2015. Journal of Arachnology 43:194–206

Survey of cuticular structures on leg IV of cosmetid harvestmen (Opiliones: Laniatores: Gonyleptoidea)

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Abstract. The legs of laniatorean harvestmen feature a considerable diversity of setae, glandular openings, sensory structures, and tubercles. In this study, we compared the morphology of cuticular structures occurring on leg IV of 12 species of cosmetid harvestmen and six species of other gonyleptoidean harvestmen representing the families Agoristenidae, Gonyleptidae (three subfamilies), and Stygnidae. Our results indicate that there is considerable interspecific variation in rough pit glands and the tarsal perforated organ among cosmetid and gonyleptoidean harvestmen. We also found interspecific variation in the relative size, number, distribution, and morphology of the tubercles on the femur, patella and tibia. We observed relatively little interspecific variation in the morphology of sensilla chaetica, trichomes, spines, single pores, tarsal aggregated pores, and slit sensilla. Additional studies of the ultrastructure of cuticular structures, especially rough pit glands, are needed to assess their functional significance. Future systematic revisions of cosmetid taxa may also benefit from the inclusion of characters based upon interspecific variation in the microanatomical structures of the legs.

Keywords: Morphology, rough pit gland, tarsal perforated organ, SEM, tubercles

Comparative studies of interspecific variation in microanatomical structures of harvestmen have the potential to yield insights into functional morphology (Spicer 1987; Murphree 1988; Willemart et al. 2007; Fernandes & Willemart 2014), identify novel characters for phylogenetic studies, and discover traits that can help make taxonomic identifications easier and more reliable (DaSilva & Gnaspini 2009; Pinto-da-Rocha & Hara 2011; Gainett et al. 2014). Taxonomic studies of cosmetid harvestmen have traditionally used characters based upon the morphology of male leg IV (e.g., the relative size, number, and distribution of tubercles on the femur and tibia and other cuticular excrescences) to differentiate species (Roewer 1912, 1923; Goodnight & Goodnight 1953). With the exception of intersexual variation in glandular openings on the metatarsus (Fernandes & Willemart 2014), variation in the microscopic anatomy of leg IV has not been examined. Males of many cosmetid species have enlarged basal tarsal segments of leg I (Townsend et al. 2010) and glandular openings on the metatarsus and tarsus (Willemart et al. 2010; Proud & Felgenhauer 2013a). In two species of *Gryne* Simon 1879, males have more numerous glandular openings on metatarsus IV and have been observed using these glands to mark the substrate (Fernandes & Willemart 2014). In contrast to tarsus I, tarsus IV lacks swollen segments, but does have tarsal aggregated pores near the base of the tarsal process (Gainett et al. 2014). The contribution of the glandular secretions from tarsal glands to intraspecific communication remains to be evaluated empirically; however, observations of gonyleptid harvestmen indicate that tarsal glands may also be used to mark substrates (Willemart et al. 2007). In many cosmetid harvestmen, the morphology of leg IV (and to a much lesser extent leg III) is sexually dimorphic (Townsend et al. 2010), with males having enlarged and more numerous tubercles on the femur, patella, tibia or metatarsus. These tubercles are generally absent from legs I and II (Townsend et al. 2010). Although male combat has not been described for any cosmetid species, in the gonyleptid harvestmen *Neosadocus maximus* (Giltay 1928), males use large tubercles on leg IV in intrasexual contests (Willemart et al. 2009b).

Recent morphological studies using scanning electron microscopy (SEM) have revealed a diverse assortment of cuticular structures on the legs of gonyleptoidean harvestmen

Table 1.—Cuticular structures on leg IV of cosmetid harvestmen. Measurements are in µm for sensilla chaetica, trichomes and slit sensilla. Slit sensilla and sensilla chaetica data are from the femur, trichomes data are from the tarsus. Meristic data are reported for the total number of glandular openings for tarsal aggregated pores (TAP) and the number of plates per row for tarsal perforated organs (TPO).

Species	Sensilla chaetica	Trichomes	Slit sensilla	TAP	TPO
Cynorta blasi	52–80	45–70	51–360	5–10	14
Cynorta dentipes	62–163	45–70	81–221	10–15	14–16
Cynorta marginalis	44–182	34–140	51–178	5–10	12–14
Cynortula granulata	38–55	53–64	50–248	10–15	13–14
Erginulus clavotibialis	75–199	42–61	111–250	5–10	17–18
Erginulus subserialis	55–124	66–116	61–296	5–10	16–18
Erginulus weyerensis	60–92	46–71	42–117	10–15	16
Eupoecilaema magnum	81–207	104–140	60–378	10–15	18–21
Holovonones compressus	65–80	47–82	120–180	5–10	6–7
Paecilaema chiriquiense	57–170	49–114	102–216	5–10	13–14
Paecilaema inglei	134–170	61–92	47–185	10–15	11–12
Vonones ornatus	56–109	41–52	87–133	5–10	12–13

including multiple types and different sizes of setae (Willemart & Gnaspini 2003; Willemart et al. 2007), rough pit glands (Willemart et al. 2007; Rodriguez et al. 2014) single and clustered glandular openings (Willemart et al. 2010; Gnaspini & Rodrigues 2011; Fernandes & Willemart 2014), slit sensilla (Willemart et al. 2007, 2009a), granulations and ridges (Murphree 1988), and different shapes, densities, and sizes of tubercles (Willemart et al. 2007, 2009a). In addition to interspecific differences in microanatomy (reviewed by Willemart et al. 2009a), variation in the distribution and morphology of setae on different legs and between leg segments has been observed (Willemart & Gnaspini 2003).

Currently, most cosmetid taxa are still defined on the basis of relatively superficial Roewerian characters related to dorsal scutal armature and the number of tarsal segments on leg I with most species descriptions also lacking characters based upon reproductive morphology (Roewer 1912, 1923, 1947). As a result, there is general consensus that cosmetid taxonomy requires major revision (Goodnight & Goodnight 1953; Perez González & Vasconcelos 2003; Kury et al. 2007). Recent studies of cosmetid harvestmen have identified a variety of structures that exhibit considerable interspecific variation. These characters include the general shape of the dorsal scutum (Kury et al. 2007), color patterns of the dorsal scutum (Kury & Barros 2014), morphology of the eye mound (Pinto-da-Rocha & Hara 2011; Rodriguez et al. 2014), distribution and shape of tubercles on the pedipalp (Pinto-da-Rocha & Hara 2011; Kury & Ferreira 2012; Rodriguez et al. 2014), relative size of male coxa IV (Pinto-da-Rocha & Hara 2011), size and number of cheliceral teeth (Rodriguez et al. 2014), penis structure (Kury et al. 2007; Pinto-da-Rocha & Hara 2011; Pinto-da-Rocha & Yamaguti 2013), microanatomy of the ovipositor (Bennett & Townsend 2013; Walker & Townsend 2014), and surface texture of the dorsal scutum and ocularium (Rodriguez et al. 2014).

In this study, we used SEM to examine the morphology of the cuticular structures occurring on leg IV of 12 cosmetid species including taxa from Central America (Belize and Costa Rica), North America (Mexico and U.S.), and the Caribbean island of Trinidad. Specifically, we investigated interspecific variation in the microanatomical features of the femur, patella, tibia, metatarsus, and tarsus. In an effort to determine if structures were unique to the cosmetid taxa being studied, we examined leg IV of select species of gonyleptoidean harvestmen representing the families Agoristenidae, Gonyleptidae, and Stygnidae.

METHODS

We used SEM to study the morphology of leg IV of at least 2 males and 3 females of the cosmetid harvestmen *Cynorta dentipes* Pickard-Cambridge 1904 (Bladen Reserve, Toledo district, Belize; July 2012), *C. marginalis* Banks 1909 (La Selva Biological Station, Costa Rica; August 2008), *Cynortula granulata* Roewer 1912 (Mt. Tamana, Trinidad, W. I., May 2008), *Erginulus clavotibialis* (Pickard-Cambridge 1905) (Tapir Mountain Reserve, Cayo District, Belize; January 2012), *E. subserialis subserialis* Pickard-Cambridge 1905 (Santa Rosa, Costa Rica; July 2010), *E. weyerensis* Goodnight & Goodnight 1977 (Bladen Reserve, Toledo District, Belize; July 2012), *Eupoecilaema magnum* Roewer 1933 (La Selva Biological Station, Costa Rica; August 2008), *Holovonones compressus* (Pickard-Cambridge 1904) (Clarissa Falls, Cayo District, Belize; January 2012), *Paecilaema chiriquiense* Goodnight & Goodnight 1943 (Rainmaker, Costa Rica; July 2010), *P. inglei* Goodnight & Goodnight 1947 (Matura, Trinidad, W.I.; May 2008), and *Vonones ornatus* (Say 1821) (Natchitoches, Louisiana, USA, collected by J.

