Comparative Morphology and Functional Significance of Setae Called Papillae on the Pedipalps of Male Camel Spiders (Arachnida: Solifugae)

PAULA E. CUSHING,[1,2] PATRICK CASTO,[1] ELIZABETH D. KNOWLTON,[3] SUZANNE ROYER,[4] DAMIEN LAUDIER,[5] DOUGLAS D. GAFFIN,[6] LORENZO PRENDINI,[7] AND JACK O. BROOKHART[1]

Ann. Entomol. Soc. Am. 107(2): 510–520 (2014); DOI: http://dx.doi.org/10.1603/AN13140

ABSTRACT Some male camel spiders (Arachnida: Solifugae) in the families Eremobatidae, Karschiidae, and Solpugidae have clusters of specialized conical or acuminate setae called papillae, on the ventral surface of the metatarsus of the pedipalps. We compared the overall structure of the papillae found on representatives of the three families using scanning electron microscopy (SEM). We examined the ultrastructure of these setae using transmission electron microscopy (TEM). We also used extracellular electrophysiological recording techniques to examine the electrical properties of these sensory structures and test the hypotheses that they function as mechanoreceptors, olfactory receptors, and chemoreceptors. We found similarities in the structure of papillae among genera within a family or distinct family-level differences in structure. Thus, the papillae are phylogenetically informative; similar within family but differing between families. TEM results demonstrated the cuticular wall of a papilla is divided into three sublayers: endo-, meso-, and exocuticle. Mechanoreceptive dendrites are evident at the base of the setal shaft. Other dendrites innervate the shaft of the papilla and penetrate through the cuticular layers near the setal apex. Two SEM images show what appear to be pores on the branches of the papillae, and we found what appears to be a pore tubule extending from the distal portion of the dendrites through the exocuticular layer. Electrophysiological data support the hypothesis that the papillae function as mechanoreceptors and provide no support for chemosensory, thermoregulatory, or hygroreceptive functions. Our data suggest that the papillae function as mechanoreceptors and may also function as chemoreceptors.

KEY WORDS solfugid, sensory seta, mechanoreceptor, chemoreceptor, electrophysiology

Camel spiders, arachnids in the order Solifugae, are important arthropod predators found in xeric and semidesert habitats worldwide except Australia. Nearly 1,100 species of Solifugae have thus far been described from the 12 families currently recognized (Harvey 2003). Much remains to be discovered about their behavior, morphology, physiology, and most aspects of their natural history. They are an extraordinarily difficult group of arachnids to study, as they are hard to find and collect, nearly impossible to keep alive in the lab for any significant length of time, and very difficult to raise from hatch through maturity (Punzo 1998a; F. Punzo, unpublished data). Only one author has successfully reared one species of solifuge, *Eremobates marathoni* Muma (Eremobatidae), through all developmental stages, and even in this study, carried out under optimal laboratory conditions, only 3% of the postembryos (24 out of 807) survived to adulthood (Punzo 1998b).

Solifuges are pugnacious predators, attacking almost any arthropod that crosses their paths (including each other; Punzo 1998a). Even the early phase of courtship and copulation in solifuges appears to have elements of aggressive interactions, with both sexes assuming agonistic postures and females often cannibalizing males either before or right after copulation (Punzo 1998b). The male initiates copulation by attacking and grasping the female with his chelicerae and pedipalps (the mating sequence is nicely described by Punzo 1998b). Copulatory behavior has been described for only a few species of Galeodidae, Solpugidae, and Eremobatidae (Heymons 1902; Junqua 1962, 1966; Muma 1966; Wharton 1987; Punzo 1997, 1998b). The chelicerae are used for insemination; the male places the spermatophore into the female's gonopore with his chelicerae. Male chelicerae of all families have evolved unique structures, called

[1] Department of Zoology, Denver Museum of Nature & Science, 2001 Colorado Blvd., Denver, CO 80205.
[2] Corresponding author, e-mail: paula.cushing@dmns.org.
[3] School of Biological Sciences, University of Nebraska, Lincoln, NE 68588.
[4] Department of Anatomy and Zoology, Colorado State University, Campus Delivery 1617, Fort Collins, CO 80523.
[5] Laudier Histology, New York, NY 10025.
[6] Department of Zoology, University of Oklahoma, Norman, OK 73019.
[7] Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th St., New York, NY 10024.

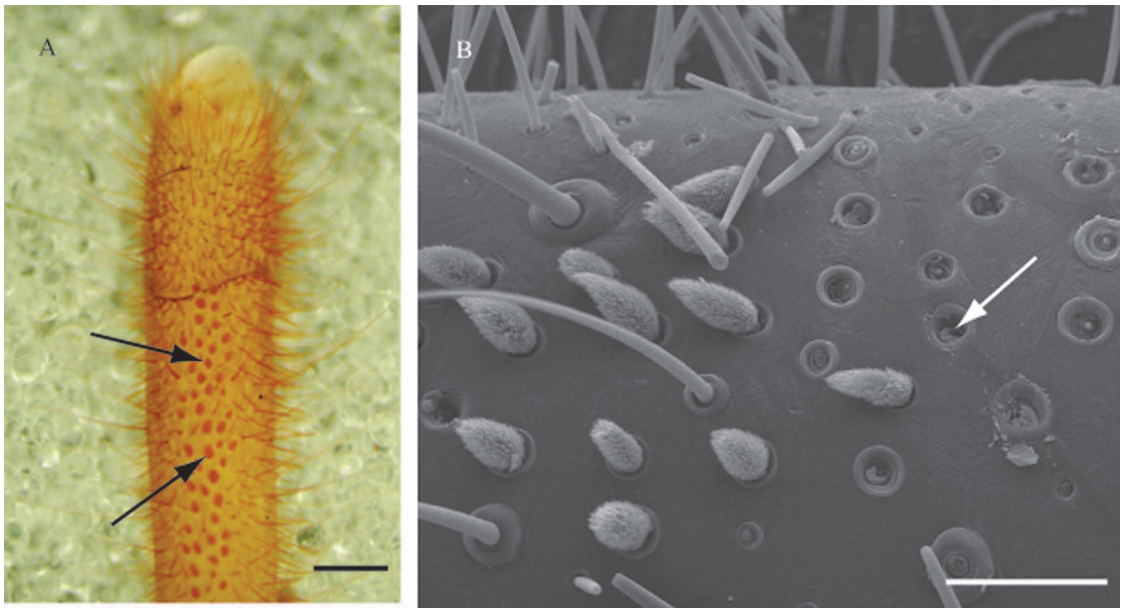

Fig. 1. Palpal papillae of *E. pallipes.* (A) Ventral view of field of papillae on male tarsal and metatarsal segments under light microscopy. Arrows point to papillae. Scale bar = 0.5 mm. (B) SEM of field of papillae on *E. pallipes* pedipalp. Scale bar = 25 μm. Arrow points to socket from which papilla emerges. (Online figure in color.)

flagella, that are presumably involved in this copulatory function; however, the exact functional nature of these cheliceral modifications is, as are so many aspects of solifuge morphology, unknown.

