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Ontogenetic differences and interspecific variation in the tarsal aggregate pores on leg IV of cosmetid harvestmen (Opiliones: Laniatores)

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Abstract. Morphological studies of harvestmen usually investigate the structures of adults, seldom including data regarding ontogenetic differences in the microanatomy of the appendages. In gonyleptoidean harvestmen, adults have clusters of pores on the distal-most tarsomeres of legs III and IV that are known as tarsal aggregate pores (TAPs). In gonyleptid harvestmen, these pores occur on the prolateral and retrolateral surfaces as groups adjacent to the tarsal claws. In this study, we used scanning electron microscopy (SEM) to compare the microanatomy of the TAPs occurring on leg IV of adults and antepenultimate nymphs for three common species of Neotropical cosmetid harvestmen, nymphs of an additional seven cosmetid morphospecies, and adults and antepenultimate nymphs of three species of gonyleptoidean harvestmen. The distal tarsomere of leg IV of adult cosmetid harvestmen features two pairs of aggregate pores including dorsal TAPs and ventrolateral vTAPs. The TAPs of cosmetid nymphs have denticulate borders and are not closely associated with trichomes. We observed interspecific variation among cosmetid harvestmen with respect to the number of pores composing the TAPs. The TAPs on the prolateral and retrolateral surfaces occur in similar positions and are generally symmetrical with respect to overall shape and the number of pores. The functional significance of the TAPs and vTAPs of cosmetid harvestmen will require additional empirical evaluation.

Keywords: Glands, Gonyleptoidea, microanatomy, nymph, setae

With more than 700 described species (Medrano & Kury 2016), the Cosmetidae Koch, 1839 represents the third largest family in the order Opiliones, and the second most diverse group in the suborder Laniatores and superfamily Gonyleptoidea (Sharma & Giribet 2011; Pinto-da-Rocha et al. 2012; Giribet & Sharma 2014). Species of cosmetid harvestmen are found from the southern U.S. to Argentina, with the greatest diversity occurring in the forested habitats of Mexico, Central America and northern South America (Kury & Pinto-da-Rocha 2007). The adults of most species are easily differentiated from other gonyleptoideans by the morphology of the pedipalps, which feature flattened femora and patellae and spoon-shaped tibiae (Kury & Pinto-da-Rocha 2007; Pinto-da-Rocha & Hara 2011). In nymphs, these same podomeres are

Table 1.—Comparison of the TAPs and vTAPs of antepenultimate nymphs and adults of gonyleptoidean harvestmen examined in the present study. CR = Costa Rica

	TAP		vTAP	
Species	No. Pores	Type of Border	No. Pores	Type of Border
Cosmetidae				
Cynortula granulata, nymph	7–9	Denticulate	Absent	n/a
Cynortula granulata, adult	8–11	Smooth	4–5	Smooth
Erginulus clavotibialis, nymph	10–13	Denticulate	Absent	n/a
Erginulus clavotibialis, adult	13–15	Smooth	13–14	Smooth
Paecilaema inglei, nymph	7–9	Denticulate	Absent	n/a
Paecilaema inglei, adult	9–11	Smooth	3–4	Smooth
Morphospecies 1 (Belize), nymph	8–9	Denticulate	Absent	n/a
Morphospecies 2 (Belize), nymph	8–10	Denticulate	Absent	n/a
Morphospecies 3 (Belize), nymph	6–8	Smooth	Absent	n/a
Morphospecies 4 (CR), nymph	7–9	Denticulate	Absent	n/a
Morphospecies 5 (CR), nymph	6–9	Smooth	Absent	n/a
Morphospecies 6 (CR), nymph	6–9	Denticulate	Absent	n/a
Morphospecies 7 (CR), nymph	9–12	Smooth	Absent	n/a
Ampycidae				
Glysterus sp., nymph	14–16	Smooth	Absent	n/a
Glysterus sp., adult	13–16	Smooth	3–4	Smooth
Manaosbiidae				
Cranellus montgomeryi, nymph	3–5	Smooth	Absent	n/a
Cranellus montgomeryi, adult	6–9	Smooth	3–4	Smooth
Stygnidae				
Stygnoplus clavotibialis, nymph	10–14	Denticulate	Absent	n/a
Stygnoplus clavotibialis, adult	6–8	Smooth	14–15	Smooth

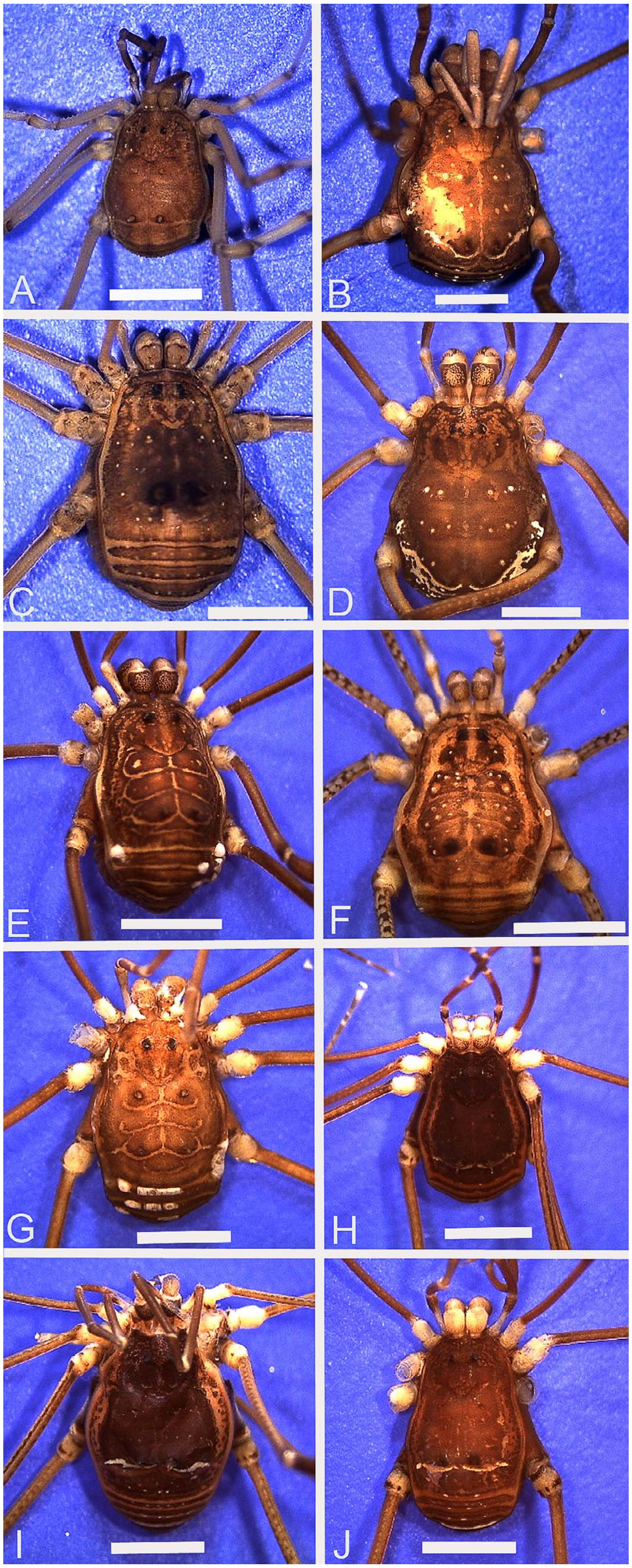

Figure 1.—Light micrographs of the dorsal habitus of cosmetid nymphs (antepenultimates) including (A) *Cynortula granulata*, (B) *Erginulus clavotibialis*, (C) *Paecilaema inglei*, (D–F) morphospecies 1–3 from Bladen Reserve, Belize, and (G-J) morphospecies 4–7 from La Selva Biological Station, Costa Rica. Scale bar = 2 mm.

cylindrical, rather than laterally compressed (Juberthie 1972; Goodnight & Goodnight 1976). The tarsus of the pedipalp of the adult is adorned with a flexible, sclerotized claw, whereas that of the nymph has a slender, elongate pretarsus with a ventral patch of setae (Wolff et al. 2016).