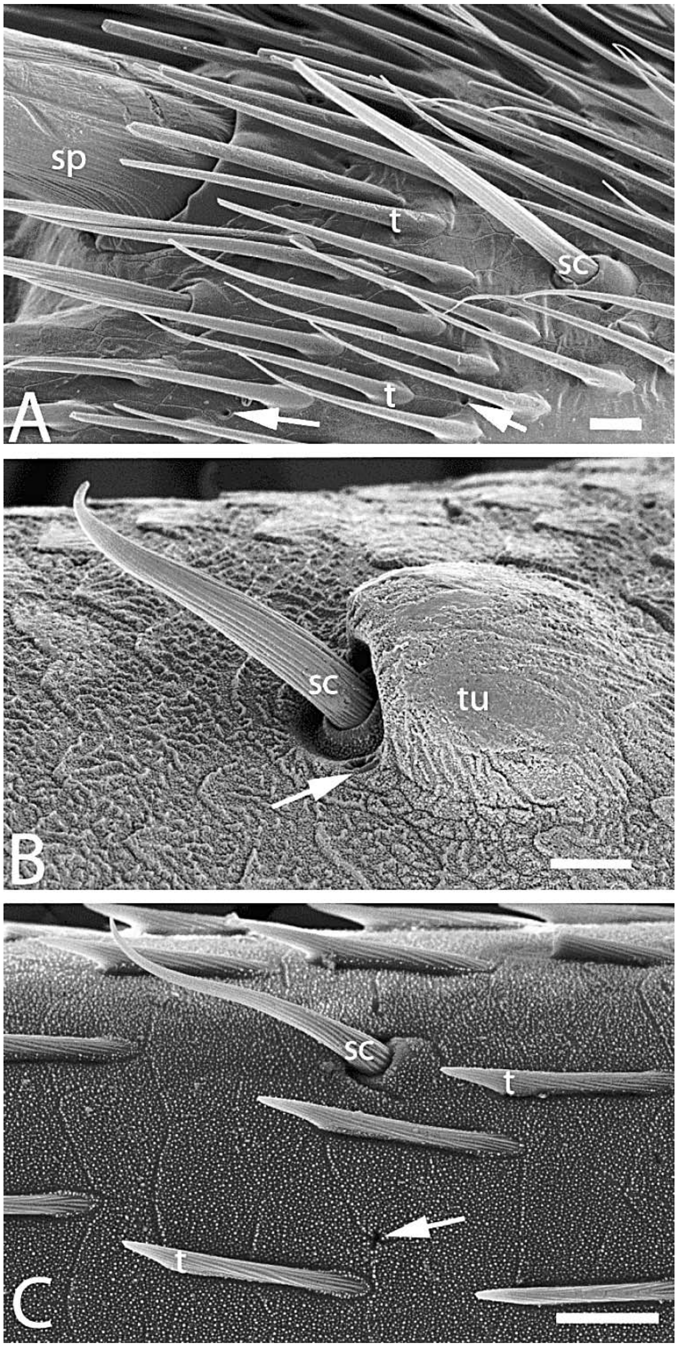

Figure 1.—Cuticular structures on metatarsus IV of cosmetid (A, B) and agoristenid (C) harvestmen. A) *Cynorta blasi* with spines (sp), trichomes (t), sensilla chaetica (sc), and glandular openings (arrows), B) *C. dentipes* displaying a tubercle (tu) with a basal sensilla chaetica and glandular opening, C) *Avima intermedia* with sensilla chaetica, trichomes, and glandular openings. Scale bar = 10 μm.

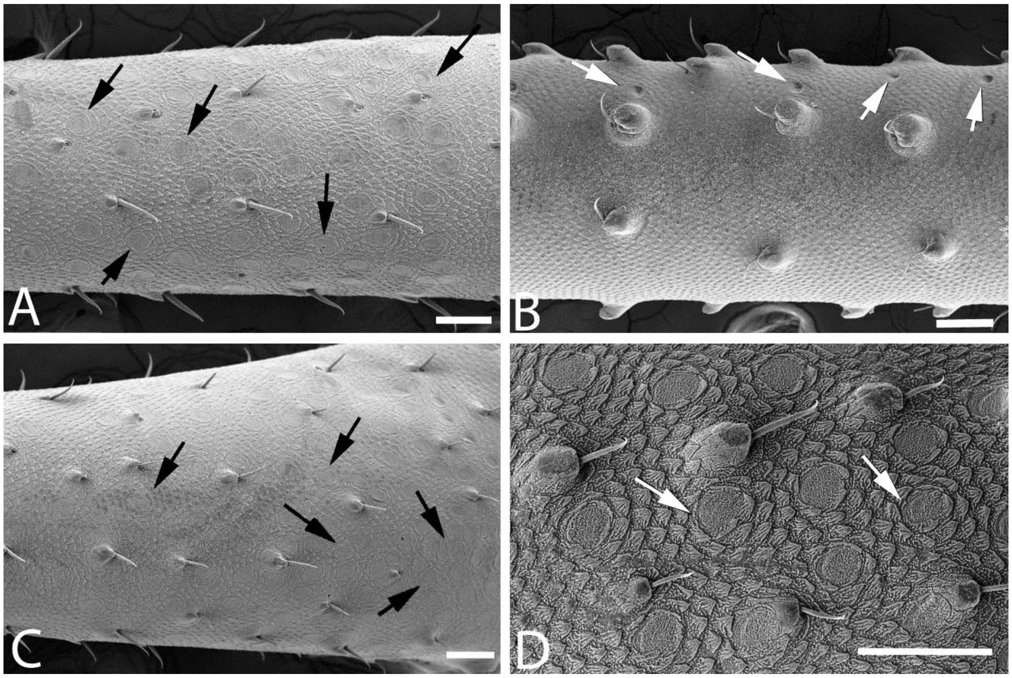

Figure 2.—Rough pit glands (arrows) on the femur and patella of leg IV of cosmetid harvestmen. A) Proximal femur of *Paecilaema inglei,* B) distal femur of *P. chiriquiense,* C) patella of *P. inglei,* D) patella of *Erginulus weyerensis*. Scale bar = 100 µm.

Akin; September 2012). For *Cynorta blasi* Goodnight & Goodnight 1953 (Puerto Vallarta, Jalisco, Mexico, collected by F. Cupul; July 2012), we were only able to examine one male and two females. In addition, we examined leg IV of multiple (2–4) adults of the agoristenid *Avima intermedia* (Goodnight & Goodnight 1947) (Petite Tacaribe, Trinidad, W.I; July 2007), the gonyleptids *Glysterus* sp. (La Selva Biological Station, Costa Rica; July 2010), *Phareicranaus calcariferus* (Cranainae) (Simon 1879) (Petite Tacaribe, Trinidad, W.I. July 2006), *Rhopalocranaus albilineatus* Roewer 1932 (Petite Tacaribe, Trinidad, WI.; May 2008) and *Zygopachylus albomarginis* Chamberlin 1925 (Parque Summit, Panama, collected by R. Miranda, September 2009), and the stygnid *Stygnoplus clavotibialis* (Goodnight & Goodnight 1947) (Petite Tacaribe, Trinidad, W.I.; May 2008). The gonyleptid harvestmen examined in this study represent the subfamilies Ampycinae (*Glysterus* sp.), Cranainae (*P. calcariferus*), and Manaosbiinae (*R. albilineatus* and *Z. albomarginis*). Specimens were captured in the field by hand and preserved in 70% ethanol. Voucher specimens were deposited in the collections of INBio, Universidad de Costa Rica, and the American Museum of Natural History.

Table 2.—Comparison of the morphology and distribution of rough pit glands (RPG) on male leg IV of the gonyleptoidean harvestmen examined in the present study; F = femur, P = patella, T = tibia.