In all families, the pedipalps are also involved in, at least, the courtship phase, and the male either strokes the female with the pedipalps or maintains contact with the female's body using his pedipalps (Amitai et al. 1962, Junqua 1966, Cloudsley-Thompson 1967, Wharton 1987, Peretti and Willemart 2007, Hrušková-Martišova et al. 2010, J. R., unpublished data). Thus, the pedipalps may be used to grasp, calm, or appease the female or may function to pick up chemical or other sensory cues from the female. Only recently have researchers initiated investigations of the sensory structures found on solifuge pedipalps to try and determine how these appendages may be involved in hunting, courtship, intra- and intersexual communication. Bauchhenss (1983) examined the morphology and ultrastructure of sensilla ampullacea on the pedipalps. These surface structures (pores with dendrites at the base) are thought to be involved in olfaction as well as thermoreception, hygroreception, or both. Cushing et al. (2005) and Klann et al. (2008) studied the structure of the suctorial organs at the distal tips of the pedipalps. These eversible organs are used for prey capture and can be used to climb up smooth surfaces (Cushing et al. 2005, Willemart et al. 2011). Cushing and Casto (2012) carried out a preliminary scanning electron microscopic (SEM) study of the setae on the pedipalps of one member of each of the 12 families of Solifugae, demonstrating that the pedipalps are covered in various sensory setae, many with apical pores that most likely have some sort of chemosensory function.

In the Old World families Karschiidae and Solpugidae and the New World family Eremobatidae, specialized setae called papillae are found on the ventral to mesoventral surface of the metatarsus of the male pedipalp (Kraepelin 1899; Roewer 1934; Muma 1951, 1970, 1989; El Hennawy 1990). In the Karschiidae, five species in the single genus *Karschia* Simon possess papillae. Some species within each of the 17 genera of Solpugidae have papillae. Some species within each of the following eremobatid genera have papillae (Roewer 1934, Muma 1951): *Chanbria* Muma, *Eremobates* Kraepelin, *Eremochelis* Roewer, and *Hemerotrecha* Banks.

Papillae are typically found in clusters on the palps of males (Fig. 1A). Under light microscopy, these structures appear to be conical to accuminate setae (Kraepelin 1899; Roewer 1934; Muma 1951, 1970, 1989; El Hennawy 1990), and under SEM the complex branching pattern of these setae is revealed (Fig. 1B). Occasionally, females have a few scattered papillae, but seldom in the density found on male palps (Fichter 1940, Brookhart and Muma 1981, Brookhart and Cushing 2004). Although the papillae have been used in solifuge classification (Roewer 1934; Muma 1951, 1970, 1989; Brookhart and Cushing 2002), the functional significance of papillae is unknown. Because they are found primarily on the pedipalps of males, we hypothesized that these structures serve as mechanoreceptors, chemoreceptors, or both, during courtship or copulation. In this study, we used SEM to determine if the structure of papillae was phylogenetically informative at the family level, differing between families but consistent in structure within them. We used transmission electron microscopy (TEM) and electrophysiological recordings to determine the possible

function of these setae and to test the hypotheses that papillae are mechanoreceptors, chemoreceptors, or both, used to detect chemical, vibrational, or other signals.

Materials and Methods

Scanning Electron Microscopy. Solifuges in the family Eremobatidae used in the study were captured in ethylene glycol pitfall traps and preserved in 75% ethanol. Karschiidae and Solpugidae species used were either collected in pitfall traps and placed in 70% ethanol or live trapped and placed directly in alcohol. Eremobatidae specimens are housed in the arachnology collection at the Denver Museum of Nature & Science (DMNS). Full collection data for the DMNS specimens can be found at http://symbiota1.acis.ufl.edu/scan/portal/ by searching the DMNS collection under the ZA# listed throughout Methods. The Karschiidae and Solpugidae specimens are housed at the American Museum of Natural History. We were only able to obtain a single karschiid for study, as they are rare in collections. The following male pedipalps were dissected from specimens and used for SEM: one male Karschia mastigofera Birula (Karshiidae); Solpugidae: *Solpugyla darlingi* (Pocock), *Solpugista bicolor* (Lawrence), *Metasolpuga picta* (Kraepelin), *Solpuguna cervina* (Purcell), *Zeria persephone* Simon; Eremobatidae: *Eremobates pallipes* (Say), *Chanbria rectus* Muma, *Eremochelis insignitus* Roewer, and *Hemerotrecha sevilleta* Brookhart and Cushing. The pedipalps were sonicated in 70% ethanol, air dried, and mounted on stubs using conductive carbon paint. The samples were sputter coated with gold. SEM observations were performed using a microscope (FEI Quanta, FEI Company, Hillsboro, OR) operating at 30 kV. The papillae illustrated in Figs. 1B and 5B were taken with a field emission gun microscope (FEI Quanta 450, FEI Company).

Histology/TEM. We used two male *Eremobates corpink* Brookhart and Cushing and one male *E. pallipes* (Eremobatidae) for TEM (voucher specimen numbers DMNS ZA.28542, ZA.28560, and ZA.29126). Both pedipalps were cut off the live specimens with microscissors at the metatarsus-tibial joint. The animal was then immediately placed in absolute EtOH, and the terminal parts of the pedipalps, 3.5 mm in length, were placed in a fixative of 2.5% glutaraldehyde–2% formaldehyde solution in 0.1 M sodium cacodylate buffer. Further processing was done while the pedipalps were submerged in the fixative. The basal quarter of the metatarsus was cut to expose the tissues just before the proximal edge of the field of papillae. The tarsus was also cut away from the metatarsus to expose the tissue near the distal end of the field of papillae.

One of the pedipalps was designated for longitudinal sections and the other for cross sections. For longitudinal sections, the length of a metatarsus was cut down to 2.5 mm, the length of the field of papillae. To ensure the best penetration of the fixatives, a longitudinal incision was made through the cuticle along the metatarsus on the side of the pedipalp opposite the papillae (the dorsolateral surface of the metatarsus). For cross sections, a metatarsus was cut in half through the middle of the field of papillae. The two halves were just over 1 mm each in length. The pedipalps were left in the fixative for 24–36 h at 4°C, and then placed in 0.1 M cacodylate buffer until secondary fixation took place.

Secondary fixation ensued with 1% OsO_4 in a 0.1 M cacodylate buffered solution (Foelix and Chu-Wang 1973, Ribi 1976, Talarico et al. 2006). The pedipalps were placed in the secondary fixative and lightly agitated for 2 h. The pedipalps were then rinsed with de-ionized water three times for 10–15 min on an agitator.

We performed an en bloc stain with a saturated aqueous uranyl acetate solution (Stempak and Ward 1964). The pedipalps were submerged in 1 ml of the solution, and agitated in darkness for 8–12 h. The pedipalps were rinsed again three times with deionized water for 10–15 min each rinse. Our specimens were slowly dehydrated with increasing concentrations of ethanol, 50, 70, 80, 90%, and twice with absolute ethanol. The pedipalps were then placed in two changes of propylene oxide before embedding (Foelix and Axtell 1972, Foelix and Chu-Wang 1973).

We used PELCO Eponate 12 as our epoxy resin to embed the pedipalps. We began with ≈1:3 ratio of Eponate 12 to propylene oxide and gradually increased the concentration of Eponate 12 over a period of 3 d. The pedipalps were then changed into 100% Eponate 12 and allowed to penetrate for 0.5–2 h. They were individually placed in separate blocks and allowed to polymerize overnight in an oven at 65°C.