The life history of harvestmen includes three distinct postembryonic stages: larva, nymph (multiple instars) and adult (reviewed by Gnaspini 2007). Although postembryonic development of relatively few species have been studied, cosmetid harvestmen have been observed to have six nymphal instars before the final molt to adulthood (Juberthie 1972; Goodnight & Goodnight 1976). Different ages of nymphs may be distinguished on the basis of body size, the morphology of the pedipalps, and the presence of an arolium (sac-like structure) and pseudonychium (small cuticular process ventral to the arolium) on tarsus III and IV (Muñoz Cuevas 1971). In gonyleptoidean harvestmen, the secondary sexual characteristics of males begin to appear during the antepenultimate nymph (Muñoz Cuevas 1971; Gnaspini 1995; Townsend et al. 2009). The penultimate nymph is distinguished by the absence of the pseudonychium and arolium and the genitalia are not fully formed and functional (Muñoz Cuevas 1971). Temperature affects the rate of growth and it appears to take 4–7 months from hatching for individuals to reach the terminal molt to adulthood (Juberthie 1972; Goodnight & Goodnight 1976; Cokendolpher & Jones 1991). Under captive conditions, adult cosmetid harvestmen have been observed to live 2–3 years (Juberthie 1972; Cokendolpher & Jones 1991).

Relatively little is known about the morphological changes that occur during postembryonic development in cosmetid harvestmen. Like other gonyleptoideans, adult cosmetid harvestmen have clusters of pores with distinct, often sculptured, borders on the distal tarsal segments of legs III and IV that are known as tarsal aggregate pores, or TAPs (Gainett et al. 2014; Rodriguez & Townsend 2015). The TAPs occur as pairs on the dorso-lateral region proximal to the tarsal process, with one cluster on the retrolateral surface and another on the prolateral surface (Willemart et al. 2007, 2009; Ramin et al. 2016). These groups of pores occur in close association with the bases of multiple trichomes (Gainett et al. 2014; Rodriguez & Townsend 2015; Ramin et al. 2016). The TAPs appear to have consistent locations on the surfaces of the distal most tarsomeres of legs III–IV, occurring in the areas bounded by three dorsal setae, termed the S0-S1-S3 triangle (Gainett et al. 2014; Ramin et al. 2016).

In gonyleptoidean harvestmen, the morphology of the TAPs is very similar between tarsi III and IV and does not differ markedly between the prolateral and retrolateral surfaces (Gainett et al. 2014; Ramin et al. 2016). The TAPs of adults are believed to be glandular (Willemart et al. 2007; Gainett et al. 2014; Ramin et al. 2016). The biological role of TAPs has been hypothesized to be that of marking the substrate, with the chemical secretions serving as an aid in navigation (Willemart et al. 2007).

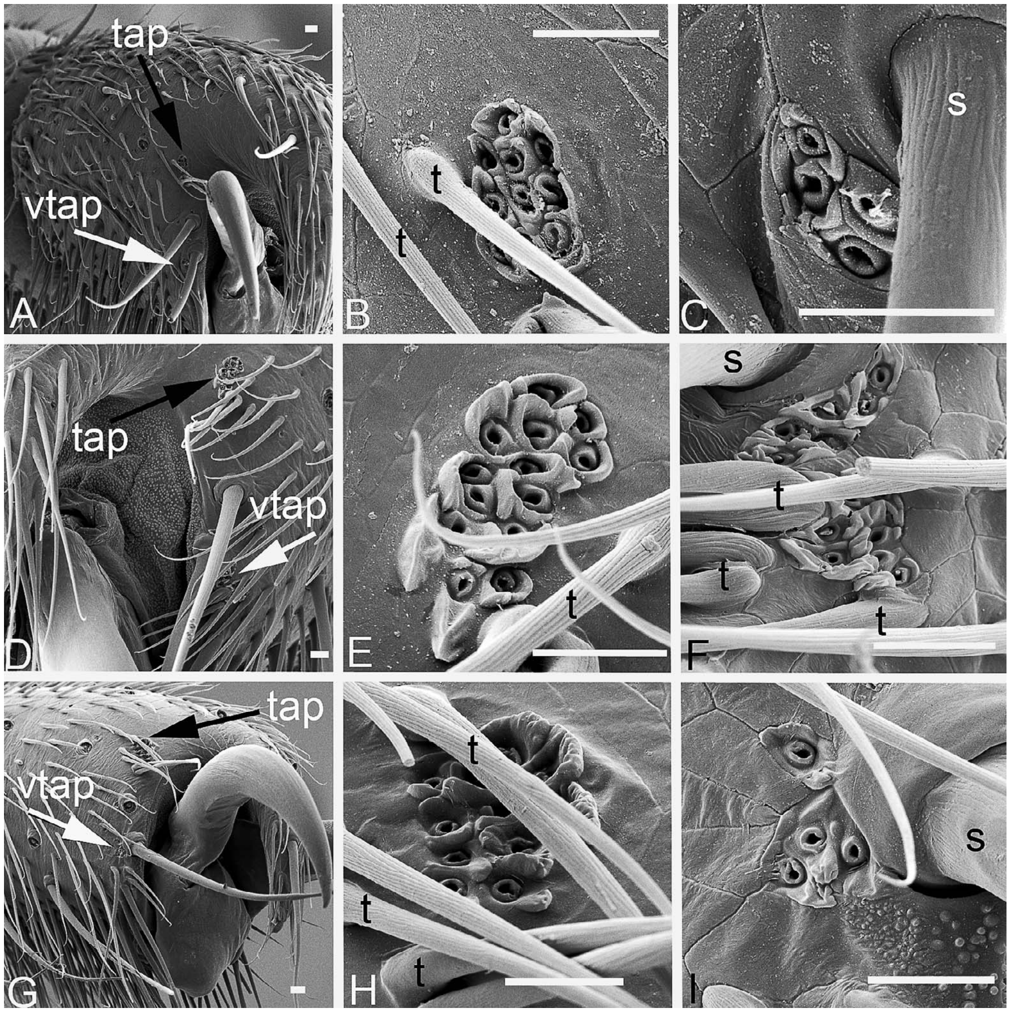

Figure 2.—SEM micrographs of the tarsal aggregate pores (TAPs) and ventral tarsal aggregate pores (vTAPs) on the distal segment of tarsus IV of adult cosmetid harvestmen. (A–C) *Cynortula granulata*: (A) tip of tarsus IV, frontal lateral view. (B) Morphology of the TAP. (C) Morphology of the vTAP. (D–F) *Erginulus clavotibialis*: (D) tip of tarsus IV, frontal view. (E) Morphology of the TAP. (F) Morphology of the vTAP. (G–I) *Paecilaema inglei*: (G) tip of tarsus, lateral view. (H) morphology of the TAP. (I) morphology of the vTAP (I). Scale bar = 10 μm. s = sensillum chaeticum; t = trichome, tc = tarsal claw.

While no sexual dimorphism has been observed in the microanatomy of the TAPs (Gainett et al. 2014; Ramin et al. 2016), ontogenetic variation in the distribution and composition of the TAPs has recently been reported for the gonyleptid harvestman *Heteromitobates albiscriptus* (Mello-Leitão, 1932) (Ramin et al. 2016). In this species, TAPs are absent from the tarsi of the first nymph. Beginning in the second nymph, the numbers of pores that constitute the TAPs increase with age (Ramin et al. 2016). Adult *H. albiscriptus* also have two distinct types of TAPs, one pair that are dorsal to the tarsal claws and another pair that are lateral to the tarsal claws and are known as vTAPs (Ramin et al. 2016). Like the more dorsal TAPs, the vTAPs are associated with trichomes and occur in a consistent location, inferior to the dorsal seta known as S2 (Ramin et al. 2016). The vTAPs have only been observed on tarsi III and IV of adults and are not known to occur on the tarsi of nymphs (Ramin et al. 2016). In adult *H. albiscriptus*, there are usually fewer pores associated with the vTAP than with the more dorsal TAP (Ramin et al. 2016). The occurrence of vTAPs in other gonyleptoidean taxa, including the Cosmetidae, has not been investigated.

In this study, we used scanning electron microscopy (SEM) to examine ontogenetic differences in TAPs among cosmetid harvestmen by comparing the morphology of these clusters on tarsi IV of antepenultimate nymphs with those of adults. Specifically, we compared the tarsal morphology of adults and nymphs for the cosmetid harvestmen *Cynortula granulata* Roewer 1912, *Erginulus clavotibialis* (Pickard-Cambridge, 1905) and *Paecilaema inglei* Goodnight & Goodnight, 1947 and those of adults and nymphs for *Cranellus montgomeryi* Goodnight & Goodnight, 1947 (Manaosbiidae), *Glysterus* sp. (Ampycidae) and *Stygnoplus clavotibialis* (Goodnight & Goodnight, 1947) (Stygnidae). For comparative purposes, we also surveyed the morphology of the TAPs on tarsi IV for seven morphospecies of cosmetid nymphs collected from field sites in Belize and Costa Rica.