Species	Family	RPG morphology	RPG distribution
Cynorta blasi	Cosmetidae	Convex, no pores	F, P, T
Cynorta dentipes	Cosmetidae	Convex with pores	F, P, T
Cynorta marginalis	Cosmetidae	Surface with pores	F, P, T
Cynortula granulata	Cosmetidae	Surface, no pores	F, P, T
Erginulus clavotibialis	Cosmetidae	Convex, no pores	F, P
Erginulus subserialis	Cosmetidae	Surface, no pores	F, P
Erginulus weyerensis	Cosmetidae	Surface with pores	F, P
Eupoecilaema magnum	Cosmetidae	Surface, no pores	F, P
Holovonones compressus	Cosmetidae	Convex, no pores	F, P, T
Paecilaema chiriquiense	Cosmetidae	Convex with pores	F, P, T
Paecilaema inglei	Cosmetidae	Convex with pores	F, P, T
Vonones ornatus	Cosmetidae	Surface, no pores	F, P, T
Avima intermedia	Agoristenidae	Absent	Absent
Glysterus sp.	Gonyleptidae, Ampycinae	Surface, no pores	F
Phareicranaus calcariferus	Gonyelptidae, Cranainae	Absent	Absent
Rhopalocranaus albilineatus	Gonyleptoidae, Manaosbiinae	Surface, no pores	F, P, T
Zygopachylus albomarginis	Gonyleptidae, Manaosbiinae	Surface, no pores	P, T
Stygnoplus clavotibialis	Stygnidae	Absent	Absent

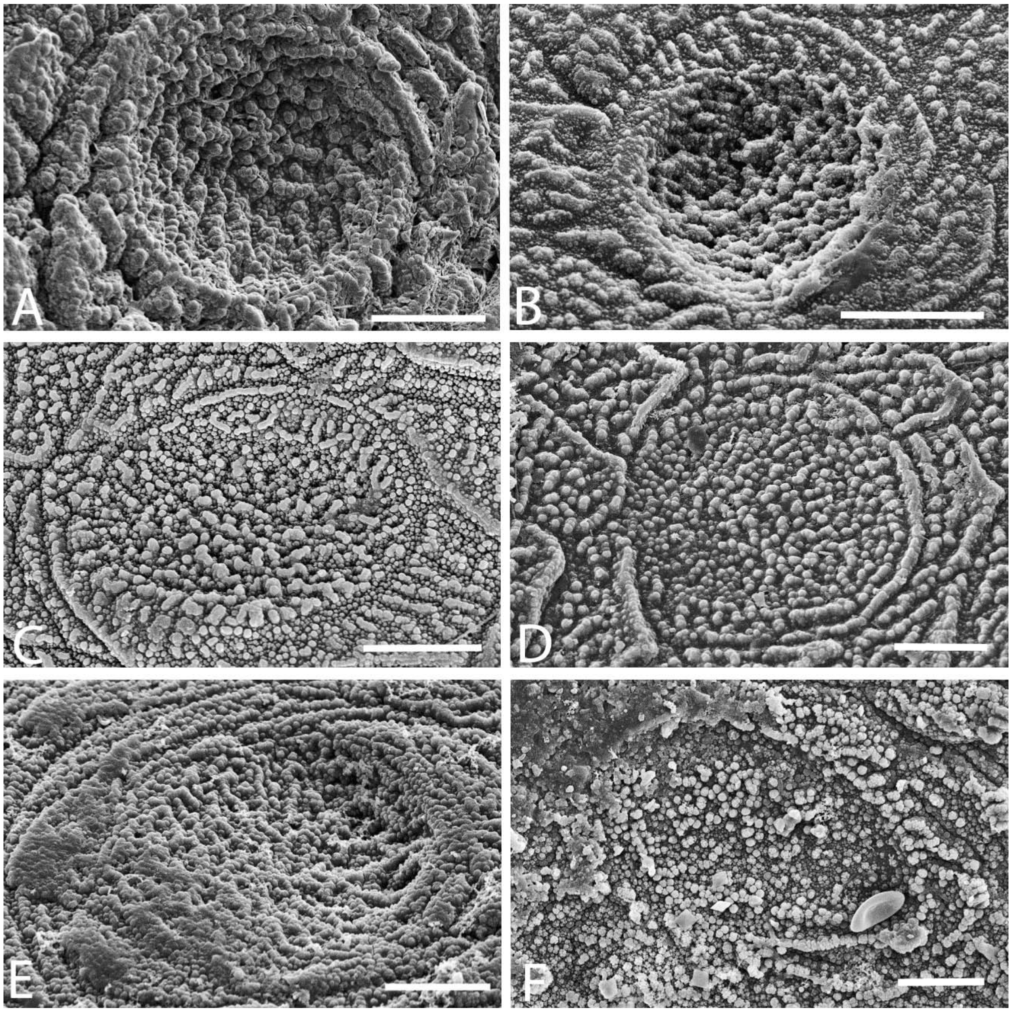

Figure 3.—Rough pit glands on femur IV of cosmetid harvestmen. A) *Cynorta blasi,* B) *C. dentipes,* C) *C. marginalis*, D) *Cynortula granulata,* E) *Erginulus clavotibialitis,* F) *E. subserialis*. Scale bar = 10 μm.

To prepare samples for SEM, a dissecting scalpel was used to carefully separate the legs (right and left) from the body of each specimen at the trochanter-coxa joint. Each leg was ultrasonicated in 70% ethanol for 2–5 min. Specimens were dehydrated in a graded ethanol series and chemically dried using hexamethyldisilazane (Nation 1983). We mounted legs on 26-mm aluminum stubs coated with colloidal silver paint or a carbon adhesive tab. Specimens were sputter-coated with 15–30 nm of gold and examined with a Hitachi S-3400N SEM at accelerating voltages of 5–10 kV. For the cosmetid species, we used image analysis software (PC SEM, Hitachi Technologies) to measure the diameter and length of most cuticular structures. Terminology used to describe cuticular structures (i.e., setae, slit sensilla, tubercles) follow the morphological descriptions of Willemart et al. (2007, 2009a), and Willemart & Giribet (2010).

RESULTS

Setae.—The segments of leg IV of cosmetid harvestmen featured three major types of setae including sensilla chaetica, trichomes and spines (Fig. 1A). The morphology and distribution of these setae were generally similar to those reported for gonyleptid harvestmen (Willemart et al. 2007). Sensilla chaetica were especially common on the lateral, dorsal, and ventral surfaces of the femur, patella, and tibia, but more sparsely distributed on the metatarsus and tarsus (Fig. 1), segments in which trichomes were abundant. Sensilla chaetica typically had well-defined surface striations and inserted at acute angles into well-developed sockets (Fig. 1B). The sensilla chaetica exhibited considerable interspecific and intraspecific variation in total length (Table 1), with the longest setae (often exceeding 100 μm) found on the lateral surfaces of the metatarsus and tarsus. On the femur, patella and tibia, the sensilla chaetica were generally shorter (most commonly 60–80 μm) and also frequently occurred on the distal borders of rounded tubercles (Fig. 1B). In contrast to sensilla chaetica, the trichomes lacked sockets (Willemart et al. 2007) and were absent from the proximal leg segments. They were the most common setal type on the metatarsus and tarsus. In general, they were slightly shorter than sensilla chaetica (Table 1) and typically smaller in diameter. Spines (Fig. 1A) were significantly larger in diameter (8–25 μm) than sensilla chaetica (3.5–9.0 μm) and trichomes (1.9–7 μm), but were much less abundant and relatively short (similar in total length to