We used a Reichert-Jung Ultracut E ultramicrotome and a diamond knife with a deionized water bath to cut our sections. Semithin sections of 1–4 μm thickness were cut from the blocks to view under the light microscope. Several semithin sections were cut and placed on a glass slide with a tiny platinum loop and stained with 0.1% toluidine blue in a 1% sodium borate solution. The slides were heated to dry the stain and then viewed with a compound microscope to evaluate what tissues we were cutting through. Once we began cutting through a papilla, we stopped cutting semithin sections and adjusted the ultramicrotome to cut ultrathin sections, measured thickness between 60–90 nm. Several ultrathin sections fit on a formvar-covered copper grid. A secondary stain was applied to these sections to increase contrast of the images. For 10 min, the grids were stained with an alcoholic uranyl acetate: 5% uranyl acetate in a solution of 50% methanol and 35% ethanol by volume. The grids were rinsed with 70% ethanol, then with de-ionized water, dried, and subsequently stained for 5 min with Reynold's lead citrate.

Our specimens were viewed with a JEOL JEM-2000EX II TEM set at 100.0 kV accelerating voltage and in high vacuum (1.2×10^{-6} torr). Micrographs were taken on photo film, and negatives were scanned into digital images.

Electrophysiology. The subject of the electrophysiological studies was one adult male *Eremobates do-*

colora Brookhart and Muma (voucher specimen # DMNS ZA.19987) with well developed papillae on the ventral surface of the pedipalps. We chilled the animal at −5°C for 2 min to slow its movements. Using double-sided adhesive tape and modeling clay, we affixed the animal, ventral side upwards, on a microscope slide. We positioned and affixed the right pedipalp to position the papillae field upward. We then inserted an indifferent electrode (silver wire) into the second coxa of a left leg, ensuring wire-to-hemolymph contact. To extracellularly record neural activity, we inserted an electrolytically sharpened tungsten electrode with a ≈1.0-μm-diameter tip (sharpened in 1 mol./liter $NaNO_2$) into the cuticular base of a single papilla. For one male, the papillae were difficult to pierce and electrodes needed to be more blunt (≈7 μm) for successful insertion. Various chemicals and distilled water were introduced to papillae (one male, n = 12 papillae) to test whether the papilla is a chemoreceptor or, in the case of water, a hygroreceptor. The following diluted chemicals were used in the test: 1 M cineole, 1 M and 0.1 M citric acid. The following undiluted (pure form) chemicals were used: methanol, ethanol, hexanol, limonene, 1-hexanol, hexanal, 4-heptanone, octane, hexane, heptane, butyric acid, and hexanoic acid. The chemicals used as possible odorants or tastants are common constituents of a variety of complex chemical stimuli present in the natural world. It has been shown that arthropod chemoreceptors are reactive to individual constituents of complex chemicals (Selzer 1981).

Chemical sensitivity was carried out in one of two ways. We used either chemically filled pipettes (glass capillary tubes pulled to a 10-μm tip opening with a Sutter Micropipette puller; pull parameters: heat = 370°C, pull = 20, velocity = 20, and time = 80 s), which were attached to a micromanipulator and brought near to, or into contact with, papillae (n = 9) or we used Pasteur pipettes (also attached to a micromanipulator). Viz. the latter method, we specifically moved the Pasteur pipette tip to within 1 cm of the recorded papilla (n = 3); before introduction, we saturated a small piece (≈2 cm^2) of Kimwipe tissue with the desired chemical and placed the tissue in the bore of the Pasteur pipette. We attached a 5 cm length of polyethylene tubing to the large end of the Pasteur pipette to which we inserted the tip of a 1,000-μl mechanical pipette. The mechanical pipette was used to deliver a consistent air stream across the saturated tissue, out the tip of the Pasteur pipette, and across the recorded preparation. To avoid cross-contamination, we used a different Pasteur pipette and polyethylene tubing connector for each chemical. The chemicals used in the tests were among those that elicit responses in scorpion peg sensilla; structures on the pectines of scorpions demonstrated to be involved in chemoreception (Gaffin and Brownell 1997a). For introducing a mechanical stimulus, we used a micromanipulator to push papillae with empty, nonchemically filled glass capillary tubes. To introduce a temperature stimulus, we manually brought a heated metal probe to individual papillae. Electrophysiology methods, including data analysis, follow Gaffin and Brownell (1997a,b) and Gaffin and Walvoord (2004).

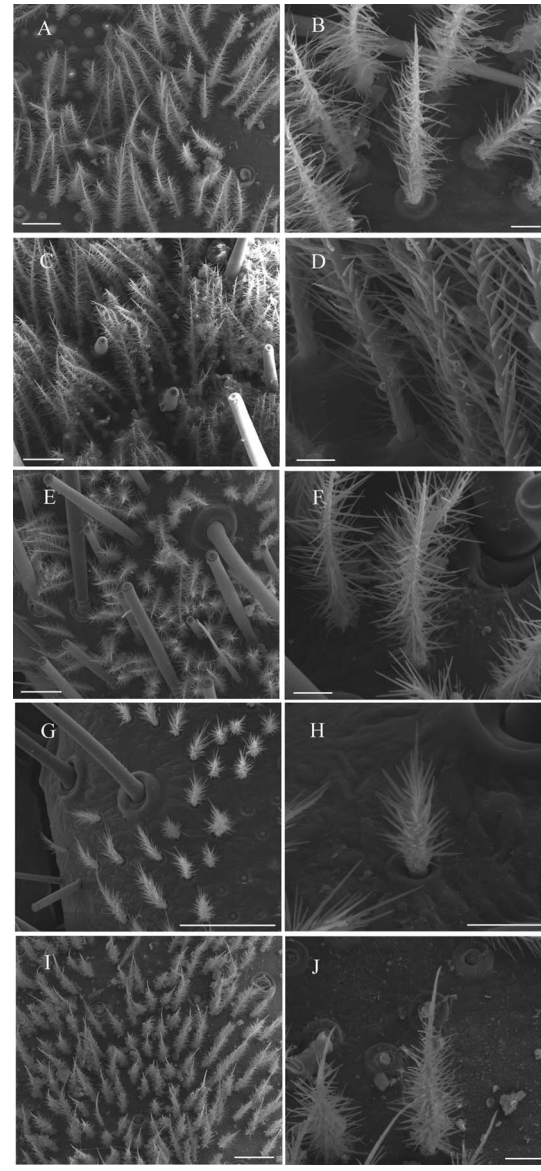

Fig. 2. Palpal papillae of Solpugidae Leach. Scale bars of lower magnification images = 100 μm; scale bars of higher magnification images = 20 μm. (A, B) *S. darlingi.* (C, D) *So. bicolor.* (E, F) *M. picta.* (G, H) *Sl. cervina.* (I, J) *Z. persephone.*

Results

Scanning Electron Microscopy. The papillae of Solpugidae, Eremobatidae, and Karschiidae all emerge from a socket (Figs. 1–4). Such socketed shafts are characteristic of setae functioning as mechanoreceptors and contact chemoreceptors (Felgenhauer 1999). In two instances, we saw what appear to be pores on individual branches of papillae (Fig. 5A–B). The single

