

Evolution of a sensory cluster on the legs of Opiliones (Arachnida) informs multi-level phylogenetic relationships

GUILHERME GAINETT^{1,2,3*}, PRASHANT P. SHARMA², NATHÁLIA FERNANDES³, RICARDO PINTO-DA-ROCHA³, GONZALO GIRIBET FLS⁴ and RODRIGO HIRATA WILLEMART^{1,3,5}

¹Laboratório de Ecologia Sensorial e Comportamento de Artrópodes, Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, Rua Arlindo Bétto, 1000, Ermelino Matarazzo, São Paulo, SP 03828-000, Brazil

²Department of Integrative Biology, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, WI, USA

³Programa de Pós-Graduação em Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 321, Travessa 14, São Paulo, SP 05508-090, Brazil.

⁴Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA

⁵Programa de Pós-Graduação em Ecologia e Evolução, Universidade Federal de São Paulo, Campus Diadema, Rua Professor Artur Riedel, 275, Jardim Eldorado, Diadema, SP 09972-270, Brazil

Received 20 September 2018; revised 16 December 2018; accepted for publication 5 June 2019

Phylogenetic relationships in Opiliones (Arachnida) at the suborder level have greatly stabilized in recent years, largely due to advances in molecular systematics. Nonetheless, identifying morphological characters in the context of well-resolved phylogenies is essential for testing new systematic hypotheses and establishing diagnostic markers. Here, we investigate with SEM a promising character system across Opiliones: the sensilla on the distalmost article of legs I and II. We identified four discrete characters and scored species of nearly all families of Laniatores (28 families, 44 species), three Dyspnoi, two Eupnoi and two Cyphophthalmi. Using a phylogenetic backbone compiled from recent and ongoing phylogenomic studies, we trace the evolution of these sensilla using ancestral state reconstruction. We discover a widespread occurrence of three sensilla (a pair of sensilla basiconica and one hooded sensillum) on the anterior legs of all families of Laniatores studied, and that comparable structures occur in the other suborders of Opiliones. Our analysis shows that this sensory field provides diagnostic information at different levels of phylogenetic relationships. We discuss the implications of the widespread occurrence of these sensilla in Opiliones, which have recently been hypothesized as hygro-/thermoreceptors and their putative homology with tarsal organs in Arachnida.

ADDITIONAL KEYWORDS: ancestral state reconstruction – chaetotaxy – Dyspnoi – Eupnoi – harvestmen – Laniatores – sensilla – tarsal organ.

INTRODUCTION

Harvestmen, arachnids of the order Opiliones, comprise four well-defined main lineages with considerable morphological disparity. The suborder Cyphophthalmi includes diminutive, inconspicuous animals (1–10 mm)

popularly known as ‘mite-harvestmen’ (Giribet *et al.*, 2012). Dyspnoi includes generally small, short-legged animals, with a characteristic pattern of fusion of opisthosomal tergites (Pinto-da-Rocha & Giribet, 2007). Eupnoi species range from short-legged forms, to the archetypal ‘daddy-long-legs’ (Pinto-da-Rocha & Giribet, 2007). The suborder Laniatores (‘armoured harvestmen’) includes sclerotized animals with

*Corresponding author. E-mail: guilherme.gainett@wisc.edu

typically raptorial pedipalps and a remarkable diversity of armature (Sharma & Giribet, 2011). Phylogenetic studies, including the use of phylogenomic data, have corroborated Cyphophthalmi as sister group to the remaining extant orders (clade Phalangida) and the monophyly of Palpatores (Eupnoi + Dyspnoi) (Giribet *et al.*, 2010). The result has been the recently settled and robustly supported Opiliones tree of life, with nearly complete sampling of all harvestman families in transcriptomic datasets (Hedin *et al.*, 2012; Garwood *et al.*, 2014; Sharma & Giribet, 2014; Fernández *et al.*, 2017; Fig. 1).

Laniatores represent more than two-thirds of the described harvestman species, comprising ~30 families, depending on the classification scheme employed (Kury, 2013; Giribet & Sharma, 2015; Derkarabetian *et al.*, 2018; Fig. 1). Relationships between the families and superfamilies have become better understood in recent years, due to the implementation of molecular phylogenetics (Giribet *et al.*, 2010; Sharma & Giribet, 2011; Fernández *et al.*, 2017; Derkarabetian *et al.*, 2018). Due to the major systematic changes in higher-level classification of harvestmen in recent years (e.g. Sharma & Giribet, 2011; Groh & Giribet, 2015; Derkarabetian *et al.*, 2018), it is imperative to identify morphological characters that are consistent with robustly recovered clades in molecular phylogenies, both for the purpose of testing newly proposed relationships and also for facilitating efficient identification of diverse harvestman groups (Sharma & Giribet, 2011; Kury, 2014; Pinto-da-Rocha *et al.*, 2014; Kury & Villarreal, 2015).

