present study, but certainly an interesting question to consider. For a general review of auditory organs in insects, see Field and Matheson (1998); Stumpner and von Helversen (2001); Yack (2004).

For the phylogenetic analysis, 13 new characters were added to the data matrix of Szumik et al. (2008), representing an increase of 6.5% in the size of the data matrix. A full analysis, with additional taxa and characters, is presently being developed and will be published elsewhere; the present results are preliminary, intended only as a first approximation of the behavior of the new characters on a phylogenetic tree. The characters added, despite their low numbers, help resolve and stabilize several inter- and intrafamily relationships. The same optimal tree was obtained from the four concavities values used (to make the results comparable to those of Szumik et al., 2008, we used $k = 4-7$). Embiididae appeared with two optimal resolutions in previous analysis (Szumik et al., 2008: figs. 13, 14); for the present matrix, the results stabilize in only one of those resolutions: the genera *Embia*, *Dihybocercus*, *Macrembia*, *Machadoembia*, and *Cleomia* as sister group of Australembiidae, and *Pseudembia*, *Metembia*, *Odontembia*, *Berlandembia*, *Dinembia*, and *Donaconethis* as sister group of the Afro-Neotropical Archembidae. The position of Oligotomidae, Teratembidae, and Anisembidae is

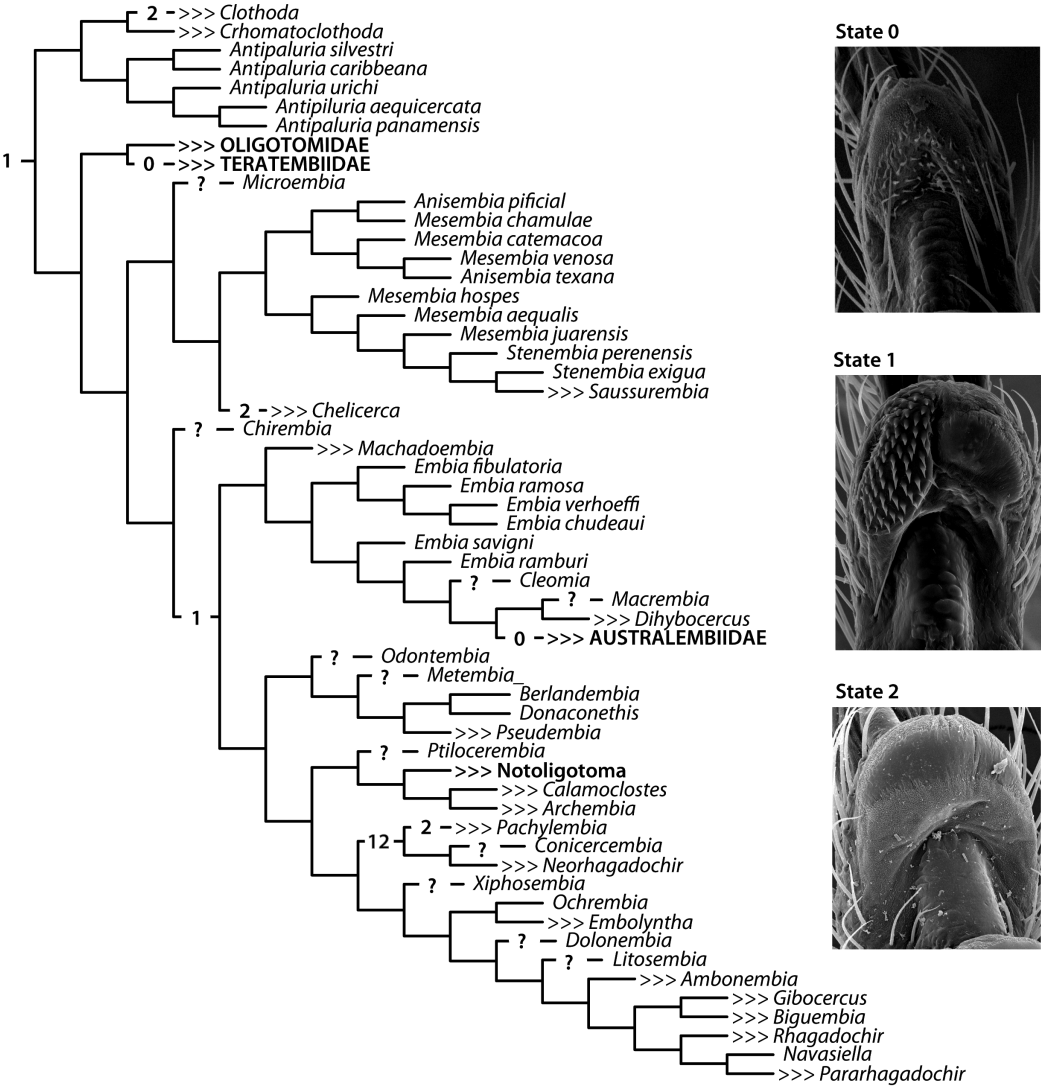


FIG. 93. Optimization of the microtrichia on apical bladder of middle tarsus: state 0, present and uniformly distributed; state 1, present but concentrated on the external side of the bladder; state 2, absent.

similar to Szumik et al. (2008: fig. 5), but there are some interesting changes: Oligotomiidae now appears as monophyletic, and the relationships of their species are almost entirely resolved (whereas Szumik et al. [2008: fig. 6] found Oligotomiidae a complete polytomy). The same occurs with the intergeneric relationships within Anisembiidae, which have additional resolution with the addition of the new characters (fig. 29). Finally, Archembiidae maintains the major groups, but the tetrachotomy in the basal part of Archembiidae (Szumik et al., 2008: fig. 16) is now resolved (fig. 92). All this shows that focusing on structures related to their lifestyle in silk tubes characteristic of Embioptera provides useful phylogenetic information to several groups and sections of the tree.

ACKNOWLEDGMENTS

We thank the Willi Hennig Society for access to the program TNT. We deeply appreciate the comments on the manuscript of James M. Carpenter and an anonymous reviewer. This study was supported thanks to PIP 010, PUE 070 CONICET, and PICT 2015-0283 (to M.J.R.).