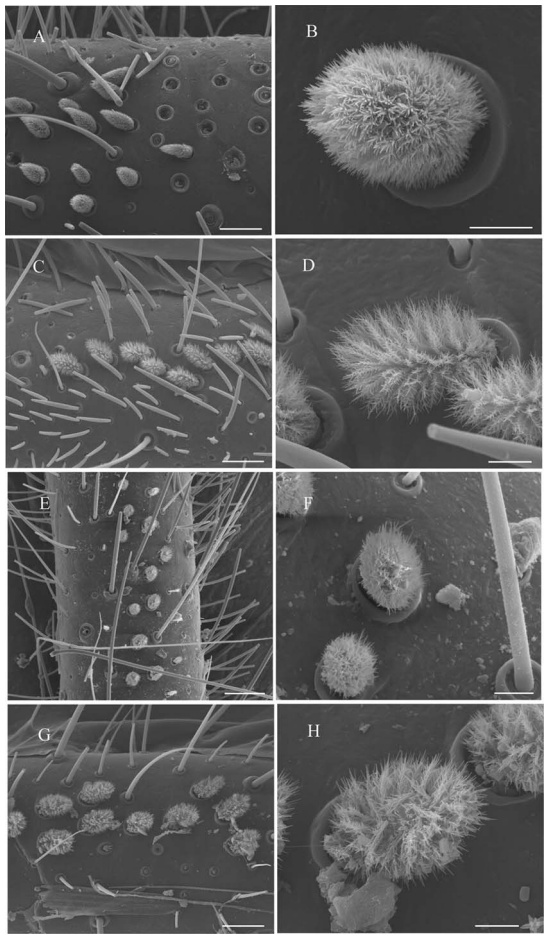

Fig. 3. Palpal papillae of Eremobatidae Kraepelin, 1899. Scale bars of lower magnification images = 100 μm; scale bars of higher magnification images = 20 μm. (A, B) *E. pallipes.* (C, D) *C. rectus.* (E, F) *Er. insignitus.* (G, H) *H. sevilleta.*

pore visible on a branch of the *Hemerotrecha cornuta* (Eremobatidae) papilla is ≈0.25 μm in diameter (Fig. 5A). The pores visible on a branch of the *E. pallipes* (Eremobatidae) papilla are ≈0.08 μm in diameter (Fig. 5B).

The general structure of papillae varies among the three families (Figs. 2–4) but is consistent within the Solpugidae and the Eremobatidae (Figs. 2 and 3). The papillae of all genera of Solpugidae examined comprise a single central trunk with pointed spiculate branches extending from the trunk (Fig. 2A–J). The branches are sparse such that the trunk is clearly evident (Fig. 2B, D, F, H, and J). All the genera of Eremobatidae examined exhibit conical papillae with extremely dense frond-like branches radiating from a central trunk (Figs. 1B and 3A–H). *K. mastigofera* exhibits papillae with branches radiating from the base of a central trunk. The apical tip of the trunk is elongated, extending above the branches and ending in a curled tip (Fig. 4B). Individual branches possess frond-like projections similar to the branches of eremobatid papillae but unlike the spiculate branches of solpugid papillae.

Histology/TEM. The shaft of the papilla is connected to the surrounding cuticle of the pedipalp by an articulating membrane at the base of the socket (am in Fig. 6A and C). Papillae have dark staining endocuticular and exocuticular layers with a lighter stained mesocuticular layer between (layers ec, mc, and ex in Fig. 6A). Seven mechanoreceptor dendritic terminals are visible at the base of the setal shaft in the articulating membrane; four terminals are surrounded by a dendritic sheath located proximal to the articulating membrane and three are surrounded by a separate dendritic sheath and located proximal to the palpal cuticle (Fig. 6C and inset; am, articulating membrane; md, mechanoreceptors; ds, dendritic sheath). The tubular bodies of the four mechanoreceptive dendritic terminals proximal to the articulating membrane are dark granulate bundles of microtubules in the cross section of Fig. 6C, inset (tb). Multiple dendrites (at least six) innervate the shaft of the papilla and go through the endo- and mesocuticular layers (Fig. 6A–D, labeled "d"). We saw evidence of the proximal end of a pore tubule in the exocuticle near the apex of the papilla (labeled "pt" in Fig. 6B). The overall structure of the papilla is presented in Fig. 7 showing the mechanoreceptors ending in the articulating membrane at the base of the socketed shaft, the dendrites extending through the shaft, and the incipient pore

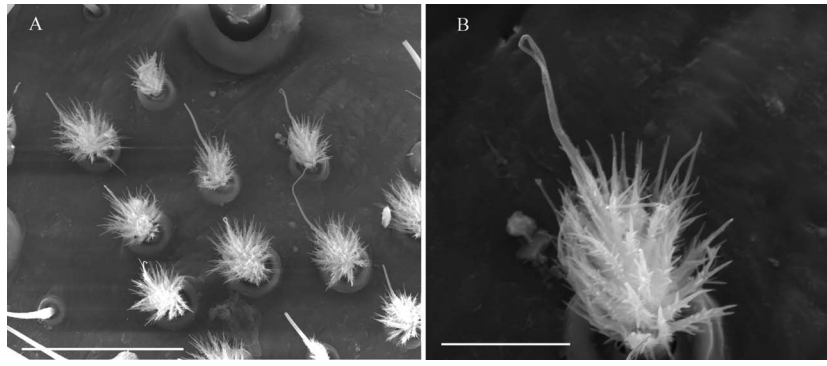

Fig. 4. Palpal papillae of *K. mastigofera.* (A) Field of papillae. Scale bar = 100 μm. (B) Single papilla. Scale bar = 20 μm.

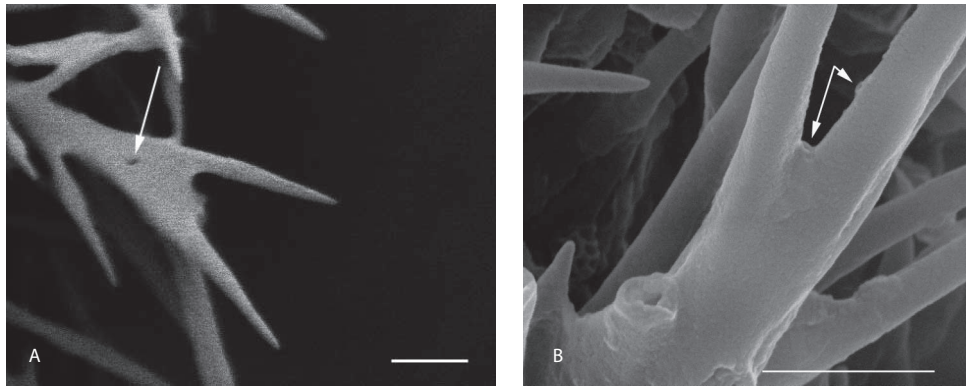

Fig. 5. Evidence of pores near the tips of papilla branches (Eremobatidae). (A) Single frond of *H. cornuta* papilla. Arrow points to pore. Scale bar = 2 μm. (B) Frond of *E. pallipes* papilla. Arrows point to pores. Scale bar = 1 μm.

tubule extending through the three cuticular layers of the papilla.

Electrophysiology. We tested for papillar sensitivity to possible odorants or tastants. Water was used to test for hygroreception, and we used a heated probe to test for thermoreception. Responses were not detected when we introduced volatile chemicals (see Electrophysiology Methods), nor were they detected when we directly contacted papillae with droplets of ethanol, hexanol, limonene, water, or citric acid. A heated metal probe brought to within microns of a papilla elicited no response. In addition, no responses were detected when we brought a pipette containing a small piece of water-drenched tissue near individual papillae.

However, our electrophysiological recordings did detect background neural activity, referred to as A activity (Fig. 8) believed to originate from motor neurons governing the eversion of the pedipalp's suctorial organ (Cushing et al. 2005, Klann et al. 2008). The animal was observed to evert the suctorial organ in correspondence with an intense electrical signal at the end of each repetitive bout of A activity. Eversion is controlled by increasing hemolymph into the pedipalp, and inversion is controlled by muscular contraction (Cushing et al. 2005, Klann et al. 2008). When we severed the pedipalp from the body in vivo, activity A disappeared, as did the reflex.