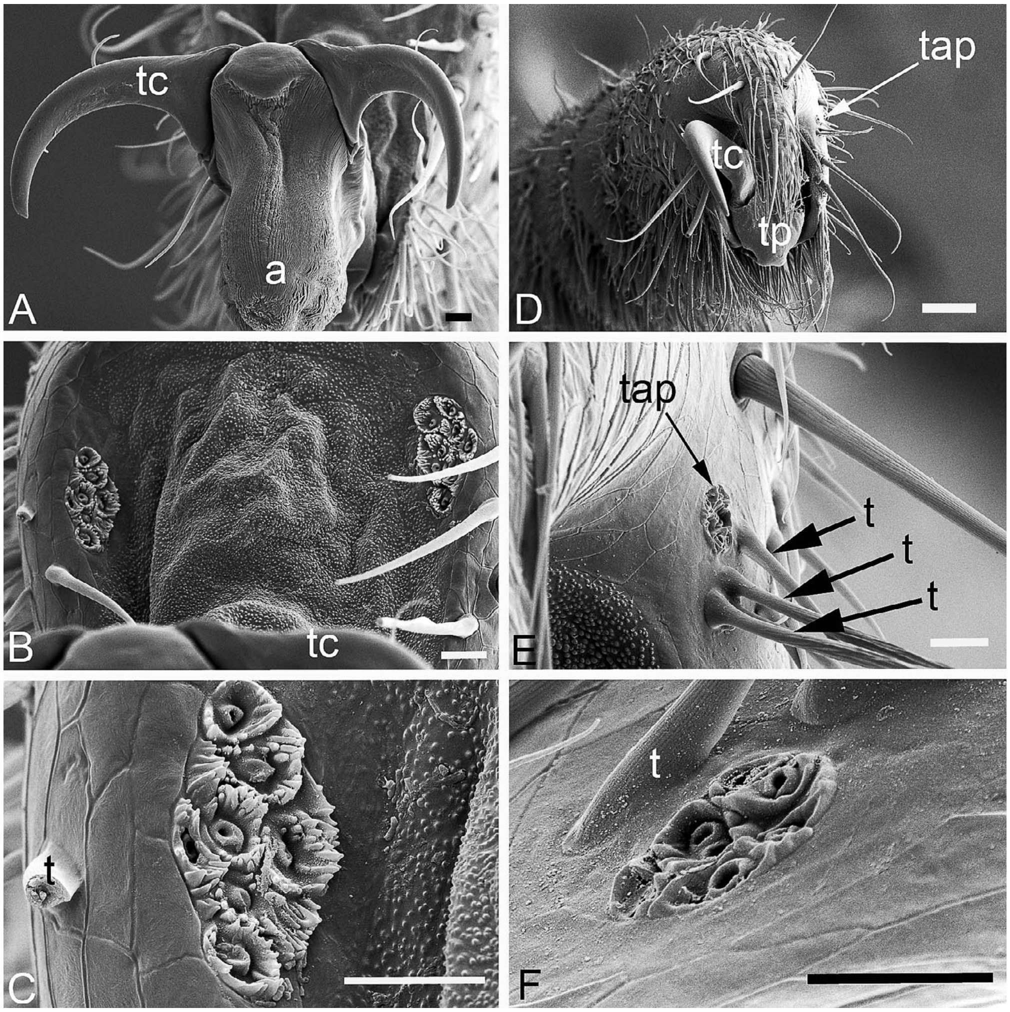

Figure 3.—SEM micrographs of tarsal aggregate pores (TAPs) on tarsus IV of penultimate nymph (A–C) and adult (D–F) *Cynortula granulata* (Cosmetidae). (A) Arolium and tarsal claws, frontal view. (B) Slightly asymmetrical TAPs (shape only) on prolateral and retrolateral surfaces. (C) Surface features of TAP; note sculpturing of borders separating pores. (D) Tarsus with broken claw, tarsal process and TAP, frontal view. (E) TAP with adjacent trichomes. (F) Surface features of TAP; note the reduced sculpturing on the borders surrounding each of pores. Scale bars = 60 μm for D; 10 μm for A–C, E; 5 μm for F. a = arolium, t = trichome, tc = tarsal claws, tp = tarsal process.

METHODS

Owing to ontogenetic variation in the coloration of the body and legs, armature of the dorsal scutum, pedipalps and legs, tarsal morphology and genitalia (present only in adults), it can be challenging to identify to genus or species, the nymphs of laniatorean harvestmen (Townsend et al. 2009), especially from samples collected in Neotropical forests with considerable biodiversity (e.g., the forests at the La Selva Biological Station in Costa Rica are home to 19 species of cosmetid harvestmen: Proud et al. 2012). In this study, we examined the microanatomy of tarsus IV for multiple nymphs (n = 3–6 individuals) and adults (n = 2–3 males and females) of *Cynortula granulata*, *Erginulus clavotibialis*, and *Paecilaema inglei*. Adults and nymphs of these species were previously collected for examination in other studies (Townsend et al. 2008; Walker & Townsend 2014; Townsend et al. 2017). The specimens of *C. granulata* and *P. inglei* were captured by hand in 2005–2007 from forested habitats in the northern and central ranges of Trinidad, West Indies (see Townsend et al. 2008 for specific locations). Adults and nymphs of *Erginulus clavotibialis* were collected from beneath logs and rocks at Clarissa Falls, Cayo District, Belize (17.116° N, 89.120° W; datum: WGS84) in 2012 (Schaus et al. 2013; Townsend et al. 2017). In addition, we examined tarsus IV for multiple individuals (n = 2–5 specimens) of three morphospecies of cosmetid nymphs (Fig. 1D–F) collected by hand from the leaf litter Bladen Reserve, Toledo District, Belize (16°29′60″ N, 88°53′9.6″ W, datum WGS84) in July 2012, and four morphospecies of cosmetid nymphs (Fig. 1G–J) that were captured from leaf litter and vegetation at La Selva Biological Station, Heredia Province, Costa Rica (10°27′20″ N, 84°0′20″ W, datum: WGS84) from 11–24 August 2015. These nymphs were similar in body size to the three known cosmetid species (Fig. 1A–C), but differed from them and each other with respect to dorsal coloration and body shape (Fig. 1D–J). We believe that they represent different species, rather than different instars of nymphs for a single species. However, in