In Arthropoda, the employment of sensillar occurrence and distribution as diagnostic characters (chaetotaxy) is a common practice that yields synapomorphies at different levels of relationships, from generic to higher groups (e.g. Insecta: Brozek & Zettel, 2014; Crustacea: Karanovic & Kim, 2014; Arachnida: Tomasiewicz & Framenau, 2005; Botero-Trujillo & Flórez, 2011; Platnick *et al.*, 2012). In Opiliones, various cuticular structures have been suggested as promising for systematics, such as microstructure of the integument (Murphree, 1988; Rodriguez *et al.*, 2014a, 2014b), glandular openings (Willemart & Giribet, 2010; Gainett *et al.*, 2014; Rodriguez & Townsend, 2015) and sensillar distribution and morphology (Willemart & Giribet, 2010; Gainett *et al.*, 2014; Kury & Villarreal, 2015; Townsend *et al.*, 2015; Wolff *et al.*, 2016). While appendicular characters generally seem to be a promising source of characters (Gainett *et al.*, 2014), the diagnostic potential of tarsal sensilla in a systematic context has not been investigated heretofore under a phylogenetic framework.

The tarsi [terminal podomere (true segment)] of the legs of harvestmen concentrate most sensillar units, often on specific tarsomeres (the subdivisions of the tarsus). In the case of Laniatores, legs I and II are used as sensory legs, and their tarsi have higher density and diversity of sensory structures (Willemart & Gnaspini, 2003; Willemart *et al.*, 2009; Gainett *et al.*, 2017a). Willemart *et al.* (2007) first described a pair of sensilla basiconica on the distalmost tarsomeres I and II of *Iporangaia pustulosa* (Mello-Leitão, 1935) and *Neosadocus maximus* (Giltay, 1928) (Laniatores, Gonyleptidae), inserted 'on an irregular depression of the cuticle'. Gainett *et al.* (2017b) then described a third sensillum on this irregular depression, the hooded sensillum, always distal to the pair of sensilla basiconica. A preliminary survey on the diverse family Gonyleptidae showed that these sensillar units were conserved in position and morphology in these legs, suggesting them as a source of new morphological characters.

We therefore searched for similar structures occurring on the corresponding region of the distalmost tarsomeres I and II of species in other laniatorean families to investigate the phylogenetic distribution of the triad (hooded sensillum and pair of sensilla basiconica). Additionally, we sampled the corresponding region on representatives of the suborders Dyspnoi, Eupnoi and Cyphophthalmi and optimized all surveyed data on a modern phylogeny of Opiliones.

MATERIAL AND METHODS

SPECIES SAMPLING

Specimens were obtained from the Invertebrate Zoology collection at the Museum of Comparative Zoology (Harvard University, USA) and the Museu de Zoologia da Universidade de São Paulo (Brazil). We sampled the tarsi I and II of 44 species representatives of 28 families of Laniatores (60 individuals), three Dyspnoi, two Eupnoi and two Cyphophthalmi. Absence of the studied structures in tarsi III and IV for some laniatorean species was inferred from the SEM data generated by Gainett *et al.* (2014) and the study of the laniatorean *Heteromitobates discolor* Sørensen, 1884 (Gainett *et al.*, 2017a, b). All SEM data of aforementioned projects and of the present study are publicly available at the MCZ database (mcz-base.mcz.harvard.edu) and upon request. The list of specimens examined is provided in Supporting Information, File S1. Additional media can be accessed in the MCZ database (mcz-base.mcz.harvard.edu).