To test whether the papillae contain mechanoreceptive elements, we pushed an individual papilla with a hollow empty glass capillary tube. Mechanostimulation resulted in the firing of one spike type M (Fig. 8A–C). Furthermore, A activity did not change during mechanical stimulation, but when we later abolished A activity on a different animal by severing the pedipalp from the body (as noted in the previous paragraph), we could still elicit papillar mechanoresponses.

Discussion

The general structure of palpal papillae is similar among genera within the family Eremobatidae and within the Solpugidae, differing considerably between the two families and between these families and the Karschiidae. Thus, the structure of papillae is phylogenetically informative at the family level. Given the consistent structure of papillae among genera within the Solpugidae and within the Eremobatidae, we predict that the papillae of other species of the single genus *Karschia* (Karschiidae) (the only genus in this family to present papillae) will show structures similar to those illustrated in Fig. 4A–B for *K. mastigofera*, although other species in *Karschia* should be examined to validate this. *Karschia* is rare in collections so specimens of other species were not available for destructive sampling.

Arthropod setal mechanoreceptors reside in a socket to which the seta is attached by an articulating membrane. The mechanoreceptive dendrites are surrounded by a dendritic sheath that is attached to the inner wall of the socket, and the dendrites terminate at the proximal base of the setal shaft (Foelix 1970, Foelix and Chu-Wang 1973, McIver 1975, Coons and Alberti 1999). In arachnid (and other arthropod) mechanoreceptors, the terminal ends of multiple dendrites are stabilized by a cuticular sheath, over the dendritic sheath, to the center or to one side of the base of the socket, and this sheath is often connected for some or most of its length to the cuticle of the setal shaft (Slifer 1961, 1968, 1970; Adams et al. 1965; Foelix 1970; Chu-Wang and Axtell 1973; Foelix and Chu-Wang 1973). Arthropod mechanoreceptors are also characterized by the presence of tubular bodies at the terminal segment of the mechanoreceptor dendrite (Thurm 1964, Barth 1971, Foelix and Chu-Wang 1972, Gnatzy and Tautz 1980).

Arthropod contact chemoreceptors, in contrast, are characterized as having multiple dendrites that extend up into the shaft of the seta that terminate in either a single apical pore or multiple pores, and which often possess mechanoreceptive dendrites terminating at the base of the socket (McIver 1975, Haupt 1982, Foelix 1985, Coons and Alberti 1999, Farley 1999, Felgenhauer 1999). Arachnid olfactory receptors are either single-walled sensilla with plugged pores, double-

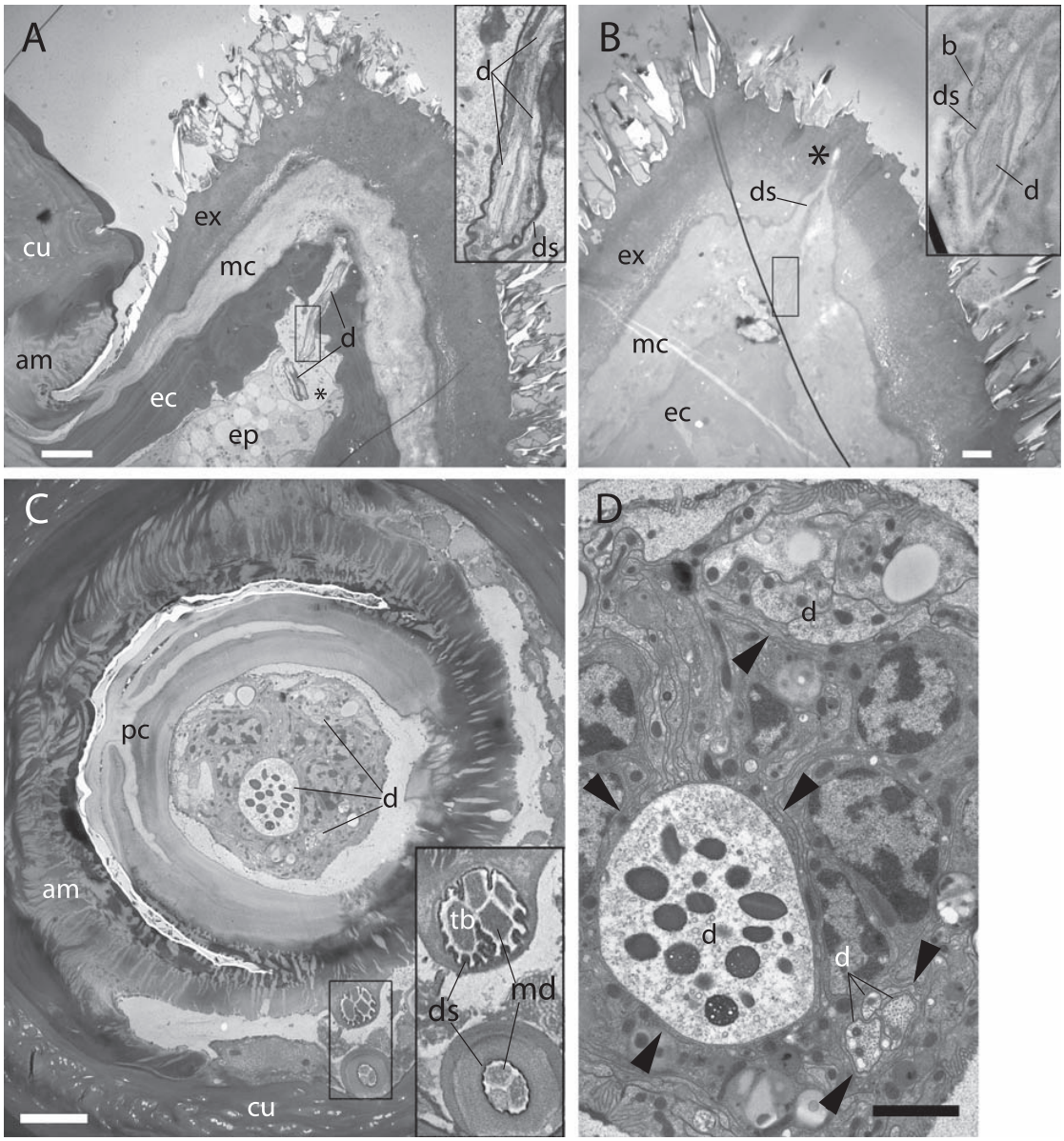

Fig. 6. TEM sections of papillae. (A) Longitudinal section through papilla of *E. corpink* showing dendrites (d) extending from the inner part of papilla through the endocuticle (ec). Magnified area shows the dendritic sheath (ds) and at least three dendrites identifiable by their longitudinal microtubule structure. (*) is the sheath cell that envelops the dendrite. am, articulating membrane; cu, pedipalp cuticle; ep, epithelial cells; ex, exocuticle; mc, mesocuticle. Scale bar = 5 μm. (B) Longitudinal section through *E. corpink* showing dendritic sheath (ds) and dendrite (d) extending through the mesocuticle (mc) and into the exocuticle (ex). *, denotes the proximal end of a pore tubule that potentially connects the dendrites to the environment near the apex of the papilla. b, cuticular boundary; ec, endocuticle. Scale bar = 2 μm. Black line extending diagonally through the micrograph is a fold in the section. (C) Transverse section through papilla of *E. pallipes* at the level of the socket showing mechanoreceptive dendrites (md) surrounded by their dendritic sheaths (ds) with tubular bodies evident (tb). In the middle of the papilla appear to be three clusters of dendrites (d). The largest of the dendrites appears to be the part of the dendrite proximal to the basal body, containing many mitochondria as electron dense bodies: am, articulating membrane; cu, pedipalp cuticle; pc, papilla cuticle. Scale bar = 5 μm. (D) Transverse section close-up of dendrites (d) shown in (C). At least two dendrites are together at the top of the image, the large dendrite proximal to the basal body below, and a cluster of three dendrites near the bottom. All arrows point to boundaries of enveloping cells around dendrites. Scale bar = 2 μm.