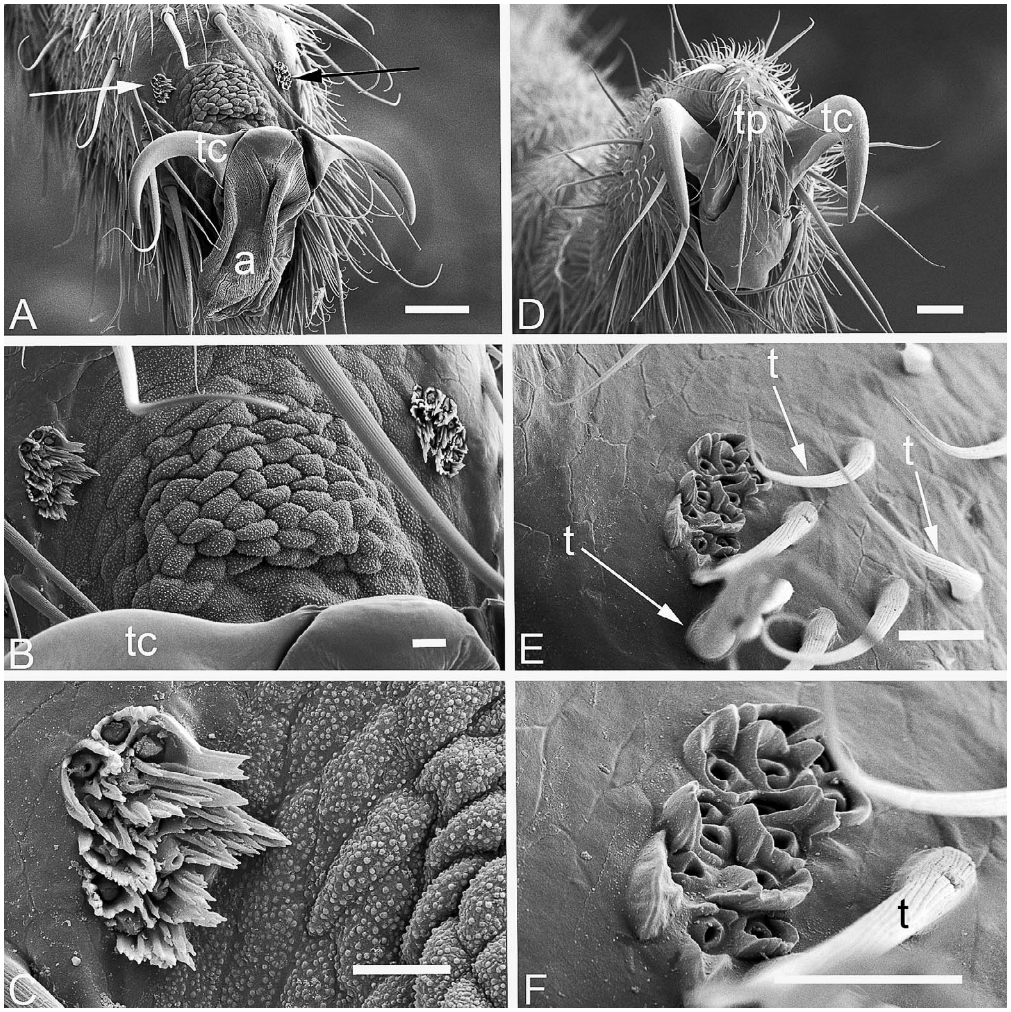

Figure 4.—SEM micrographs of tarsal aggregate pores (TAPs) on tarsus IV of antepenultimate nymph (A–C) and adult (D–F) *Erginulus clavotibialis* (Cosmetidae). (A) Arolium, tarsal claws and TAP (arrows), dorso-frontal view. (B) Asymmetrical TAPs on prolateral and retrolateral surfaces. (C) Surface features of TAP; note morphology of the borders of the pores. (D) Tarsal claws and tarsal process, frontal view. (E) TAP with adjacent trichomes. (F) Surface features of TAP; note increased number of pores and reduction in the size of the borders surrounding the pores. Scale bars = 60 μm for A, D; 15 μm for D–E; 10 μm for C, F. a = arolium, t = trichome, tc = tarsal claws, tp = tarsal process.

the absence of adult characters, we were unable to identify them to species or genus.

Specimens of *Glysterus* sp. were captured by hand from cover objects and from the leaf litter at the La Selva Biological Station, Heredia Province, Costa Rica (10°27′20″ N, 84°0′20″ W, datum: WGS84) in July 2010. Adults and nymphs of *Cranellus montgomeryi* and *Stygnoplus clavotibialis* were collected from Trinidad, West Indies in 2008 (data for specific locations is provided in Rodriguez et al. 2014).

On the basis of their relative body size, dorsal coloration, and the presence of a prominent arolium and pseudonychium on tarsus III and IV (Muñoz Cuevas 1971), we inferred that the cosmetid nymphs that we examined were likely antepenultimate, 5th instars (Fig. 1). Similarly, tarsus III and IV of the nymphs of the non-cosmetid taxa each had a prominent arolium and a well-developed pseudonychium. The degree of their dorsal coloration and relative body size were consistent with 4th or 5th instar nymphs (assuming these species have six nymphal instars, as there are no published postembryonic studies for these taxa). Prior to the removal of leg IV, we photographed nymphs and adults with a Leica EZ4 D stereomicroscope. These images were subsequently processed with Leica Application Suite (LAS) software, version 3.2.1 and Adobe Photoshop CS4 extended software, version 11.0.2.

The right and left tarsi of leg IV of each harvestman were examined by SEM following the process described in Townsend et al. (2009) and Rodriguez et al. (2014). Each leg was removed and ultrasonicated for 1–2 min prior to dehydration. The legs were chemically dried with hexamethyldisilazane (Nation 1983). We dissected each leg at the metatarsus-tibia joint and mounted the distal segments vertically on aluminum stubs with the distal tips of the tarsi visible. The specimens were sputter-coated with 15–30 nm of gold and photographed at accelerating voltages of 5–15 kV with the Hitachi S-3400N SEM on the campus of Virginia Wesleyan College. Unless noted in the figure legend, all SEM micrographs depict the

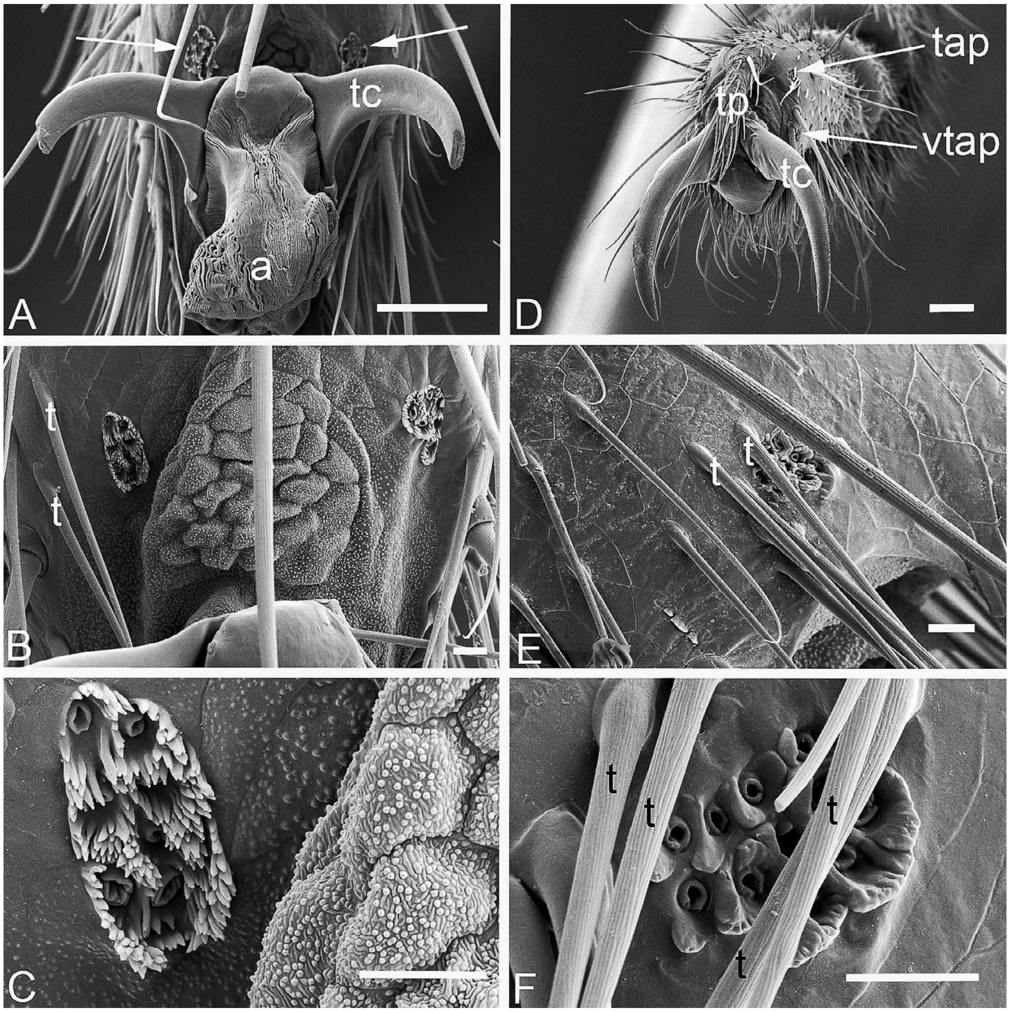

Figure 5.—SEM micrographs of tarsal aggregate pores (TAPs) on tarsus IV of antepenultimate nymph (A–C) and adult (D–F) *Paecilaema inglei* (Cosmetidae). (A) Arolium, tarsal claws and TAPs (arrows), frontal view. (B) Asymmetrical TAPs on prolateral and retrolateral surfaces and their proximity to trichomes. (C) Surface features of TAP; note the texture of the borders of the pores. (D) Tarsal claws, tarsal process, TAP and vTap, frontal view. (E) TAP with adjacent trichomes. (F) Surface features of TAP, note increase in the number of pores and change in the texture of the borders surrounding the pores. Scale bars = 60 µm for A, D; 10 µm for B–C, E–F. a = arolium, t = trichome, tc = tarsal claws, tp = tarsal process.

morphology of the right tarsus. Following the methods of Ramin et al. (2016), we attempted to provide reasonable estimates regarding the numbers of visible pores that comprise the TAPs (and vTAPs) when possible. Owing to the presence of trichomes and sensilla chaetica, we were not always able to view the complete surface of the TAPs, thus our meristic data may slightly under represent the actual number of pores present (Table 1).