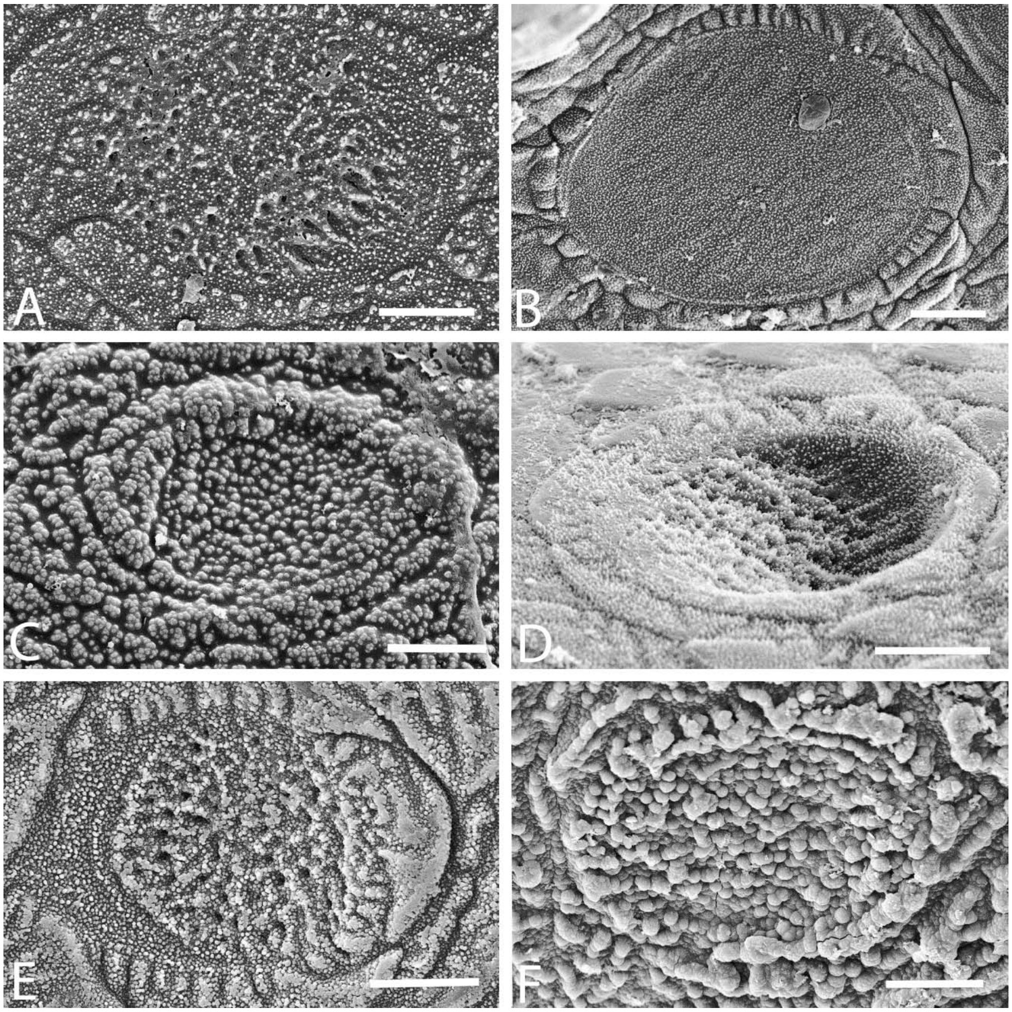

Figure 4.—Rough pit glands on femur IV of cosmetid harvestmen. A) *Erginulus weyerensis*, B) *Eupoecilaema magnum*, C) *Holovonones compressus*, D) *Paecilaema chiriquiense*, E) *P. inglei*, F) *Vonones ornatus*. Scale bar = 10 µm.

sensilla chaetica). The spines resembled sensilla chaetica in having well-developed sockets. When present, they generally occurred as isolated setae or in pairs on the ventro-lateral surfaces of the distal ends of the femur, tibia and metatarsus. Among the gonyleptoidean species, the distribution of sensilla chaetica, trichomes and spines exhibited a similar pattern to that observed for cosmetid harvestmen.

Single glandular openings.—Leg IV of each species of cosmetid and gonyleptoidean harvestmen commonly featured scattered single pores on the dorsal and lateral surfaces (Fig. 1). We did not observe any significant intraspecific or interspecific variation in the overall density of pores.

Rough pit glands.—We observed rough pit glands on the femur and patella of each cosmetid species that we examined (Table 2). We also found rough pit glands on the tibiae of most species, with the exception of *Erginulus* spp. and *Eupoecilaema magnum* (Table 2). These structures were especially common on the dorsal region of the leg (Fig. 2), but also occurred on the lateral and ventral surfaces. Rough pit glands varied in size (20–50 µm in diameter). In relation to the cuticle, these structures displayed consistent interspecific variation (Table 2) and were either convex (Figs. 3A, B, 3E, 4C, D) or on the surface (Figs. 3C, D, 3F, 4A, B, 5A, B). The centers of the rough pit glands typically had small granules that were of similar size and shape. Several rough pits glands also had prominent pores (Table 2). Among the gonyleptoidean taxa, rough pit glands were only present on the leg segments of gonyleptid harvestmen including the femur of *Glysterus* sp., the femur, patella, and tibia of *Rhopalocranus albilineatus*, and the patella and tibia of *Zygopachylus albomarginis* (Table 2; Fig. 5). The rough pit glands of these three species were on the surface of the cuticle. The rough pit glands of *Glysterus* sp. were most similar to those of cosmetid species in being formed by small granules similar in size and shape to those of the surrounding cuticle. In contrast, the surface textures of the rough pit glands of *R. albilineatus* and *Z. albomarginis* were only slightly granulated (Fig. 5) and lacked pores.

Tarsal aggregated pores.—These structures occurred on the most distal tarsal segment of leg IV near the base of the tarsal process on the prolateral and retrolateral surfaces. Each grouping of pores was surrounded by 3–-5 relatively long trichomes (Fig. 6). The tarsal aggregated pores were observed

Table 3.—Comparison of the external morphology of sexually dimorphic armature on male leg IV of cosmetid harvestmen examined in the present study. When present, the occurrences of the three major types of tubercle are indicated by Roman numerals (I, II and III).

Species	Femur	Patella	Tibia	Metatarsus
Cynorta blasi	Unarmed	Unarmed	I, II	I, II
Cynorta dentipes	I, III	Unarmed	Unarmed	Unarmed
Cynorta marginalis	I (very few)	Unarmed	Unarmed	Unarmed
Cynortula granulata	I	Unarmed	Unarmed	Unarmed
Erginulus clavotibialis	I	I, III	I, III	Unarmed
Erginulus subserialis	I, III	I	I	I
Erginulus weyerensis	I, III	I	I	Unarmed
Eupoecilaema magnum	I, II	I	Unarmed	Unarmed
Holovonones compressus	I	Unarmed	Unarmed	Unarmed
Paecilaema chiriquiense	I	I	I	Unarmed
Paecilaema inglei	I (very few)	Unarmed	Unarmed	Unarmed
Vonones ornatus	I	I	I	Unarmed

in all cosmetid species examined (Table 1). The number of pores exhibited interspecific variation, ranging from 5–15 per cluster (Fig. 6). The trichomes nearest the pores were generally longer than the other trichomes on the rest of the tarsus. Among the gonyleptoidean species examined, tarsal aggregated pores were observed in *Glysterus* sp., *Phareicranaus calcariferus*, *Rhopalocranus albilineatus*, *Stygnoplus clavotibialis* and *Zygopachylus albomarginis*. The number of pores in an aggregation varied from ~12 in *Z. albomarginis* to over 20 in *P. calcariferus*.

Tarsal perforated organ.—We observed these structures occurring on the ventral surface of the most proximal tarsomere and they were composed of two parallel rows of polygonal plates (Fig. 7). These plates were not smooth, but were granulated or irregularly pitted and lacked setae, unlike the surrounding cuticle (Fig. 7). Tarsal perforated organs were observed in all cosmetid species examined. The number of plates varied among species, ranging from 12–21 plates per row (Table 1). We did not observe any pores or evidence of glandular secretions in association with the plates (Fig. 8). The tarsal perforated organs of the gonyleptoidean taxa were similar in morphology to those of cosmetid harvestmen. The tarsal perforated organs of *S. clavotibialis* and *Z. albomarginis* were composed of two rows of 18 plates each.

Slit sensilla.—Slit sensilla occurred on the femur (Figs. 9A–C), tibia, metatarsus (Fig. 9D), and tarsus of most cosmetid species. These structures were generally located on the dorsal and lateral surfaces of the proximal and distal ends of leg segments, ranging in size from 40–360 μm (Table 1). Slit sensilla occurred as single slits (Figs. 9C, D), in pairs (Fig. 9B), or in groups of three (Fig. 8A). We observed the metatarsal paired slits identified by Gainett et al. (2014) in all species that we examined. The midpoint of each slit sensillum usually was slightly wider than the rest of the structure (Figs. 9A, C). Two single pores were observed on each side of the midpoint of most slit sensilla (Fig. 9C). Slits were positioned perpendicular to the long axis of the leg, as described by Willemart et al. (2007). We observed several slits covered by partially broken membranes (Fig. 9D). The distribution and morphology of the slit sensilla of the legs of the gonyleptoidean species exhibited a similar pattern to those of cosmetid harvestmen.

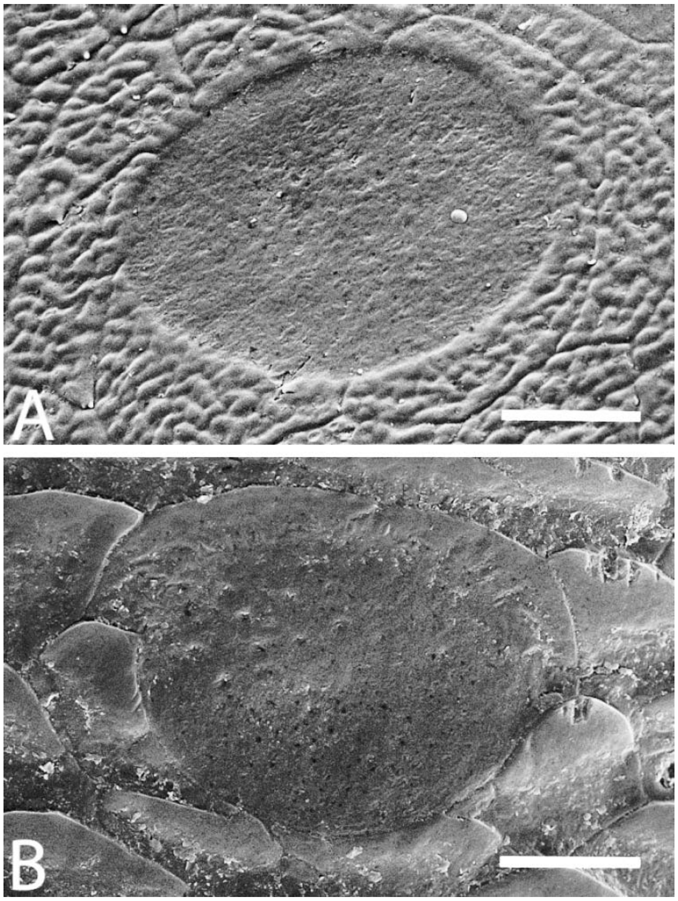

Figure 5.—Rough pit glands on leg IV of manaosbiine harvestmen. A) Patella of *Rhopalocranaus albilineatus*, B) tibia of *Zygopachylus albomarginis*. Scale bar = 10 μm.