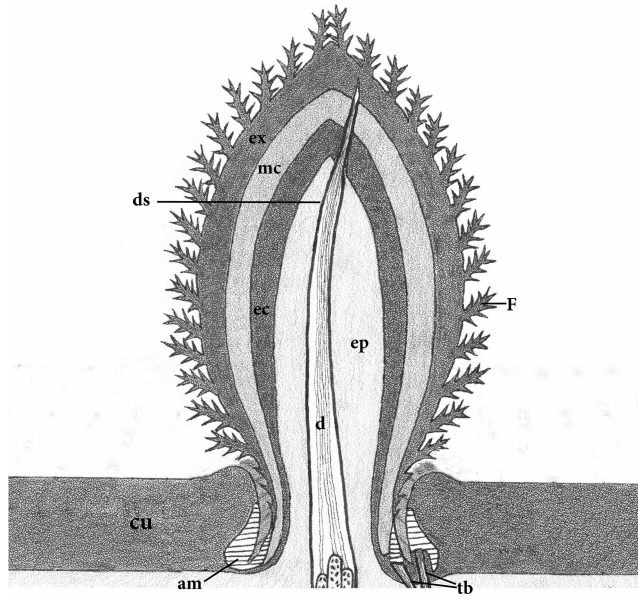

Fig. 7. Schematic diagram of an Eremobatidae papilla showing the tubular bodies (tb) of the mechanoreceptor dendrites ending in the articulating membrane (am) of the socket; other dendrites (d) extending through the epithelial (ep), endocuticular (ec), mesocuticular (mc), and exocuticular (ex) layers. The dendrites are enclosed by a dendritic sheath (ds). cu, cuticle of papilla; F, frond.

walled sensilla with spoke canals, or single-walled sensilla with pore openings (Tichy and Barth 1992, Hallberg and Hansson 1999).

The ultrastructure of papillae suggests that these structures function as mechanoreceptors and chemoreceptors. The papilla articulates within a socket to which the seta is attached by an articulating membrane. The papilla has distinct mechanoreceptor dendrites surrounded by dendritic sheaths; the mechanoreceptors terminate at the base of the setal shaft (Fig. 6C) and these seven mechanoreceptors have clearly defined tubular bodies characteristic of arthropod

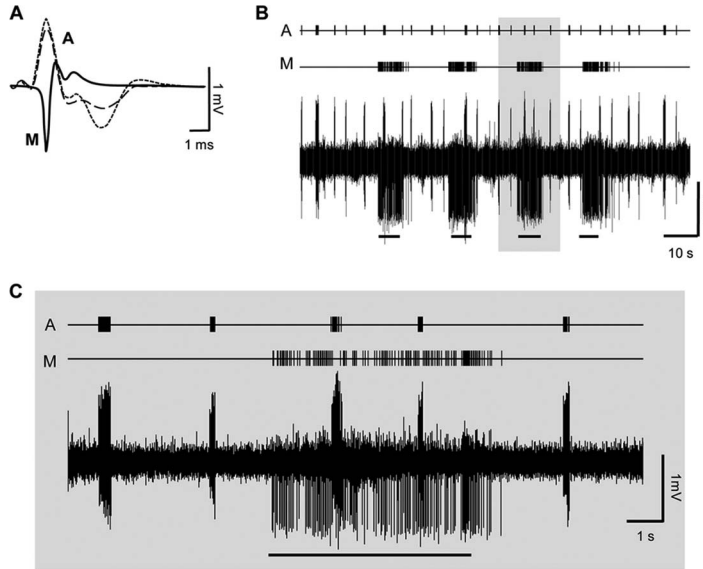

Fig. 8. (A–C) Mechanical deflection of papilla. (A) Averaged waveform of spike type M superimposed on the averaged waveforms of two classes of type A spikes from the recording shown in B. (B) Deflections of papilla elicit a barrage of type M spikes amid type A spikes. Bottom trace shows raw, composite record; firing times of type A spikes and type M spikes are segregated in the upper two traces. Lines below indicate the duration of the four mechanical deflections. (C) Expanded time view of third deflection.

mechanoreceptors (Fig. 6C, inset). Dendrites (at least six) are visible in cross section in the center of the setal shaft (Fig. 6C and D) and extend up into the shaft through the endo-, meso-, and exocuticular layers (Fig. 6A and B). An incipient pore tubule was visible in a single longitudinal section (Fig. 6B) and pores were seen using SEM on the frond-like branches of the papillae (Fig. 5A and B). The presence of such pores and pore tubules is characteristic of arthropod chemoreceptors (Foelix 1970, Slifer 1970, Foelix and Chu-Wang 1972, Harris and Mill 1973, Zacharuk 1980, Barth 2001, Talarico et al. 2006). Previous studies of insect and arachnid chemoreceptors have shown that these pores are often difficult to see owing to their small size (often in the range of 0.03–0.2 μm), even under SEM, or because they may be plugged with extruded sensillar fluid (Adams et al. 1965, Slifer 1970, Foelix and Axtell 1971, Zacharuk 1980, Foelix and Schabronath 1983, Akkerhuis et al. 1985, Tichy and Barth 1992, Guffey et al. 2000). This may explain why we did not see these pores in more papillae during the SEM survey. However, the pores we did see on the branches of papillae of *H. cornuta* and *E. pallipes* are within the correct size range for pores of arthropod chemoreceptors.

Our electrophysiological data support the hypothesis that papillae function as mechanoreceptors. Mechanostimulation of an individual papilla resulted in an unambiguous electrical signal (spike type M, Fig. 8). The papillae did not respond to any chemical stimuli nor did they respond to humidity or heat. Thus, the electrophysiological data provide no support for a chemoreceptor, hygroreceptor, or thermoreceptor function but do support the hypothesis that the papillae function as mechanoreceptors. The lack of any response to either a moistened tissue or heated probe argues against a hygroreceptive function for the papillae. This is because any change of temperature coincidentally and inversely changes humidity; it is often possible to stimulate hygroreceptors via changes in heat or cold (Tichy and Loftus 1990). The lack of a clear response to any of the chemical cues presented is not proof against a chemoreceptive function, as the suite of chemicals presented may not have been biologically meaningful for this particular sensory seta. At the time of the electrophysiological part of this study, no living female was available for testing; thus, we could not test the male response to chemical extracts from females of the species. Such extracts might elicit a positive response when presented to a live male.

When papillae are present, their number varies among conspecific males (Muma 1951, 1970, 1989; Brookhart and Muma 1981; Brookhart and Cushing 2004), which may be a function of development. Papillae are typically absent on conspecific females. Male wolf spiders have three times as many chemosensitive setae on their pedipalps as their female counterparts (Tietjen and Rovner 1980). In some wandering spiders, contact chemoreceptive setae on the male's appendages, particularly on his pedipalps, allow the male to follow pheromones the female deposits in her dragline silk (Tietjen 1977, Foelix 1985, Barth 2001). The TEM and electrophysiological data support the hypothesis that papillae function as mechanoreceptors. The TEM data, but not the electrophysiological data, support the hypothesis that these specialized setae function as chemoreceptors. Further tests with additional chemical stimulants would have to be carried out to demonstrate the chemoreceptivity of these setae.