Voucher specimens will be deposited into the collections of the American Museum of Natural History (AMNH) and the Universidad de Costa Rica.

RESULTS

TAPs and vTAPs among cosmetid harvestmen.—Adult cosmetid harvestmen have paired TAPs and vTAPs on the distal tarsomere of leg IV (Fig. 2). In *Cynortula granulata* and *Paecialema inglei*, there was a considerable difference in the number of pores between the TAPs and vTAPs (Table 1). In *Erginulus clavotibialis*, we observed a similar number of pores (13–15) present in both clusters. The TAPs of *Cynortula granulata* (Fig. 2A–C) had 8–11 pores (Fig. 2B) and vTAPs had 4–5 pores (Fig. 2C). The TAPs of *Erginulus clavotibialis* (Fig. 2D-F) featured 13–15 pores (Fig. 2E) and the vTAPs had 13–14 pores (Fig. 2F). In *Paecilaema inglei* (Fig. 2G–I), the TAPs were composed of 9–11 pores (Fig. 2H), and there were 3–4 pores associated with vTAPs (Fig. 2I). We observed no striking differences between retrolateral and prolateral clusters with respect to the number of pores constituting the TAPs and vTAPs (Figs. 4B, 5B, 6B). Similarly, we did not observe intersexual variation in the distribution or morphology of the TAPs or vTAPs in the three cosmetid species that we examined.

Ontogenetic variation in TAPs and vTAPS among cosmetid harvestmen.—Tarsus IV of the antepenultimate cosmetid nymph has an arolium (Figs. 3A, 4A, 5A) and pseudonychium between the tarsal claws, whereas tarsus IV of the adult has a

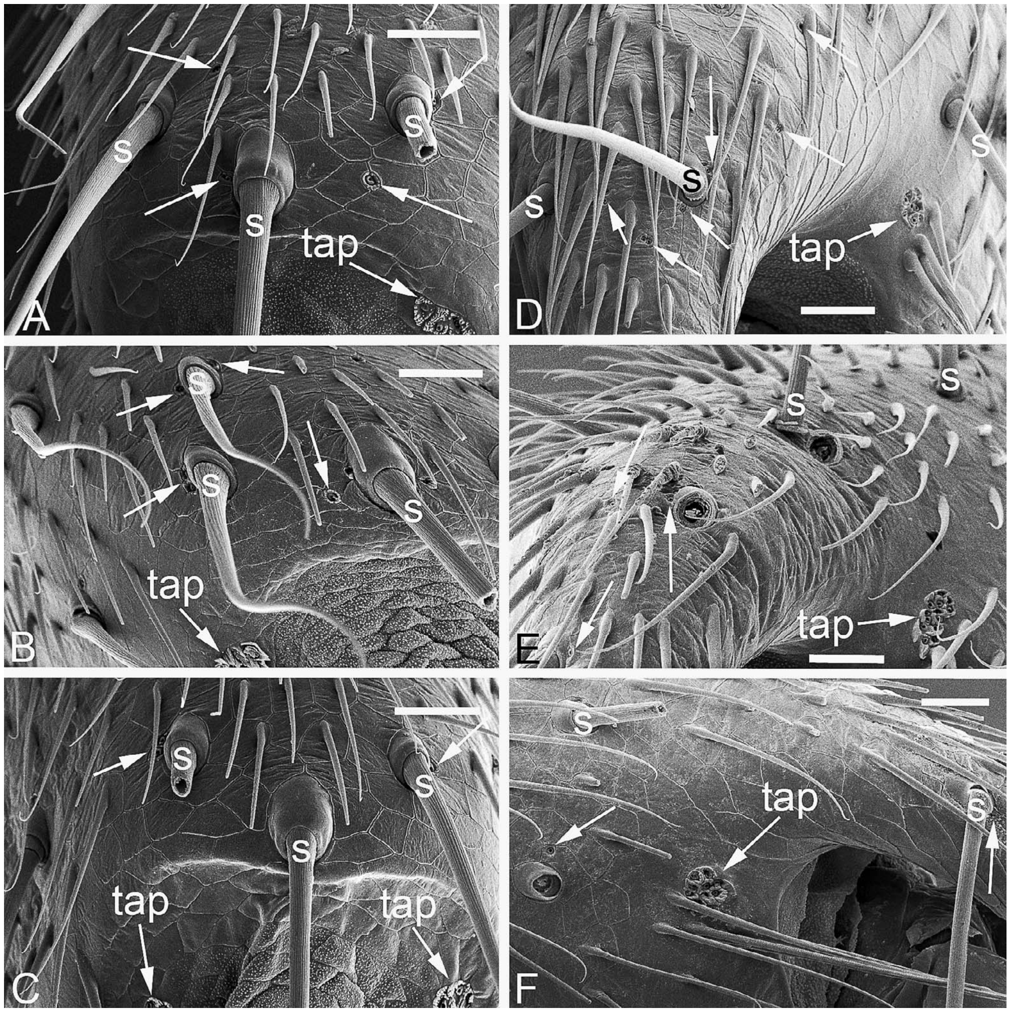

Figure 6.—SEM micrographs of the dorsal surfaces of tarsi IV revealing distribution of the simple pores (arrows), sensilla chaetica (s) and the tarsal aggregated pores (TAPs) on antepenultimate nymph (A-C) and adult (D-F) cosmetid harvestman. *Cynortula granulata*, nymph (A) and adult (D). *Erginulus clavotibialis*, nymph (B) and adult (E). *Paecilaema inglei*, nymph (C) and adult (F) Scale bars = 25 μm.

process that originates on the dorsal surface and projects inferiorly between the tarsal claws (Figs. 3D, 4D, 5D). The distal tarsomeres of nymphs feature TAPs on both the prolateral and retrolateral surfaces (Figs. 3–5). In contrast to adults (Fig. 2), vTAPs are absent from the tarsi of nymphs (Table 1). The TAPs of nymphs (Figs. 3B, 4B, 5B) are also not closely associated with the bases of trichomes as they are in the adults (Figs. 3E, F, 4E, F, 5E, F). The pores composing the TAPs of nymphs have sculptured borders (Figs. 3C, 4C, 5C), whereas the borders of the pores of adults (Figs. 3F, 4F, 5F) are much smoother, lacking the irregular, scalloped edges present in nymphs.

Occurrence of single tarsal pores among cosmetid harvestmen.—In addition to the presence of TAPs, we also compared the distribution and morphology of single pores on the distal surfaces of tarsi IV for nymphs and adults of all three species of cosmetid harvestmen (Fig. 6). These pores resemble those found in TAPs and vTAPs with respect to their relative size and the presence of a distinct border. However, in contrast to TAPs and vTAPs, the borders of the single pores were only slightly elevated (Fig. 6A, F) with respect to the surrounding surface of the cuticle and were generally smooth (and not scalloped or denticulate). We found that single pores were most frequently associated with the sensilla chaetica on the dorsal surfaces of the tarsi of nymphs (Fig. 6A–C) and the sensilla chaetica and tarsal processes of adults (Fig. 6D–F). We did not observe any ontogenetic differences between nymphs (Fig. 6A–C) and adults (Fig. 6D–F) with respect to the numbers or distribution of single pores.