REFERENCES

- Alberti, G., and V. Storch. 1976. Transmissions- und rasterelektronenmikroskopische Untersuchung der Spinndrüsen von Embien (Embioptera, Insecta). *Zoologischer Anzeiger* 197: 179–186.
- Barth, R. 1954. Untersuchungen an den Tarsaldrüsen von *Embolynta batesi* Mac Lachlan, 1877 (Embioida). *Zoologische Jahrbücher (Anatomie)* 74: 172–188.
- Büsse, S., T. Hornschemeyer, K. Hohu, D. Mcmillan, and J.S. Edgerly. 2015. The spinning apparatus of webspinners – functional-morphology, morphometrics and spinning behaviour. *Scientific Reports* 5: 9986.
- Choe, J.C. 1994. Communal nesting and subsociality in a webspinner, *Anisembia texana* (Insecta: Embiidina: Anisembiidae). *Animal Behaviour* 47: 971–973.
- Cocroft, R.B. 2001. Vibrational communication and the ecology of group-living, herbivorous insects. *American Zoologist* 41: 1215–1221.
- Cocroft, R.B., and R.L. Rodríguez. 2005. The behavioral ecology of insect vibrational communication. *BioScience* 55: 323–334.
- Collin, M.A., E. Camama, B.O. Swanson, J.S. Edgerly, and C.Y. Hayashi. 2009. Comparison of embiopteran silks reveals tensile and structural similarities across taxa. *Biomacromolecules* 10: 2268–2274.
- Davis, C. 1936. Studies of Australian Embioptera. Part II: Further notes on systematics. *Proceedings of the Linnean Society of New South Wales* 61: 254–258.
- Dubitzky, A., and R.R. Melzer. 1999. Untersuchung des Spinnvorgangs bei *Haploembia solieri* (Rambur) im REM (Insecta, Embioptera, Oligotomidae). *Nachrichtenblatt der Bayerischen Entomologen* 48: 97–103.
- Eberhard, M.J.B., et al. 2010. Structure and sensory physiology of the leg scolopidial organs in Mantophasmatodea and their role in vibrational communication. *Arthropod Structure and Development* 39: 230–241.
- Edgerly, J.S. 1987. Colony composition and some costs and benefits of facultatively communal behavior in a Trinidad wespinner, *Clothoda urichi* (Embiidina, Clothodidae). *Annals of the Entomological Society of America* 80: 29–34.
- Edgerly, J.S., J.A. Davilla, and N. Schoenfeld. 2002. Silk spinning behavior and domicile construction in webspinners. *Journal of Insect Behavior* 15: 219–242.
- Edgerly, J.S., S. Busse, and T. Hornschemeyer. 2012. Spinning behaviour and morphology of the spinning glands in male and female *Aposthonia ceylonica* (Enderlein, 1912) (Embioptera: Oligotomidae). *Zoologischer Anzeiger* 251: 297–306.
- Field, L.H., and T. Matheson. 1998. Chordotonal organs of insects. *Advances in Insect Physiology* 27: 1–228.
- Goloboff, P. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15: 415–428.
- Goloboff, P., and S. Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* 32: 221–238.