Tubercles.—In many species of cosmetid harvestmen, the segments of leg IV (femur, patella, tibia or metatarsus) are sexually dimorphic, with males possessing one or more large tubercles, often arranged into longitudinal rows (Table 3). Metatarsus IV of male *C. blasi* had parallel rows of small rounded tubercles separated by a single row of larger tubercles (Fig. 10A). Femur IV of male *C. dentipes* had a single, large proximal tubercle and a few smaller tubercles on the femur (Fig. 10B). In contrast, femur IV of *C. marginalis* and *Cynortula granulata* were not sexually dimorphic and had relatively few (Fig. 10C) or considerably many, rounded tubercles (Fig. 10D). In male *Erginulus clavotibialis*, there was a single row of large, conical tubercles on both the

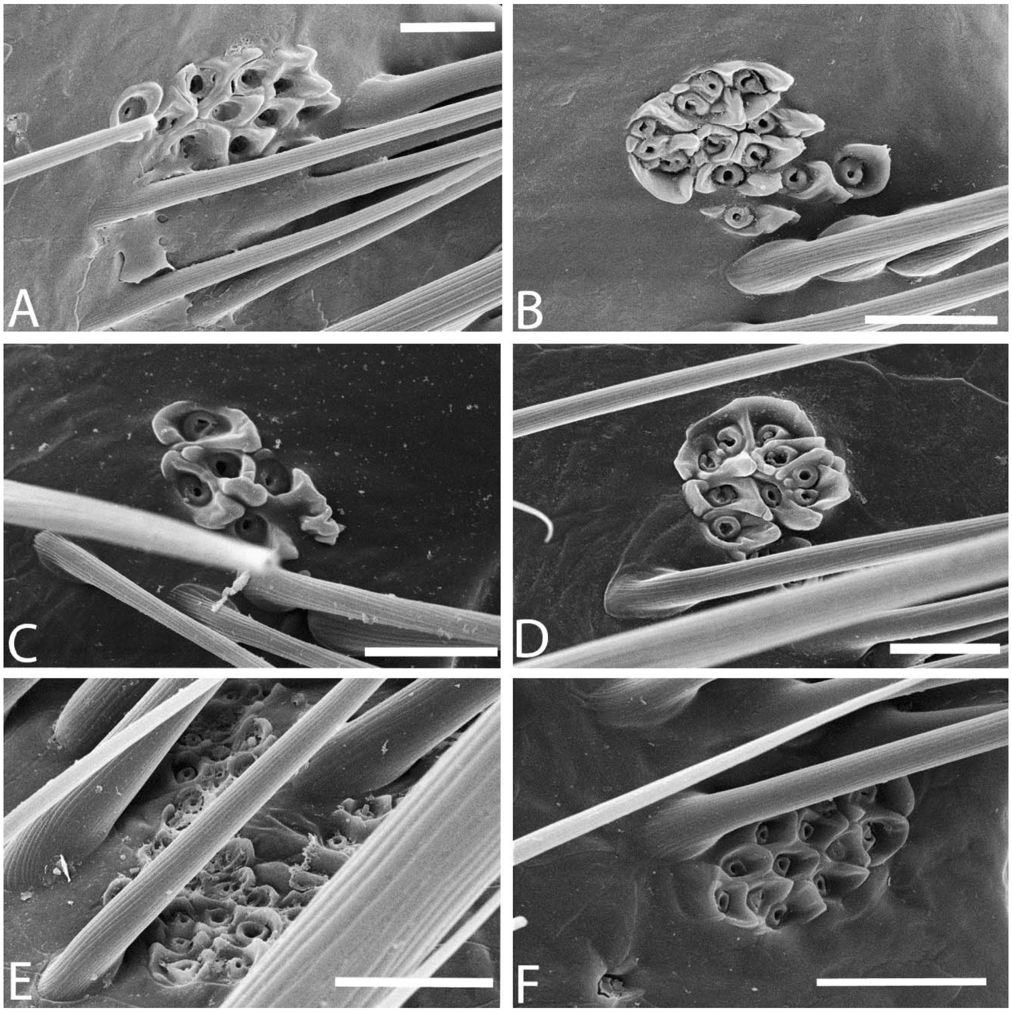

Figure 6.—Tarsal aggregated pores on leg IV of gonyleptoidean harvestmen. A) *Cynorta dentipes* (Cosmetidae), B) *Erginulus weyerensis* (Cosmetidae), C) *Holovonones compressus* (Cosmetidae), D) *Paecilaema inglei* (Cosmetidae), E) *Phareicranaus calcariferus* (Gonyleptidae, Cranainae), F) *Zygopachylus albomarginis* (Gonyleptidae, Manaosbiinae). Scale bar = 10 µm.

prolateral and retrolateral surfaces (Fig. 10E). The femora of *Erginulus subserialis* and *E. weyerensis* had large conical tubercles (Figs. 10F, 11A). There were also abundant, enlarged rounded tubercles on femur IV in male *Eupoecilaema magnum* (Fig. 11B). Femur IV of male *H. compressus* had several small tubercles along the retrolateral surface (Fig. 11 C). The femora of *P. chirquiense*, *P. inglei*, and *V. ornatus* were not sexually dimorphic and varied with respect to the number and size of the rounded tubercles present on the dorsal and lateral surfaces (Figs. 11D–F). With respect to leg IV tubercles on the other gonyleptoidean harvestmen, tubercle number and morphology is sexually dimorphic in *Glysterus* sp., *Phareicranaus calcariferus*, and *Stygnoplus clavotibialis*, but not in *Avima intermedia*, *Rhopalocranus albilineatus*, or *Zygopachylus ablomarginis* (Fig. 12).

We observed three major types of tubercles on leg IV segments (Table 3). Type I tubercles were low, rounded structures with relatively large sensilla chaetica inserting into sockets on the distal surfaces. When present, these tubercles were generally the most abundant type of tubercle on a leg segment. They were observed in nine out of 12 cosmetid species (Figs. 10, 11) and four of the other gonyleptoidean harvestmen (Fig. 12). Type II tubercles were larger, rounded asymmetric structures that either had a small subapical sensilla chaetica or did not have a seta associated with it (Figs. 10A, 11B). These tubercles were observed in multiple species. Type III tubercles were large, conical symmetrical structures with small, subapical setae (Figs. 10B, 10E, F, 11A) and were only observed in four cosmetid species (Table 3).

DISCUSSION

Our study of the cuticular structures occurring on leg IV of 18 gonyleptoidean species revealed relatively little interspecific variation in the general morphology of sensilla chaetica, trichomes and slit sensilla. These observations are consistent with similar descriptions of these structures in other morphological studies (e.g., Willemart et al. 2007). We observed variation in the relative number, size and distribution of these structures between leg segments and found interspecific variation in relative length or overall size of several structures (Table 1). However, we did not find any consistent differences