Interspecific variation in TAPs among nymphs of cosmetid harvestmen.—We examined the distal tarsomeres of leg IV for seven distinct morphospecies of cosmetid nymphs (Fig. 1D–J), including three morphospecies from Belize (Fig. 7) and four additional morphospecies from Costa Rica (Fig. 8). Individuals of each of these cosmetid harvestmen had TAPs on the prolateral and retrolateral surfaces (Figs. 7A, C, 8A, C, G) that were generally symmetrical in appearance. The TAPs of these nymphs had 6-12 pores in each cluster (Table 1). There was considerable interspecific variation with regards to the sculpturing of the elevated borders surrounding the pores (Figs. 7B, D, F, 8B, D, F, H). In several species (Figs. 7B, D, 8B), the borders had a complex texture of ridges giving the

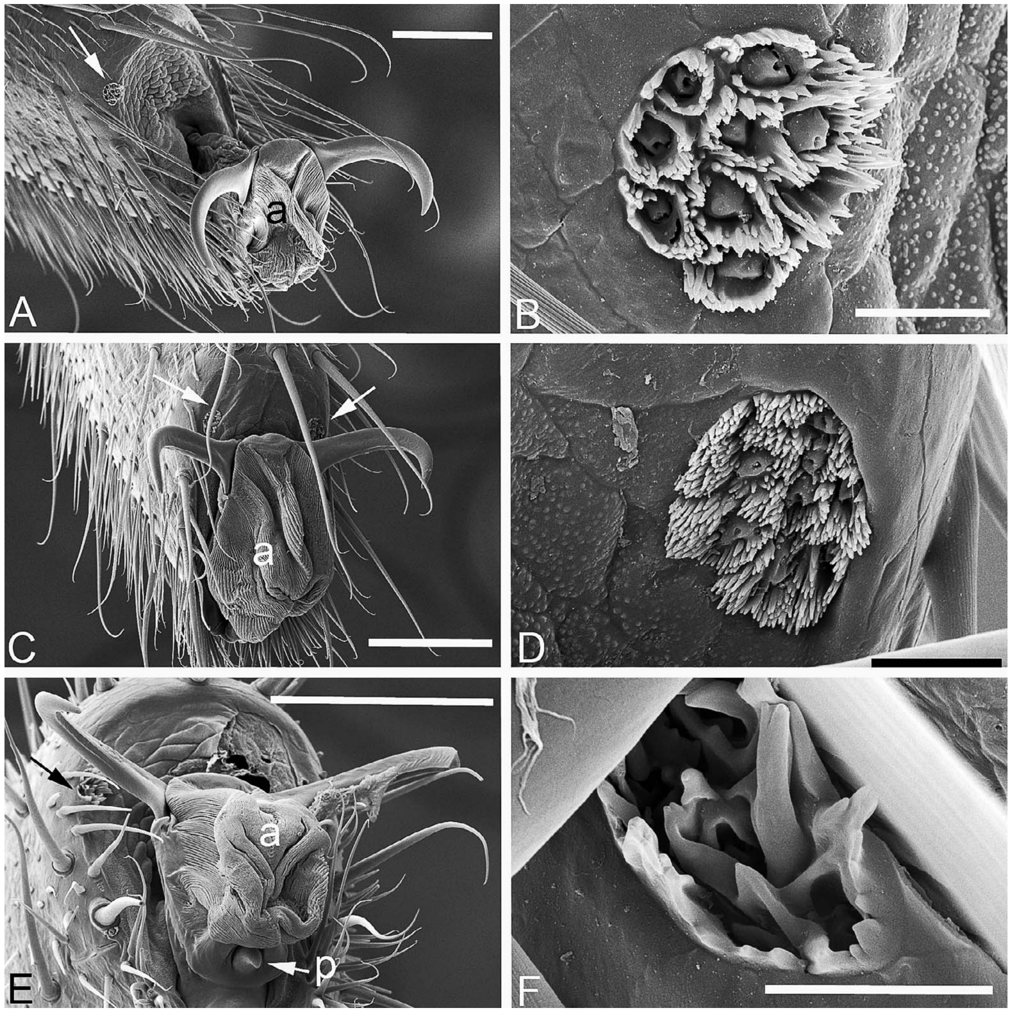

Figure 7.—SEM micrographs of arolium (a) and tarsal aggregate pores (TAPs) on tarsus IV of three morphospecies of cosmetid nymphs from Bladen Reserve, Belize. (A–B) Morphospecies 1: (A) arolium (a), distal tip of tarsus and TAP (arrow), latero-dorsal frontal view. (B) surface features of TAP. (C–D) Morphospecies 2: (C) distal tip of tarsus, frontal view. (D) TAP. (E–F) Morphospecies 3: (E) distal tip of tarsus, arolium, pseudonychium (p), ventro-frontal view. (F) TAP. Scale bars = 100 µm for A, C, E and 10 µm for B, D, F.

TAP a denticulate appearance. In other morphospecies, the borders around the pores were relatively smooth and less irregular (Figs. 7F, 8D, H).

Ontogenetic and interspecific variation in TAPs, single tarsal pores and vTAPs among Gonyleptoidea.—Tarsus IV of the nymphs and adults of *Cranellus montgomeryi* (Manaosbiidae), *Glysterus* sp. (Ampycidae), and *Stygnoplus clavotibialis* (Stygnidae) all had TAPs and single pores, but only those of adults had vTAPs (Figs. 9–11). Single tarsal pores occurred on the dorsal surface of the tarsi of nymphs and were usually associated with the bases of the sockets of large sensilla chaetica (Figs. 9B, C, 10B, 11B). In adults, single tarsal pores were also associated with the bases of large sensilla chaetica (Figs. 9F, 10F) or they occurred near the bases of trichomes on the tarsal process (Fig. 11F). We observed fewer pores in association with the TAPs of nymphs (Fig. 9D) than with those of adults (Fig. 9G) in *C. montgomeryi* (Table 1), but only a slight ontogenetic difference in pore numbers for TAPs between nymphs (Figs. 10D, 11D) and adults (Figs. 10G, 11G) in *Glysterus* sp. and *S. clavotibialis* (Table 1). In *C. montgomeryi* and *Glysterus* sp., there were only 3–4 pores present in the vTAPs (Figs. 9H, 10H), whereas in *S. clavotibialis*, there were 14–15 pores comprising the vTAPs (Fig. 11H). In addition, we found no ontogenetic difference in the morphology of the elevated borders that surrounded the pores between the nymphs (Figs. 9D, 10D) and adults (Figs. 9G, 10G) of *C. montgomeryi* and *Glysterus* sp. The TAPs of the nymphs and adults of *Glysterus* sp. (Figs. 10C, G) as well as those of adult *C. montgomeryi* (Fig. 9G) were associated with the bases of trichomes, whereas those of the nymphs of *C. montgomeryi* were not (Fig. 9C, D). In *S. clavotibialis*, the TAPs were partially obscured by the tarsal process (Fig. 11E) owing to a more medial position of the TAPs than in other gonyleptoidean taxa. This location may have led us to undercount the number of pores associated with the TAP as this was the only species that we examined in which we observed more pores in the TAPs of the nymph than in the adult (Table 1). In this species, there was also an ontogenetic difference in the sculpturing of the elevated borders surrounding the pores of the TAPs (Fig. 11C-H). In the nymphs (Fig.

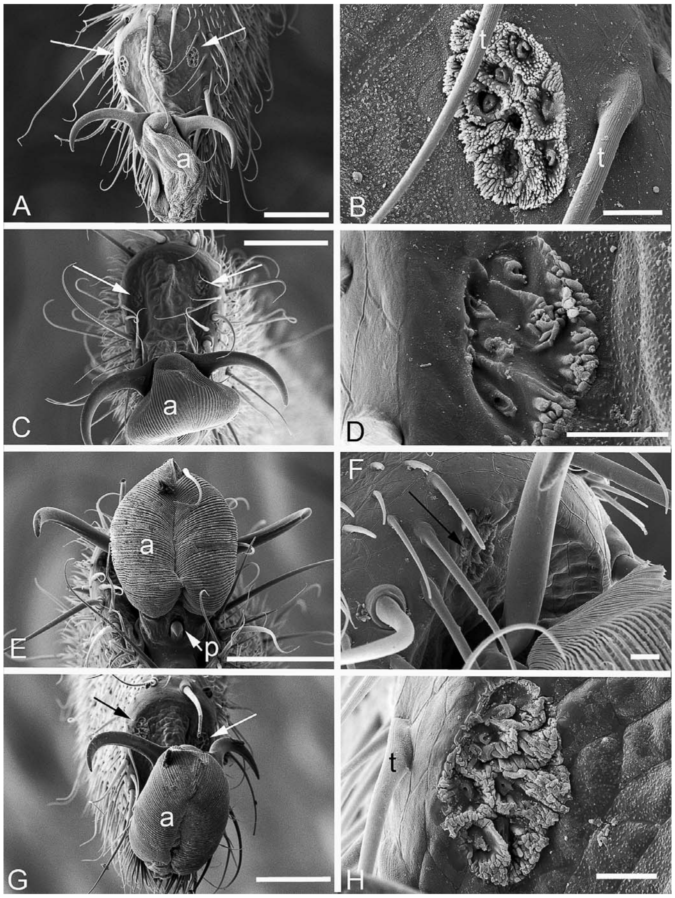

Figure 8.—SEM micrographs of arolium (a) and tarsal aggregate pores (TAPs) on tarsus IV of four morphospecies of cosmetid nymphs from La Selva Station, Costa Rica. (A–B) Morphospecies 4: (A) arolium, distal tip of tarsus and position of retrolateral and prolateral TAPs (arrows), frontal view. (B) Surface features of TAP. (C–D) Morphospecies 5: (C) arolium, distal tip of tarsus and position of retrolateral and prolateral TAPs (arrows), frontal view. (D) Surface features of TAP. (E–F) Morphospecies 6: (E) arolium and pseudonychium (p), ventro-frontal view. (F) TAP (arrow). (G–H) Morphospecies 7: (G) arolium, distal tip of tarsus and position of retrolateral and prolateral TAPs, frontal view. (H) Surface features of TAP. Scale bars = 100 µm for A, C, E, G; 10 µm for B, D, F, H.