- Goloboff, P., J.S. Farris, and K. Nixon. 2003. TNT: Tree Analysis using New Technology. Program and documentation available from the authors (www.lillo.org.ar/phylogeny/tnt).
- Goloboff, P., J.S. Farris, and K. Nixon. 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.
- Grassi, B., and A. Sandias. 1894. Costituzione e sviluppo della società dei Termitidi. Osservazione sui loro costumi con un'appendice sui protozoi parassiti dei Termitidi e sulla famiglia delle Embidine. Appendice II. Contribuzione allo studio delle Embidine. Memoria 1. Atti della Accademia Gioenia di Scienze Naturali in Catania 7: 1–76.
- Greenfield, M.D. 2016. Evolution of acoustic communication in insects. In G.S. Pollack, A.C. Mason, A.N. Popper, and R.R. Fay (editors), *Insect hearing*: 17–47. New York: Springer International Publishing.
- Hoy, R.R., and D. Robert. 1996. Tympanal hearing in insects. *Annual Review of Entomology* 41: 433–450.
- Keil, T.A. 1997. Functional morphology of insect mechanoreceptors. *Microscopy Research and Technique* 39: 506–531.
- Kershaw, J.C. 1914. Development of an Embiid. *Journal of the Royal Microscopical Society* 1914: 24–27.
- McMillan, D., K. Hohn, and J.S. Edgerly. 2016. Choreography of silk spinning by webspinners (Insecta: Embioptera) reflects lifestyle and hints at phylogeny *Biological Journal of the Linnean Society* 118: 430–442.
- Melander, A.L. 1902. Two new Embiidae. *Biological Bulletin of the Marine Biology Laboratory* 3: 16–26.
- Miller, K.B., C. Hayashi, M.F. Whiting, G.J. Svenson, and J.S. Edgerly. 2012. The phylogeny and classification of Embioptera (Insecta). *Systematic Entomology* 37: 550–570.
- Mills, H.B. 1932. The life history and thoracic development of *Oligotoma texana* (Mel.) (Embiidina). *Annals of the Entomological Society of America* 25: 648–652.
- Mukerji, S. 1927. On the morphology and bionomics of *Embia major* sp. n., with special reference to its spinning organs. *Records of the Indian Museum* 29: 253–282.
- Nagashima, T., N. Niwa, S. Okajima, and T. Nonaka. 1991. Ultrastructure of silk gland of webspinner, *Oligotoma japonica* (Insecta, Embioptera). *Cytologia* 56: 679–685.
- Okada, S., et al. 2008. An Australian webspinner species makes the finest known insect silk fibers. *International Journal of Biological Macromolecules* 43: 271–275.
- Proaño, C.B., S. Cruz, D.M. McMillan, and J.S. Edgerly. 2012. Exploration of substrate vibrations as communication signals in a webspinner from Ecuador (Embioptera: Clothodidae). *Neotropical Entomology* 41: 196–203.
- Rimsky-Korsakow, M. 1905. Beitrag zur Kenntnis der Embiiden. *Zoologischer Anzeiger* 29: 433–442.
- Rimsky-Korsakow, M. 1910. Über das Spinnen der Embiiden. *Zoologischer Anzeiger* 36: 153–156.
- Rimsky-Korsakow, M. 1914. Über den Bau und die Entwicklung des Spinnapparates bei Embien. *Zeitschrift für Wissenschaftliche Zoologie* 108: 499–519.
- Ross, E.S. 1943. Métodos de recolección, crianza y estudio de los Embiópteros (Ins. Embioptera). *Revista de Entomologia (Rio de Janeiro)* 14: 441–446.
- Ross, E.S. 2000. Origin, relationships and integumental anatomy of the insect order Embiidina. *Embia*, part 1. *Occasional Papers of the California Academy of Sciences* 149: 1–53.
- Silvestri, F. 1912. Embiidae from Java and Krakatau. *Tijdschrift voor Entomologie* 55: 333–335.
- Silvestri, F. 1921. Description of a new species of Embiidae from southern Nigeria. *Transactions of the Entomological Society of London* 1921: 449–452.
- Slifer, E.H., and S.S. Sekshon. 1978. Sense organs on the antennal flagellum of two species of Embioptera. *Journal of Morphology* 139: 211–226.