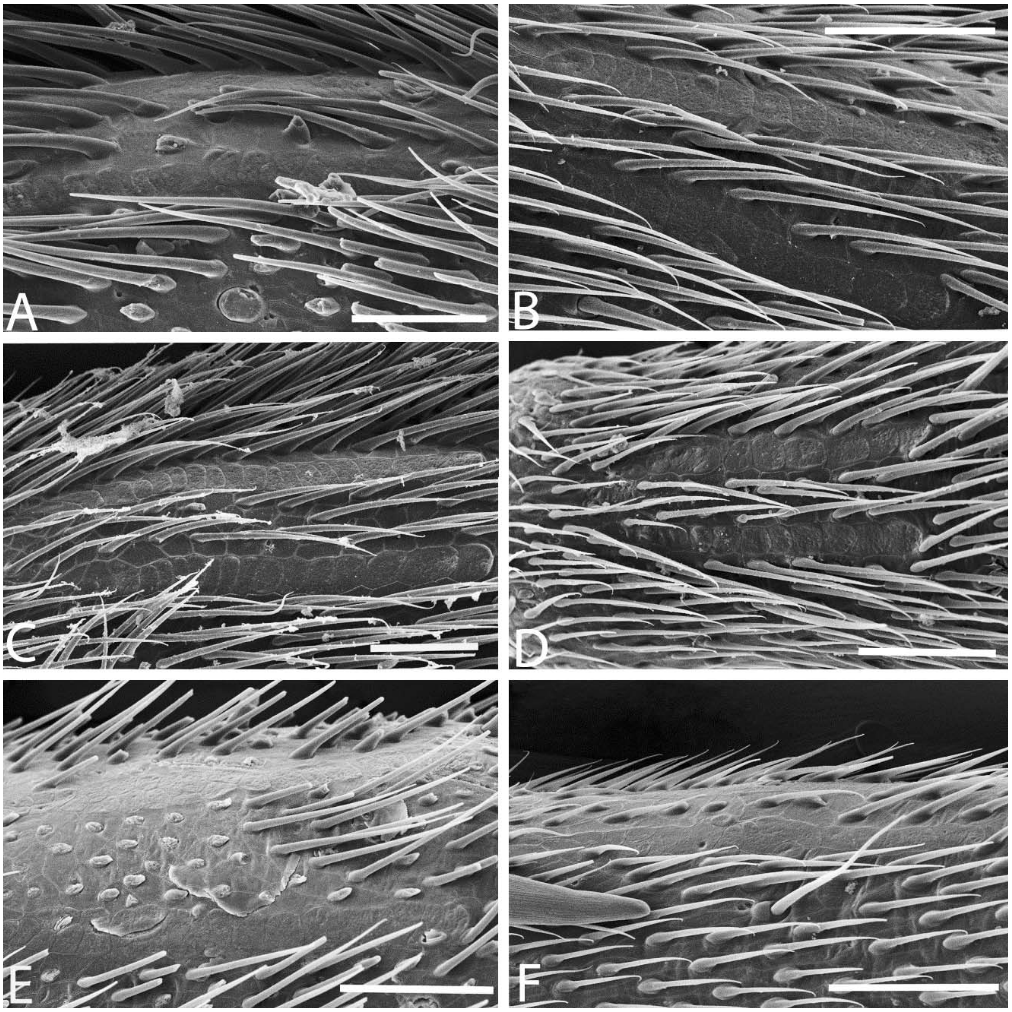

Figure 7.—Tarsal perforated organs on leg IV of gonyleptoidean harvestmen. A) *Cynorta blasi* (Cosmetidae), B) *Cynortula granulata* (Cosmetidae), C) *Erginulus clavotibialis* (Cosmetidae), D) *Holovonones compressus* (Cosmetidae), E) *Stygnoplus clavotibialis* (Stygnidae), F) *Zygopachylus albomarginis* (Gonyleptidae, Manaosbiinae). Scale bar = 50 μm.

between the three genera (*Cynorta* Koch 1839, *Erginulus* Roewer 1912 and *Paecialaema* Koch 1839) for which we were able to survey multiple species.

We observed considerable interspecific variation in the morphology of rough pit glands among cosmetid species and in comparison to taxa representing other gonyleptoidean families (Table 2). In cosmetid harvestmen, rough pit glands were relatively abundant and commonly occurred on the femur and patella, and in several species, on the tibia. They exhibited interspecific variation with respect to occurring on the surface of the cuticle and in the prominence of the pores (Table 2). Rodriguez et al. (2014) found rough pit glands to be common on the ocularium and anterior region of the dorsal scutum in these same species of cosmetid harvestmen, but absent from other gonyleptoidean harvestmen, with the notable exceptions of agoristenid and gonyleptid species. In our study, rough pit glands were generally absent from the legs of agoristenid harvestmen, but present on the legs of most gonyleptids (*Glysterus* sp. and the manaosbiine species). Willemart et al. (2007) reported rough pit glands from the trochanters of the gonyleptid harvestmen *Iporangaia pustulosa* Mello-Leitão 1939 and *Neosadocus* sp. The functional significance of rough pit glands has not been investigated (Willemart et al. 2007). The prominence of the pores associated with rough pit glands in several species (Table 2) indicates that these structures may be glandular (as hypothesized by Willemart et al. 2007) or could be chemosensory. An ultrastructural study of porous and non-porous rough pit glands is needed to resolve this issue. On the basis of their relatively high abundance on multiple leg segments (present study) and the dorsal scutum (especially the ocularium: Rodriguez et al. 2014), we hypothesize that rough pit glands may represent phylogenetically informative structures at the familial level. Within the Cosmetidae, the usefulness of rough pit glands (morphology or distribution) as characters for identifying genera or species is less clear. We did not observe any consistent differences in rough pit glands between species in the three genera (*Cynorta*, *Erginulus* and *Paecilaema*) for which we were able to examine multiple species (Table 2).

With respect to other cuticular structures on the legs, we observed variation in the number of plates comprising the

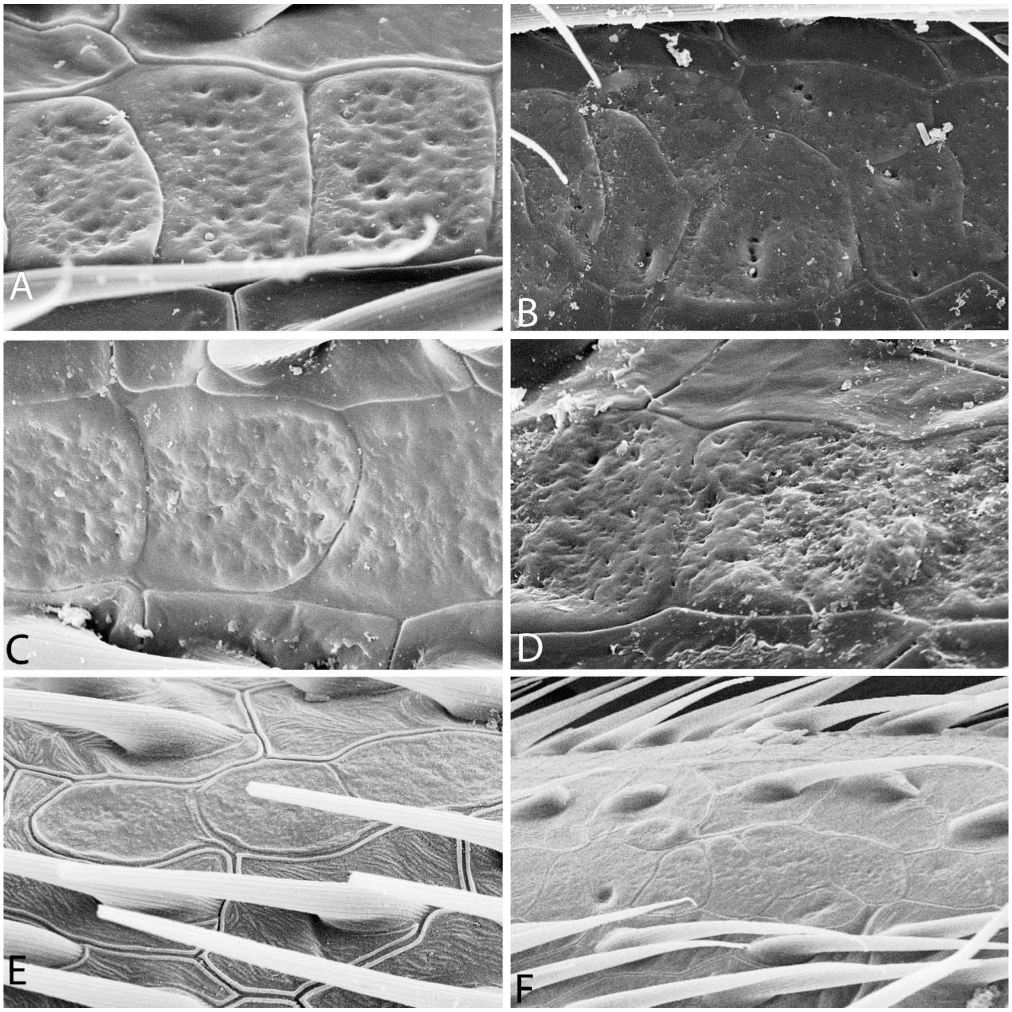

Figure 8.—Surface features of the tarsal perforated organs on leg IV of gonyleptoidean harvestmen. A) *Erginulus clavotibialis* (Cosmetidae), B) *Eupoecilaema magnum* (Cosmetidae), C) *Holovonones compressus* (Cosmetidae), D) *Vonones ornatus*, E) *Avima intermedia* (Agoristenidae), F) *Zygopachylus albomarginis* (Gonyleptidae, Manaosbiinae). Scale bar = 5 μm.

tarsal perforated organ. Willemart et al. (2007) first described the tarsal perforated organ as glandular structures composed of 10–20 plates and observed them occurring on the tarsi of all four legs of two gonyleptid species. Proud & Felgenhauer (2013b) examined the ultrastructure of the tarsal perforated organ in several gonyleptoidean harvestmen including cosmetid species and found no evidence to support either a glandular or a sensory function. Proud & Felgenhauer (2013b) hypothesized that the tarsal perforated organ likely aids in the molting process by providing a large attachment site for tendinous cells which maintain a connection with the old cuticle during the premolt stage. They hypothesized that it is the one place on the tarsus that serves as a final anchoring site immediately prior to exuviation. Our observations revealed interspecific variation in the number of plates comprising the tarsal perforated organ (Table 1) but we did not observe any pores or glandular products in association with individual plates, and thus our observations are consistent with the functional hypothesis proposed by Proud & Felgenhauer (2013b).