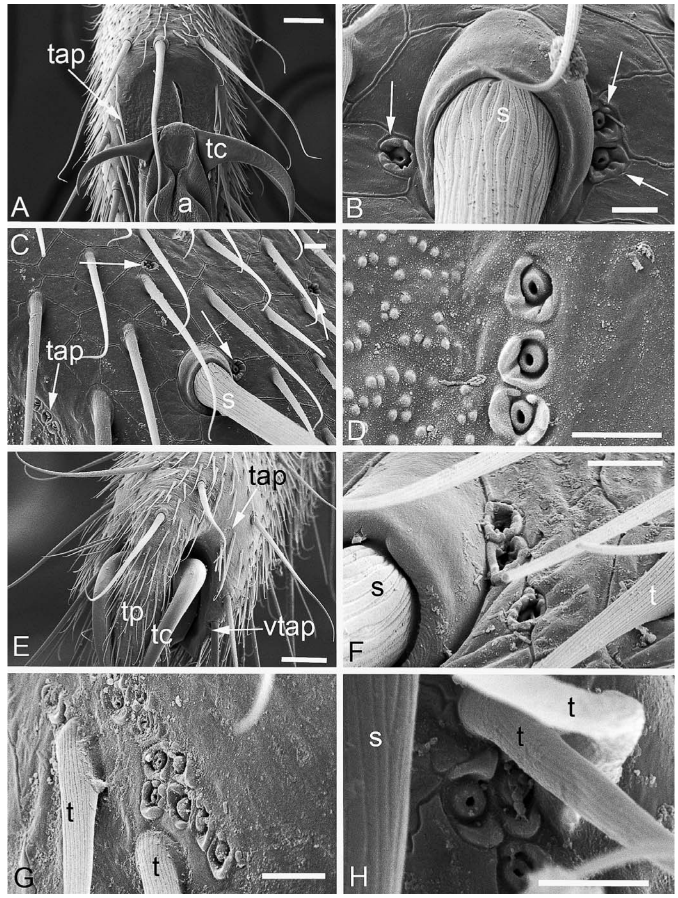

Figure 9.—SEM micrographs of tarsal aggregate pores (TAPs) on tarsus IV of nymph (A–D) and adult (E–H) of *Cranellus montgomeryi*. (A) Arolium and tarsal claws on the distal tip of tarsus, frontal view. (B) Simple pores (arrows) associated with sensillum chaeticum. (C) Trichomes, sensillum chaeticum, simple pores (arrows) and TAP. (D) Surface features of TAP. (E) Tarsal claws, tarsal process, TAP and vTAP, latero-frontal view. (F) Pores associated with sensillum chaeticum on tarsal process. (G) Surface features of TAP and associated trichomes. (H) Surface features of vTAP. Scale bars = 50 µm for A, E; 5 µm for B–D, F–H. a = arolium, s = sensillum chaeticum, t = trichome, tc = tarsal claws, tp = tarsal process.

11C, D), the borders were larger and more pointed than those of the adults (Fig. 11G).

We observed no differences between retrolateral and prolateral clusters with respect to the number of pores constituting the TAPs and vTAPs in nymphs and adults of all three non-cosmetid species. Similarly, we did not observe any intersexual differences in the distribution or morphology of the TAPs or vTAPs in these three gonyleptoidean species.

DISCUSSION

Comparative studies of sensory structures (Willemart et al. 2009), pedipalps (Wolff et al. 2016), metatarsal and tarsal sensilla (Gainett et al. 2014, 2017), tegumental glands (Willemart et al. 2010; Proud & Felgenhauer 2011, 2013), ozopores (Gnaspini & Rodrigues 2011), penises (Kury & Villarreal 2015; Kury 2016), and ovipositors (Martens et al. 1981; Walker & Townsend 2014; Townsend et al. 2015; Brooks et al. 2017) have either provided considerable new insights into the natural history of harvestmen or identified novel and informative characters for phylogenetic studies. Among harvestmen, the occurrences of metatarsal paired slits, a proximal tarsomeric gland and tarsal aggregate pores (TAPs) were recognized as diagnostic synapomorphies for the suborder Laniatores (Willemart et al. 2009; Gainett et al. 2014). Recently, Ramin et al. (2016) reported ontogenetic variation in TAPs for the gonyleptid harvestman *Heteromitobates albiscriptus* and also described a new cluster of tarsal pores, the vTAP, which occurs in the adult, but not the nymph. In addition to the Gonyleptidae, our results indicate the vTAPs occur in harvestmen that are members of the families Ampycidae, Cosmetidae, Manaosbiidae and Stygnidae and that vTAPs are also lacking in nymphs of the species that we surveyed. Additional phylogenetic surveys of other gonyleptoidean harvestmen are needed to determine if the

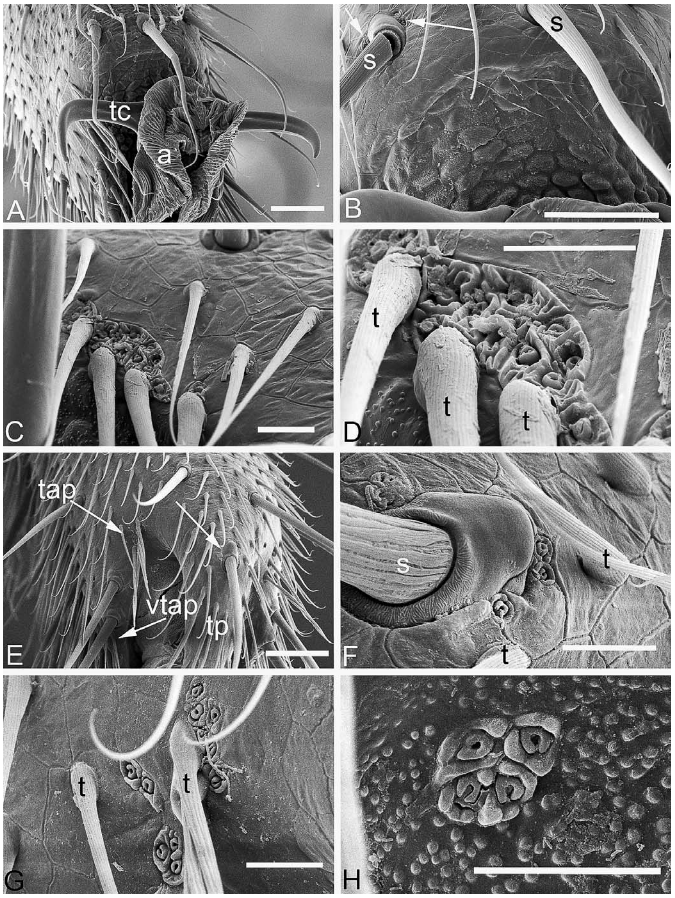

Figure 10.—SEM micrographs of tarsal aggregate pores (TAPs) on tarsus IV of nymph (A–D) and adult (E–H) of *Glysterus* sp. (A) Arolium and tarsal claws on the distal tip of tarsus, latero-frontal view. (B) Simple pores (arrows) associated with sensilla chaetica. (C) Trichomes associated with TAP. (D) Surface features of TAP and associated trichomes. (E) Tarsal claws and tarsal process, dorso-frontal view (F) Pores associated with sensillum chaeticum on tarsal process. (G) Surface features of TAP. (H) Surfaces features of vTAP (ventral tarsal aggregate pores). Scale bars = 50 µm for A, B, E; 10 µm for C–D, F–H. a = arolium, s = sensillum chaeticum, t = trichome, tc = tarsal claws, tp = tarsal process.