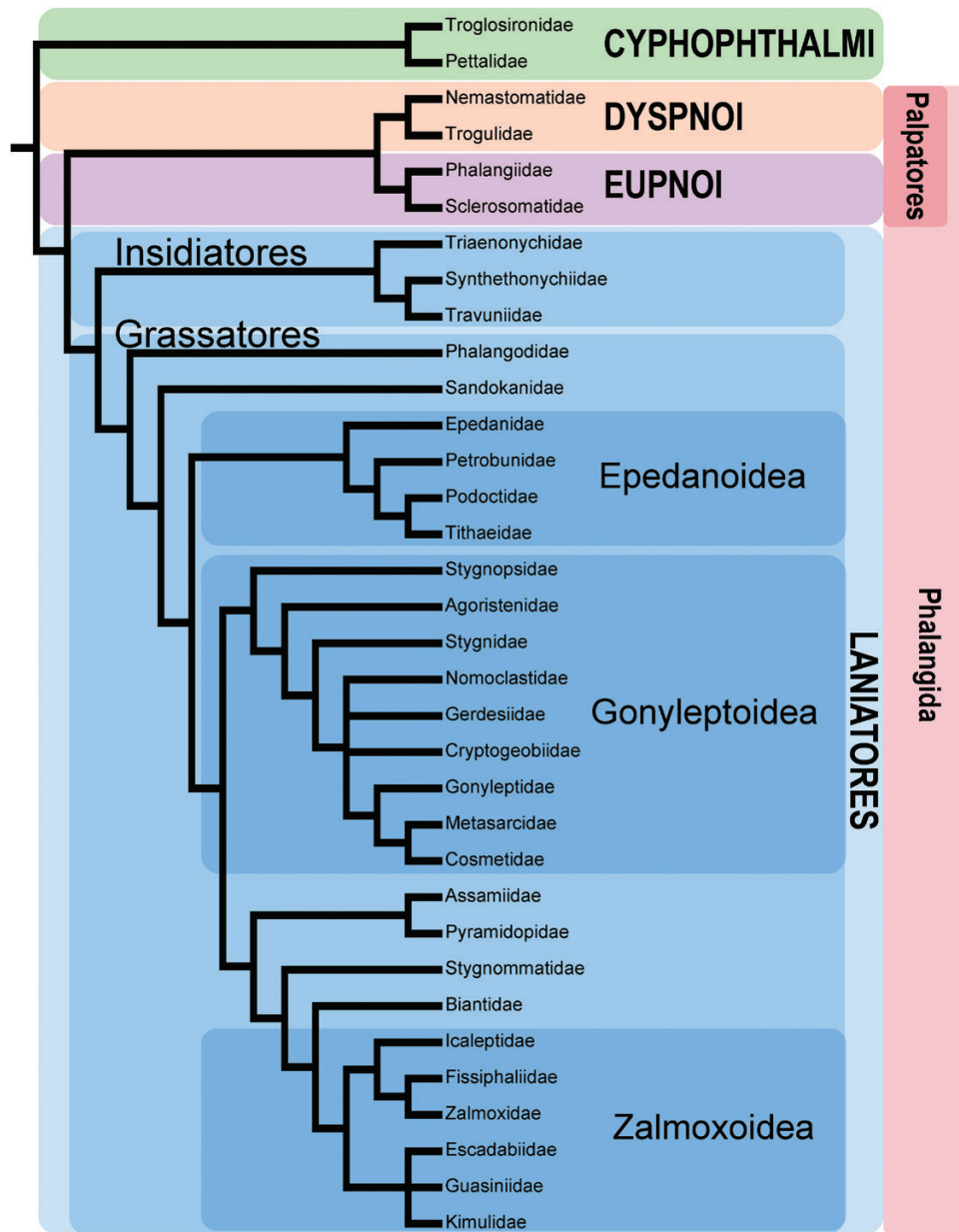


Figure 1. Classification of Opiliones, showing the most supported hypothesis of relationship among the suborders Cyphophthalmi, Eupnoi, Dyspnoi and Laniatores and relationships between the families used in this study. Compiled phylogeny is based on Giribet & Sharma (2015), with updated relationships of most inclusive clades of Laniatores, after a transcriptome-based phylogeny by Fernández *et al.* (2017). Families under 'Gonyleptidae' are *s.l.*, because Gonyleptidae is broadly defined to include Cranainae and Manaosbiinae in the newly proposed hypothesis.

SCANNING ELECTRON MICROSCOPY

Tarsi were cut with micro-scissors and submitted to three rounds of ultrasound cleaning (Branson 200): in distilled water, in a 1:10 detergent solution (Alconox) and in distilled water again. The duration of each round was determined empirically. Most specimens were critical point dried (Tousimis 931 GL), being dehydrated in graded ethanol series. Otherwise, they

were immersed in 100% acetone and then air dried. Samples were mounted on stubs with carbon adhesive tabs (Electron Microscopy Science, Hatfield, PA, US) and sputter coated with Pt-Pd targets (EMS 300T D Dual Head Sputter Coater). Photographs were taken using a Zeiss Ultra-Plus FESEM or a Zeiss Supra FESEM (field emission scanning electron microscope) at the Center for Nanoscale Systems (Harvard

University) or in a Zeiss DSM 940 at Instituto de Biociências (Universidade de São Paulo).