With respect to tubercles, we observed considerable interspecific variation in the number, size and shape of tubercles (Table 3). In addition, we discovered significant differences in their microanatomy including variation in the shape as well as the relative position of associated setae (sensilla chaetica). We noted three major classes of tubercles (types I, II and III). While we observed types I and II on leg segments of cosmetid and other gonyleptoidean harvestmen, we observed type III tubercles only on the leg segments of four cosmetid species (*Cynorta dentipes*, *Erginulus* spp.). Although we only examined 12 species of cosmetid harvestmen, we believe that tubercle type and distribution could provide useful characters for differentiating genera and identifying species.

The results of our study indicate that there is considerable interspecific variation in the cuticular structures associated with the cosmetid leg. The inclusion of characters based upon appendicular morphology along with others derived from SEM-based examinations (Rodriguez et al. 2014, Fernandes & Willemart 2014) should be considered in future systematic revisions of cosmetid harvestmen. DaSilva & Gnaspini (2009)

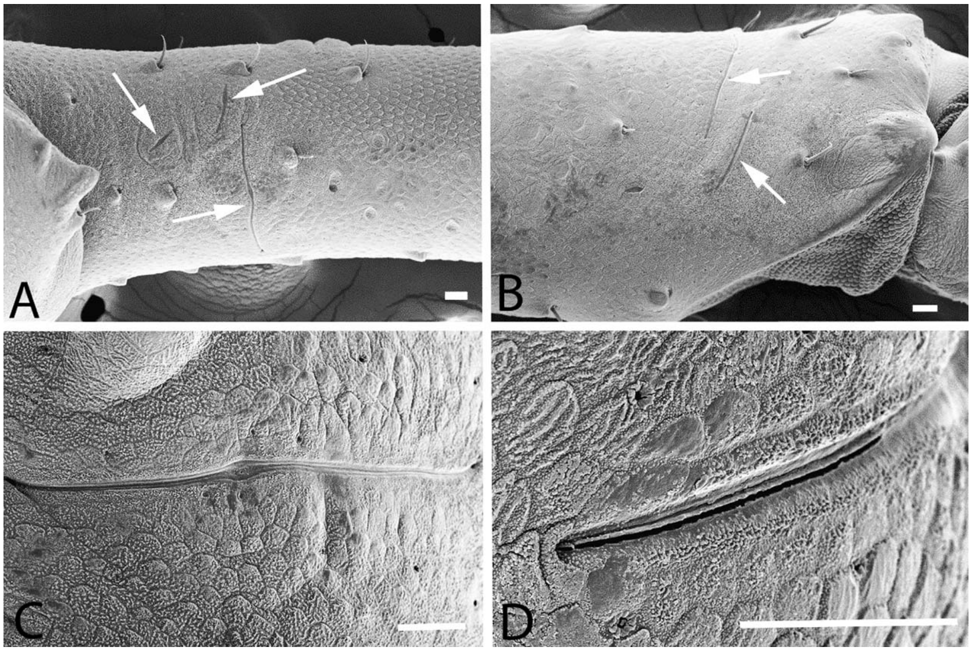

Figure 9.—Slit sensilla (arrows) on leg IV of the cosmetid harvestman *Cynorta dentipes*. A) low magnification, proximal femur, B) distal femur, C) high magnification, femur, D) metatarsus. Scale bar = 30 μm.

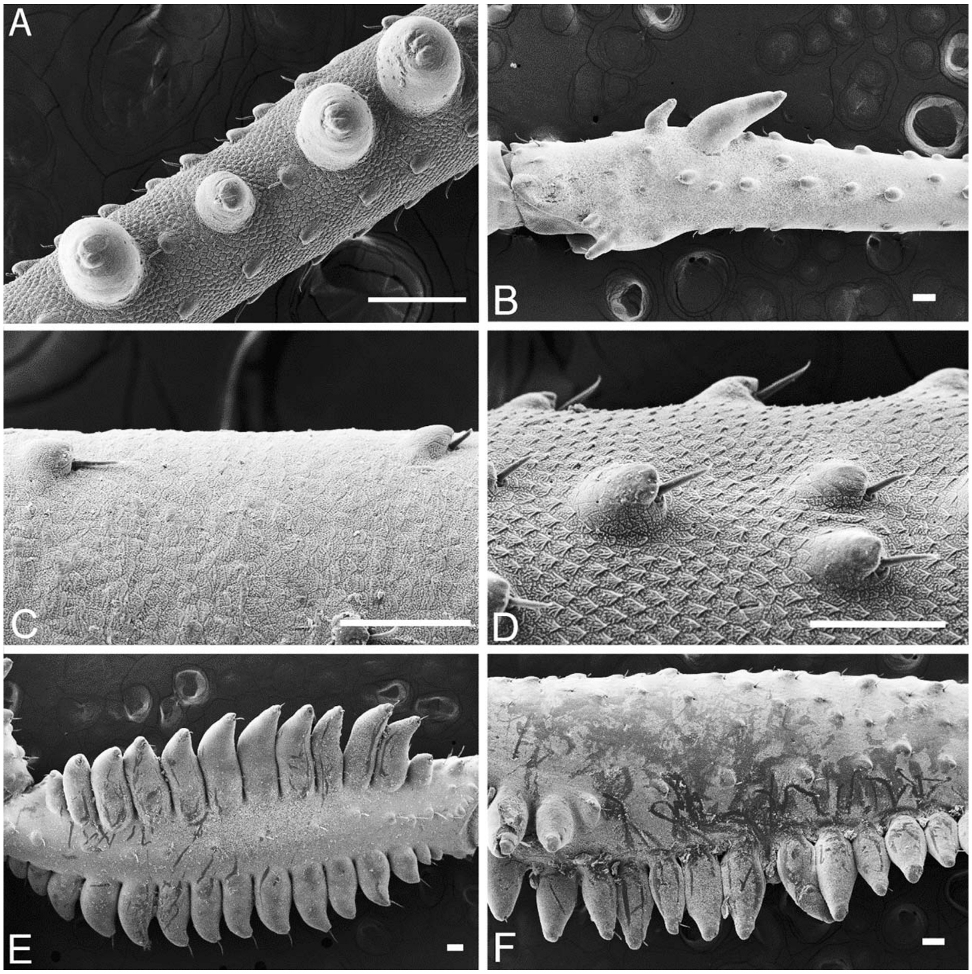

Figure 10.—Armature of leg IV of male cosmetid harvestmen. A) Metatarsus of *Cynorta blasi*, B) distal femur of *C. dentipes*, C) femur of *C. marginalis*, D) femur of *Cynortula granulata*, E) tibia of *Erginulus clavotibialis*, F) femur of *E. subserialis*. Scale bar = 100 μm.