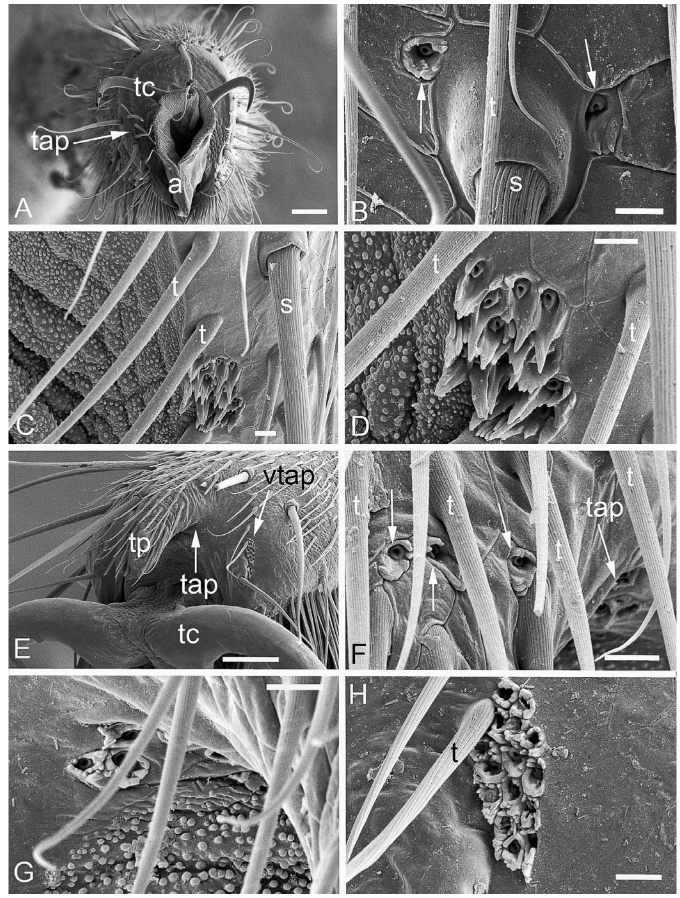

Figure 11.—SEM micrographs of tarsal aggregate pores (TAPs) on tarsus IV of nymph (A–D and adult (E–H) of *Stygnoplus clavotibialis*. (A) Arolium and tarsal claws on the distal tip of tarsus, frontal view. (B) Simple pores (arrows) associated with sensillum chaeticum. (C) Trichomes associated with TAP and ventral tarsal aggregate pores (vTAP). (D) Surface features of TAP. (E) Tarsal claws, tarsal process, TAP and vTAP, dorso-frontal view. (F) Simple pores (arrows) associated with sensilla chaetica on tarsal process. (G) Surface features of TAP. (H) Surface features of vTAP. Scale bars = 50 µm for A, E; 5 µm for B–D, F–H. a = arolium, s = sensillum chaeticum, t = trichome, tc = tarsal claws, tp = tarsal process.

morphology and occurrence of vTAPs are informative characters like TAPS as well as if these clusters of pores only occur in adults of other harvestmen.

The results presented by Ramin et al. (2016) also raise interesting questions concerning the sensory ecology, functional morphology and natural history of the nymphs of harvestmen, highlighting the current paucity of knowledge concerning the biology of this important life history stage. In our study, we observed considerable interspecific and ontogenetic variation in the morphology of the tarsal pores (TAPs and vTAPs) among nymphs and adults of cosmetid harvestmen. As with nymphs of the gonyleptid harvestman *Heteromitobates albiscriptus* (Ramin et al. 2016), cosmetid nymphs lack vTAPS and each TAP has fewer pores than in conspecific adults. In the nymphs of several species, the pores comprising each TAP were generally separated by elevated borders that were denticulate or featured irregular scalloped edges. In contrast, cosmetid adults have a TAP and vTAP on both the prolateral and retrolateral surfaces. The TAPs and vTAPs of cosmetid harvestmen generally occur in the same anatomical locations and are associated with the same setae (S0-S1-S3 and S2, respectively) as those reported for the gonyleptid harvestmen, *H. albiscriptus* (Ramin et al. 2016). As in *H. albiscriptus*, we did not observe any striking asymmetries between tarsal surfaces nor did we observe sexual dimorphism. With the exception of *Erginulus clavotibialis*, there were more pores in the TAP (13–15) than in the vTAP (3–5). The borders of the pores of the TAPs of adults were reduced in prominence and much smoother than those of the nymphs. The functional significance of this ontogenetic difference is not clear, however, this type of variation was not observed for the TAPs of the gonyleptid *H. albiscriptus* (Ramin et al. 2016) or for those of the ampycid *Glysterus* sp. (Table 1) or the manaosbiid *Cranellus montgomeryi* (Table 1). In addition, we also discovered ontogenetic variation with respect to the association of TAPs with trichomes. In adult cosmetid harvestmen, the TAPs and vTAPs were situated near the bases of multiple trichomes, however, in nymphs, there were no setae in close proximity to the TAPs.

Gainett et al. (2014) reported the widespread occurrence of TAPs among harvestmen in the superfamily Gonyleptoidea. The results of our study indicate that vTAPs may also be common among these harvestmen. In addition to their occurrence in cosmetid harvestmen, we also observed vTAPs on tarsus IV of adults for the ampycid *Glysterus* sp., the manaosbiid *C. montgomeryi* and the stygnid *Stygnoplus clavotibialis*. In *Glysterus* and *C. montgomeryi*, there were more pores in the TAPs than in the vTAPs. However, in *S. clavotibialis*, the vTAPs were more developed and had a much larger number of pores (comparable to those in the TAPs). In *S. clavotibialis*, the pores of the TAPs had elevated, denticulate borders in the nymph that were much shorter in the adult (similar to the pattern that we observed in cosmetid species). In *Glysterus* and *C. montgomeryi*, there was only a subtle difference in the morphology of the border of the pores in the TAPs of nymphs and adults. As with the cosmetid taxa that we observed, the TAPs of nymphs also had fewer pores than those of adults. Unfortunately, our survey featured only 10 species of cosmetid harvestmen and did not include penultimate nymphs, so we are unable to comment on the morphology of the TAPs of these instars. In the gonyleptid *H. albiscriptus*, penultimate nymphs no longer have an arolium or pseudonychium on tarsi III or IV, but also lack vTAPs on their tarsi (Ramin et al. 2016). Additional studies are needed to determine if the same ontogenetic pattern is present in cosmetid harvestmen and other laniatorean taxa.

In addition to clusters of pores (TAPs and vTAPs), we also observed the occurrence of single tarsal pores on the leg IV of all of the gonyleptoidean harvestmen that we surveyed including cosmetid and non-cosmetid taxa and nymphs and adults stages. The morphology of these pores resembles those comprising the TAPs and vTAPs, especially with respect to relative size and the presence of a well-developed, slightly elevated border. We also found that single tarsal pores occurred in close proximity to the sockets and bases of sensilla chaetica and trichomes in both nymphs and adults. The functional significance of these pores is unclear as is the relative contributions of the various tarsal glands of the nymph (single pores and TAPs) and adult (single pores, TAPs and vTAPs) to the chemical marking ability of individuals. Additional studies similar to those of Proud & Felgenhauer (2011, 2013) and Wolff et al. (2016) are needed to investigate the ultrastructure of the tarsal glands on legs III and IV and chemical composition of their secretions, respectively.

There is relatively little published data as to the functional significance of the tarsal glands on legs III and IV of gonyleptoidean harvestmen. Adults have been hypothesized to use glandular secretions to mark the substrate, thereby permitting more efficient navigation through the environment (Ramin et al. 2016). Recent studies have revealed that harvestmen have olfactory setae on the tarsi of leg I–IV (Gainett et al. 2017) and may use chemical cues in conspecific interactions (Willemart & Hebets 2012; Fernandes & Willemart 2014; Murayama & Willemart 2015). Additional ecological experiments are needed to assess how adults and nymphs employ their tarsal glands in the environment and to determine if there is any relationship between ontogenetic differences in the morphology of the tarsal glands and the behavior of nymphs and adults.

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