Coding, ancestral state reconstruction and tests of phylogenetic signal

Characters and character states were proposed following a reductive coding scheme to explore the informativeness of the hierarchical variation observed in less inclusive clades (Strong & Lipscomb, 1999; Brazeau, 2011). Ancestral state reconstructions were performed in MESQUITE v.3.31 (Maddison & Maddison, 2011) under equal weights parsimony and under maximum likelihood using a one-parameter Markov model (Supporting Information, File S4). The tree topology used was a compiled phylogeny of Opiliones based on Giribet & Sharma (2015), with updated relationships of most inclusive clades of Laniatores, after the phylogenomic analysis of Fernández *et al.* (2017) and Derkarabetian *et al.* (2018) (Fig. 1). The main outcomes of adding the data of Fernández *et al.* (2017) were in Laniatores, with (1) the reinstatement of Insidiatores as the monophyletic sister group to Grassatores and (2) the placement of the family Sandokanidae as part of a grade with Phalangodidae at the root of Grassatores (Fig. 1). In ambiguous optimizations, we did not favour specific transformation schemes, discussing both ACCTRAN and DELTRAN reconstructions.

A permutation tail probability (PTP) analysis (Faith & Cranston, 1991) was performed to test the phylogenetic signal of each character using MESQUITE v.3.31 (Maddison & Maddison, 2011). The PTP value may be interpreted as the probability of obtaining a tree of a given length under a null model of random character evolution (Faith & Cranston, 1991). Character states in each character were randomly shuffled 500 times and tree lengths mapped onto the original tree topology (see above). The resulting null distributions of tree lengths were compared to the empirical character cost, with a cut-off left tail of 0.05. A low PTP value (PTP < 0.05) indicates a highly structured character state distribution and phylogenetic signal.

RESULTS

MORPHOLOGICAL SURVEY USING SCANNING ELECTRON MICROSCOPY

Laniatores

All investigated species of Laniatores, in 28 families, possess three distinct sensilla on the distal-third end of the distalmost tarsomere of leg pairs I and II, roughly aligned along the sagittal axis (Fig. 2).

The only exception is *Sandokan truncatus* Thorell, 1891 (Sandokanidae), in which only the distal sensillum was clearly identified in the corresponding position. The distal sensillum has been termed the 'hooded sensillum' (Gainett *et al.*, 2017b) and the mid and proximal sensilla have been termed 'sensilla basiconica' (after Willemart *et al.*, 2007, 2009). Below, we describe the variation observed in the morphology and topology of these three sensilla on the distalmost tarsomeres I and II.

Distal sensillum: hooded sensillum

In all species studied, for both males and females (15 species), the hooded sensillum was sexually monomorphic. Moreover, no difference in shaft morphology was detected between legs I and II. The hooded sensillum is inserted dorsally to dorsolaterally on the tarsi (Fig. 2), in a flat and oval-shaped membrane (Fig. 3B–D). The membrane has no protuberance, which is different from the socket of surrounding sensilla chaetica (see Fig. 3B for comparison with sensillum chaeticum). The seta wall bears continuous longitudinal grooves along the shaft (Figs 4–6). The basal portion of the shaft is much wider than the apex, tapering gradually (Figs 2–6). The hooded sensillum is always longer than the sensilla basiconica (Figs 2, 3). In *Erebomaster flavescens flavescens* Cope, 1872 (Travuniidae) it appears to be the longest seta in the distalmost tarsomere (Fig. 2A). The apical portion has the shape of a hood, with variation in the length and shape of the hood between groups. One of the sides of the hood is morphologically similar to the rest of the shaft, bearing grooves (Figs 4B, D, H, L, 5B). The opposite side has an oval, subapical concavity with a smooth surface, with a pore-like structure that frequently appears clogged with some material (Figs 4A, C, E, G, I, J, K, M, 5A, C, E, 6C, E). Some samples appear to have two pore-like structures, the most distal one being smaller in diameter (Figs 4A, 5A, E), but in the majority of samples only one was seen (Fig. 4C, G, I). This difference might be an artefact, probably due to clogging of the pore by dirt particles. Three types of hood morphology can be recognized: terminal swelling, with no tapering after the swollen region (spoon-shaped) (Fig. 4E, L, M); subterminal swelling, with tapering after the swollen region ('death's hood' shape) (Figs 4A, B, C, F, G, K, 5A–E, 6B–E); and gradual tapering, with no swelling (Fig. 4H–J; Supporting Information, File S2).