We hypothesize that the papillae on the pedipalps of some solifuge males may nevertheless be involved in some aspect of courtship or copulation. Behavioral observations have demonstrated that males use the pedipalps to keep constant contact with the female's opisthosoma (Amitai et al. 1962, Junqua 1966, Cloudsley-Thompson 1967, Wharton 1987, Peretti and Willemart 2007, Hrušková-Martišova et al. 2010, J. R., unpublished data). This observation lends further support to the hypothesis that the papillae may serve an important sensory function during mating. Other studies also showed that stimulation from the male's pedipalps during the copulatory sequence triggered the adoption of a submissive posture by the female (Junqua 1966, Muma 1966, Wharton 1987, Hrušková-Martišova et al. 2010). The extensive branching of these specialized setae may provide a unique mechanism for transduction of mechanical stimuli during courtship or mating in solifuges, perhaps stimulating the female or triggering her to accept her pugnacious suitor.

Acknowledgments

This project was supported by National Science Foundation grants BIO-DEB 0640245 and BIO-DEB 0228699 awarded to Cushing and Prendini. It was also supported by a grant from the Metropolitan State University of Denver Undergraduate Research Program and a Sigma Xi Grant-in-aid-of-Research for Undergraduates awarded to Casto. We thank Richard Medville for his extraordinary patience and enthusiasm working on preliminary histology for this project. Thanks also to Al Jassim Mowasak and Bobby To at the National Renewable Energy laboratory in Golden, CO, for assistance with earlier SEM work and to Gary Zito of the Colorado School of Mines for his help with the other SEMs used in this paper. Figures 1B and 5B were taken at the USGS Denver Microbeam Lab; thanks to Heather Lowers for her help with the use of this equipment. Thanks to Gary W. Mierau and Luann Goin at The Children's Hospital, Denver, for earlier TEM work (not included in this paper). Thanks to Kathy Honda for tracking down citations for this research. Thanks to Aaron Spriggs, Amanda Ladigo, Kyle Conrad, Zach Thomas, Danielle Bobb, Albert Frechette, Bailey Trierweiler, and Gary Olds for help in the field. Thanks also to Bruce Cutler and Rainer Foelix for reviewing earlier drafts of this manuscript and to anonymous reviewers whose comments greatly strengthened this paper.

References Cited

Adams, J. R., P. E. Holbert, and A. J. Forgash. 1965. Electron microscopy of the contact chemoreceptors of the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). Ann. Entomol. Soc. Am. 58: 909–917.

Akkerhuis, G.J.O., M. W. Sabelis, and W. F. Tjallingii. 1985. Ultrastructure of chemoreceptors on the pedipalps and first tarsi of *Phytoseiulus persimilis.* Exp. Appl. Acarol. 1: 235–251.

Amitai, P., G. Levy, and A. Shulov. 1962. Observations on mating in a solifugid *Galeodes sulfuripes* Roewer. Bull. Res. Counc. Isr. Sect. B Zool. 11: 156–159.

Barth, F. G. 1971. Der sensorische Apparet der Spaltsinnesorgane (*Cupiennius salei* Keys. Araneae). Zeitschrift für Zellforschung 112: 212–246.

Barth, F. G. 2001. XII Chemoreception, pp. 145–150. *In* A Spider's World: Senses and Behavior. Springer, Berlin, Heidelberg, New York, NY.

Bauchhenss, E. 1983. Morphology and ultrastructure of sensilla ampullacea in Solifugae (Chelicerata: Arachnida). Int. J. Insect Morphol. Embryol. 12: 129–138.

Brookhart, J. O., and P. E. Cushing. 2002. New species of Eremobatidae (Arachnida, Solifugae) from North America. J. Arachnol. 30: 84–97.

Brookhart, J. O., and P. E. Cushing. 2004. The systematics of the *Eremobates scaber* species-group (Solifugae, Eremobatidae). J. Arachnol. 32: 284–312.

Brookhart, J. O., and M. H. Muma. 1981. The *pallipes* species group of *Eremobates* Banks (Solpugida: Arachnida). Fla. Entomol. 64: 283–308.

Chu-Wang, I. W., and R. C. Axtell. 1973. Comparative fine structure of the claw sensilla of a soft tick, *Argas* (*Persicargas*) *arboreus* Kaiser, Hoogstraal, and Kohls, and a hard tick, *Amblyomma americanum* (L.). J. Parasitol. 59: 545–555.

Cloudsley-Thompson, J. L. 1967. Reproduction in *solifugae.* Turtox News 45: 212–215.

Coons, L. B., and G. Alberti. 1999. Acari: ticks, pp. 267–514. *In* Microscopic Anatomy of Invertebrates, vol. 8B: Chelicerate Arthropoda. Wiley-Liss, Inc., New York, NY.

Cushing, P. E., J. O. Brookhart, H.-J. Kleebe, G. Zito, and P. Payne. 2005. The suctorial organ of the Solifugae (Arachnida, Solifugae). Arthropod Struct. Dev. 34: 397–406.

Cushing, P. E., and P. Casto. 2012. Preliminary survey of the setal and sensory structures on the pedipalps of camel spiders (Arachnida, Solifugae). J. Arachnol. 40: 123–127.

El Hennawy, H. K. 1990. Key to solpugid families (Arachnidae: Solpugida). Serket 2: 20–27.

Farley, R. D. 1999. Scorpiones, pp. 117–222. *In* Microscopic anatomy of invertebrates, vol. 8A: Chelicerate Arthropoda. Wiley-Liss, Inc., New York, NY.

Felgenhauer, B. E. 1999. Araneae, pp. 223–266. *In* Microscopic anatomy of invertebrates, vol. 8A: Chelicerate Arthropoda. Wiley-Liss, Inc., New York, NY.

Fichter, E. 1940. Studies of North American Solpugida, I. The true identity of *Eremobates pallipes* (Say). Am. Midl. Nat. 24: 351–360.

Foelix, R. F. 1970. Structure and function of tarsal sensilla in the spider *Araneus diadematus.* J. Exp. Zool. 175: 99–124.

Foelix, R. F. 1985. VII Mechano- and chemoreceptive sensilla, pp. 118–137. *In* F. G. Barth (ed.), Neurobiology of arachnids. Springer, Berlin, Heidelberg, New York, NY, Tokyo, China.

Foelix, R. F., and R. C. Axtell. 1971. Fine structure of tarsal sensilla in the tick *Amblyomma americanum* (L.). Zeitschrift für Zellforschung und mikroskopische Anatomie 114: 27–37.

Foelix, R. F., and R. C. Axtell. 1972. Ultrastructure of Haller's organ in the tick *Amblyomma americanum* (L.). Zeitschrift für Zellforschung und mikroskopische Anatomie 124: 275–292.

Foelix, R. F., and I. W. Chu-Wang. 1972. Fine structural analysis of palpal receptors in the tick *Amblyomma americanum* (L.). Zeitschrift für Zellforschung und mikroskopische Anatomie 129: 548–560.

Foelix, R. F., and I. W. Chu-Wang. 1973. The morphology of spider sensilla. I. Mechanoreceptors. Tissue Cell 5: 451–460.

Foelix, R. F., and J. Schabronath. 1983. The fine structure of scorpion sensory organs. I. Tarsal sensilla. Bull. Br. Arachnol. Soc. 6: 53–67.

Gaffin, D. D., and P. H. Brownell. 1997a. Response properties of chemosensory peg sensilla on the pectines of scorpions. J. Comp. Physiol. 181: 291–300.