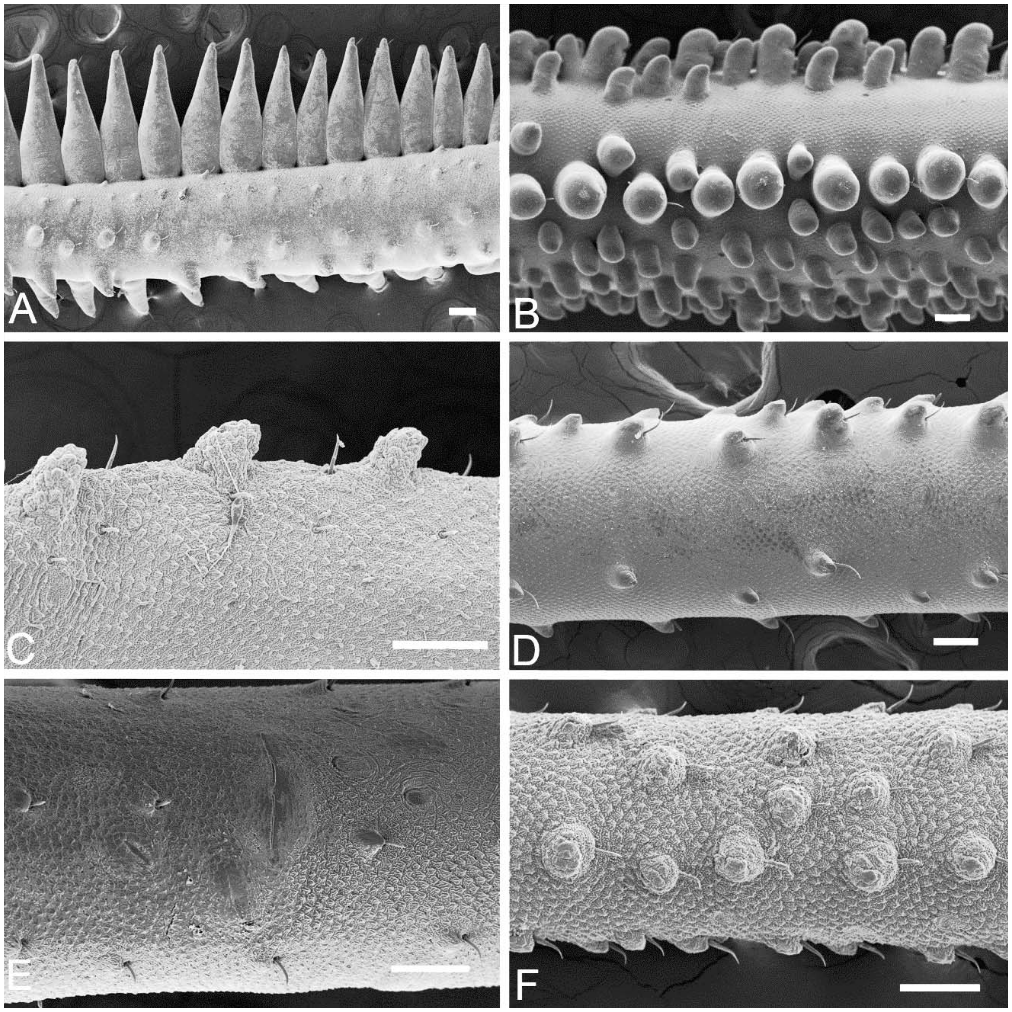

Figure 11.—Armature of leg IV of male cosmetid harvestmen. A) Femur of *Erginulus weyerensis*, B) distal femur of *Eupoecilaema magnum,* C) femur of *Holovonones compressus,* D) femur of *Paecilaema chiriquiense,* E) femur of *P. inglei,* F) femur of *Vonones ornatus*. Scale bar = 100 µm.

identified several informative characters based upon comparisons of the density, distribution and relative size of tubercles on the legs of gonyleptid harvestmen, but did not consider interspecific variation in microanatomy. Gainett et al. (2014) identified three discrete appendicular characters that were useful in refining relationships at the superfamilial level among laniatorean harvestmen. These structures included metatarsal paired slit sensilla, proximal tarsomeric gland, and tarsal aggregated pores. The results of our study indicate that rough pit glands and tubercle morphology are important features of the cosmetid surface anatomy and could represent useful characters for understanding phylogenetic relationships. In addition, future studies of the ultrastructure of cuticular structures that are common and abundant in cosmetid harvestmen (e.g., rough pit glands and tubercles) should provide major insights into the sensory ecology and natural history of these poorly known, yet amazingly diverse, harvestmen.

ACKNOWLEDGMENTS

We thank M. Schaus and J. Illinik for assistance with collecting harvestmen in Belize; S. Broadbridge, M. Moore, J. Toraya, and D. Proud for help with field work in Trinidad; C. Víquez, P. Van Zandt, and D. Proud for aid in sampling in Costa Rica. We also thank J. Akin for collecting specimens in Louisiana. We are grateful to B. Damron, R. Miranda, and F. Cupul for loans of specimens collected in Honduras, Panama and Mexico, respectively. We are grateful to D. Proud for critically reviewing an earlier draft of this manuscript. This research was supported by VWC Summer Faculty Development Grants (VRT) in 2006, 2008 and 2010, the VWC Scanning Electron Microscopy Laboratory (acquired through NSF grant DBI-1125927), and the VWC Science Undergraduate Research Fund (ALR).

Specimens were legally collected and exported under the following permits 001284 (2006), 001131 (2007), and 001339 (2008) from the Forestry Division of the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago; permit #CD/60/3/12 (1) (2012) from Forest Department of the Ministry of Natural Resources and the Environment, Belize; and scientific passport no. 01607 (2008, 2010) from the Ministerio del Ambiente, Energia y Telecomunicaciones, Costa Rica.

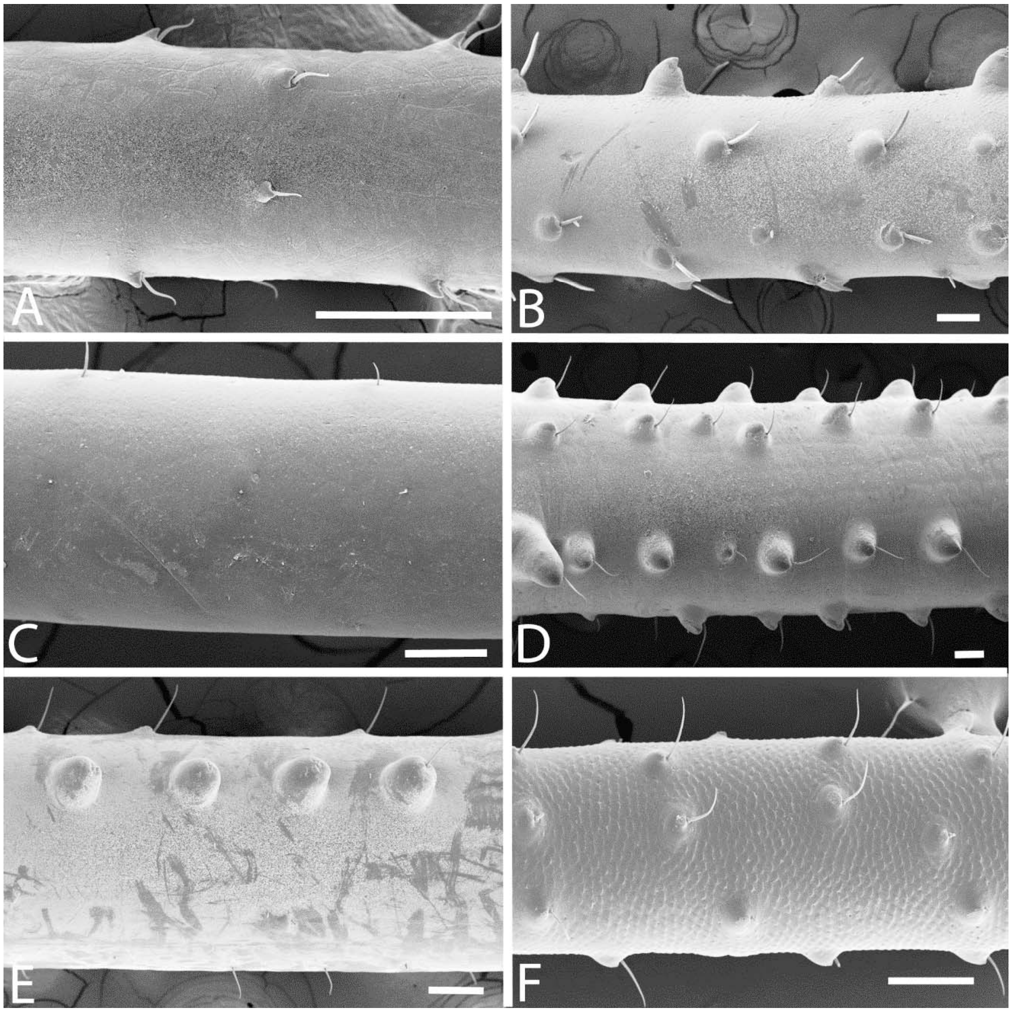

Figure 12.—Armature—of leg IV of male gonyleptoidean harvestmen. A) *Avima intermedia* (Agoristenidae), B) *Glysterus* sp. (Gonyleptidae, Ampycinae), C) *Rhopalocranaus albilineatus* (Gonyleptidae, Manaosbiinae), D) *Phareicranaus calcariferus* (Gonyleptidae, Cranainae), E) *Stygnoplus clavotibialis* (Stygnidae), F) *Zygopachylus albomarginis* (Gonyleptidae, Manaosbiinae). Scale bar = 100 μm.

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Manuscript received 8 December 2014, revised 22 April 2015.