- Strauß, J., and R. Lakes-Harlan. 2014. Evolutionary and phylogenetic origins of tympanal hearing organs in insects. In B. Hedwig (editor), *Insect hearing and acoustic communication*: 5–26. Berlin: Springer-Verlag.
- Stumpner, A., and D. Von Helversen. 2001. Evolution and function of auditory systems in insects. *Naturwissenschaften* 88: 159–170.
- Szumik, C. 1994. *Oligembia vetusta*, a new fossil Teratembiid (Embioptera) from Dominican amber. *Journal of the New York Entomological Society* 102: 7–73.
- Szumik, C. 1996. The higher classification of the order Embioptera: a cladistic analysis. *Cladistics* 12: 41–64.
- Szumik, C. 1997. Two new neotropical genera of Embiidae (Embioptera, Insecta). *Journal of the New York Entomological Society* 105: 140–153.
- Szumik, C. 2004. Phylogenetic systematics of ArchembIIDae (Embiidina, Insecta). *Systematic Entomology* 29: 215–237.
- Szumik, C., J.S. Edgerly, and C. Hayashi. 2008. Phylogeny of Embiopterans (Insecta). *Cladistics* 24: 993–1005.
- Szumik, C., R. Gandolfo, and V. Pereyra. 2017. *Gibocercus* Szumik and *Biguembia* Szumik (Embioptera, ArchembIIDae): new species and the potentiality of female traits. *Zootaxa* 4317: 338–354.
- Yack, J.E. 2004. The structure and function of auditory chordotonal organs in insects. *Microscopy Research and Technique* 63: 315–337.

APPENDIX 1

LIST OF THE CHARACTERS ADDED TO THE DATA MATRIX OF SZUMIK ET AL., 2008

Data matrix available online (<http://www.lillo.org.ar/phylogeny/published/>).

- 186 Forefemur tympanal organ: **0**, semicircular with well-defined edges; **1**, a curved, slender band, elongated along the femoral axis; **2**, a tiny triangle.
- 187 Middle femur tympanal organ: **0**, semicircular but with the basal end diffuse and expanded; **1**, a long band extended almost to the basal half of the femur; **2**, a tiny triangle.
- 188 Hind femur tympanal organ: **0**, semicircular; **1**, small, disklike depressed area; **2**, absent.
- 189 Silk ejectors Type II: **0**, absent; **1**, present.
- 190 Length of ejectors Type I: **0**, short ejectors (less than 80 µm); **1**, long ejectors (more than 100 µm).
- 191 Jagged setae, hind tarsus basal segment: **0**, absent; **1**, present.
- 192 Slender hook setae, hind tarsus basal segment: **0**, present; **1**, absent.
- 193 Sculptured bladder: **0**, present; **1**, absent.
- 194 Mechanoreceptor on medial bladder: **0**, present; **1**, absent.
- 195 Mechanoreceptor on apical bladder: **0**, present; **1**, absent.
- 196 Microtrichia medial bladder: **0**, present; **1**, absent.
- 197 Microtrichia apical bladder basal segment: **0**, present; **1**, absent.
- 198 Microtrichia apical bladder apical segment: **0**, present, uniformly distributed **1**, present, on antero-lateral face; **2**, absent.

All issues of *Novitates* and *Bulletin* are available on the web (<http://digitallibrary.amnh.org/dspace>). Order printed copies on the web from:

<http://shop.amnh.org/a701/shop-by-category/books/scientific-publications.html>

or via standard mail from:

American Museum of Natural History—Scientific Publications
Central Park West at 79th Street
New York, NY 10024

Ⓢ This paper meets the requirements of ANSI/NISO Z39.48-1992 (permanence of paper).