Gaffin, D. D., and P. H. Brownell. 1997b. Electrophysiological evidence of synaptic interactions within chemosensory sensilla of scorpion pectines. J. Comp. Physiol. 181: 301–307.

Gaffin, D. D., and M. E. Walvoord. 2004. Scorpion peg sensilla: are they the same or are they different? Euscorpius 17: 7–15.

Gnatzy, W., and J. Tautz. 1980. Ultra structure and mechanical properties of an insect mechanoreceptor: stimulus-transmitting structures and sensory apparatus of the cercal filiform hairs of *Gryllus.* Cell Tissue Res. 213: 441–463.

Guffey, C., V. R. Townsend, Jr., and B. E. Felgenhauer. 2000. External morphology and ultrastructure of the prehensile region of the legs of *Leiobunum nigripes* (Arachnida, Opiliones). J. Arachnol. 28: 231–236.

Hallberg, E., and B. S. Hansson. 1999. Arthropod sensilla: morphology and phylogenetic considerations. Microsc. Res. Techniq. 47: 428–439.

Harris, D. J., and P. J. Mill. 1973. The ultrastructure of chemoreceptor sensilla in *Ciniflo* (Araneida, Arachnida). Tissue Cell 5: 679–689.

Harvey, M. S. 2003. Catalogue of the smaller arachnid orders of the world: Amblypygi, Uropygi, Schizomida, Palpigradi, Ricinulei and Solifugae. Csiro Publishing, Collingwood, Victoria, Australia.

Haupt, J. 1982. Hair regeneration in a solifugid chemotactile sensillum during molting (Arachnida: Solifugae). Wilhelm Roux's Arch. 191: 137–142.

Heymons, R. 1902. Biologische Beobachtungen an asiatischen Solifugen nebst Beiträgen zur Systematik derselben. Abhandlungen der Königlich Preussischen Akademie der Wissenschaftern 190: 1–65.

Hrušková-Martišova, M., S. Pekár, and T. Bilde. 2010. Coercive copulation in two sexually cannibalistic camel-spider species (Arachnida: Solifugae). J. Zool. 282: 91–99.

Junqua, C. 1962. Données sur la reproduction d'un solifuge: *Othoes saharae* Panouse. Comptes Rendus Académie des Sciences (Paris) 255: 2673–2675.

Junqua, C. 1966. Recherches biologiques et histo-physiologiques sur un solifuge saharien *Othoes saharae* Panouse. Mémoires du Muséum National d'Histoire Naturelle Paris, n.s. 43: 1–124.

Klann, A. E., A. V. Gromov, P. E. Cushing, A. V. Peretti, and G. Alberti. 2008. The anatomy and ultrastructure of the suctorial organ of Solifugae (Arachnida). Arthropod Struct. Dev. 37: 3–12.

Kraepelin, K. 1899. Zur Systematik der Solifugen. Mitteilungen aus dem Naturhistorischen Museum in Hamburg Jahrgang 16: 197–259.

McIver, S. B. 1975. Structure of cuticular mechanoreceptors of arthropods. Ann. Rev. Entomol. 20: 381–397.

Muma, M. H. 1951. The arachnid order Solpugida in the United States. Bull. Am. Mus. Nat. Hist. 97: 34–141.

Muma, M. H. 1966. Mating behavior in the solpugid genus *Eremobates* Banks. Anim. Behav. 14: 346–350.

Muma, M. H. 1970. A synoptic review of North American, Central American, and West Indian Solpugida (Arthropoda, Arachnida). Arthropods Fla. Neighboring Land Areas 5: 1–62.

Muma, M. H. 1989. New species and records of Solpugida (Arachnida) from the United States, pp. 1–60. Privately published for the author by Douglas Print Shop, Douglas, AZ.

Peretti, A. V., and R. H. Willemart. 2007. Sexual coercion does not exclude luring behavior in the climbing camel-spider *Oltacola chacoensis* (Arachnida, Solifugae, Ammotrechidae). J. Ethol. 25: 29–39.

Punzo, F. 1997. Dispersion, temporal patterns of activity, and the phenology of feeding and mating behavior in *Eremobates palpisetulosus* Fichter (Solifugae, Eremobatidae). Bull. Br. Arachnol. Soc. 10: 303–307.

Punzo, F. 1998a. The Biology of Camel-Spiders (Arachnida, Solifugae). Kluwer Academic Publishers, Boston, MA.

Punzo, F. 1998b. Natural history and life cycle of the solifuge *Eremobates marathoni* Muma & Brookhart (Solifugae, Eremobatidae). Bull. Br. Arachnol. Soc. 11: 111–118.

Ribi, W. A. 1976. A golgi-electron microscope method for insect nervous tissue. Stain Technol. 51: 13–16.

Roewer, C. F. 1934. Solifugae, Palpigradi, pp. 161–480. *In* H. G. Bronn (ed.), vol. 3–5, Klassen und Ordnungen des Tierreichs. 5: Arthropoda. IV Arachnoidea, Akademische Verlagsgesellschaft M.B.H., Leipzig, Germany.

Selzer, R. 1981. The processing of a complex food odor by antennal olfactory receptors of *Periplaneta americana.* J. Comp. Physiol. 144: 509–519.

Slifer, E. H. 1961. The fine structure of insect sense organs. Int. Rev. Cytol. 11: 125–159.

Slifer, E. H. 1968. Sense organs on the antennal flagellum of a giant cockroach, *Gromphadorhina portentosa,* and a comparison with those of several other species (Dictyoptera, Blattaria). J. Morphol. 126: 19–30.

Slifer, E. H. 1970. The structure of arthropod chemoreceptors. Annu. Rev. Entomol. 15: 121–142.

Stempak, J. G., and R. T. Ward. 1964. An improved staining method for electron microscopy. J. Cell Biol. 22: 697–701.

Talarico, G., J. G. Palacios-Vargas, M. Fuentes Silva, and G. Alberti. 2006. Ultrastructure of tarsal sensilla and other integument structures of two *Pseudocellus* species (Ricinulei, Arachnida). J. Morphol. 267: 441–463.

Thurm, U. 1964. Mechanoreceptors in the cuticle of the honey bee: fine structure and stimulus mechanism. Science 145: 1063–1065.

Tichy, H., and F. G. Barth. 1992. Fine structure of olfactory sensilla in myriapods and arachnids. Microsc. Res. Tech. 22: 372–391.

Tichy, H., and R. Loftus. 1990. Response of moist-air receptor on antenna of the stick insect, *Carausius morosus,* to step changes in temperature. J. Comp. Physiol. A 166: 507–516.

Tietjen, W. J. 1977. Dragline-following by male lycosid spiders. Psyche 84: 165–178.

Tietjen, W. J., and J. S. Rovner. 1980. Trail-following behavior in two species of lycosid spiders: sensory and etho-ecological concomitants. Anim. Behav. 28: 735–741.

Wharton, R. A. 1987. Biology of the diurnal *Metasolpuga picta* (Kraepelin) (Solifugae, Solpugidae) compared with that of nocturnal species. J. Arachnol. 14: 363–383.

Willemart, R. H., R. D. Santer, A. J. Spence, and E. A. Hebets. 2011. A sticky situation: solifugids (Arachnida, Solifugae) use adhesive organs on their pedipalps for prey capture. J. Ethol. 29: 177–180.

Zacharuk, R. Y. 1980. Ultrastructure and function of insect chemosensilla. Annu. Rev. Entomol. 25: 27–47.

Received 11 September 2013; accepted 27 January 2014.