Mid and proximal sensilla: sensilla basiconica

Two sensilla basiconica occur proximal to the distal sensillum (hooded sensillum), which we refer to as the mid and proximal sensilla (numbers 2 and 3 in

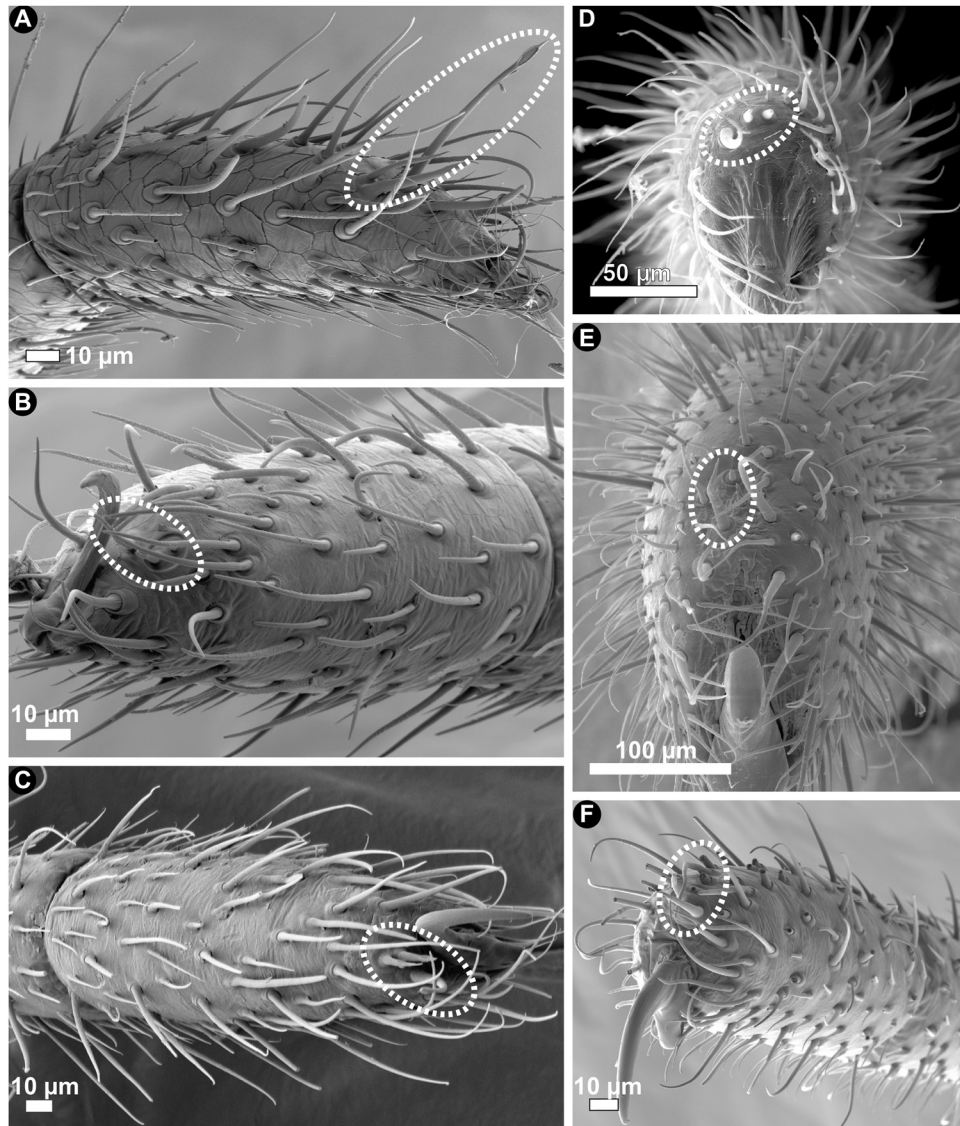


Figure 2. Laniatores. Triad on the sensory field formed by two sensilla basiconica (proximal and middle sensilla) and one hooded sensillum (distal sensillum) (dotted area) on the distal-most tarsomeres I of selected families. A, *Erebomaster flavescens flavescens* (Travuniidae), undetermined sex. B, *Remyus* sp. (Phalangodidae), female. C, *Pseudoepedanus doiensis* (Epedanidae), male. D, *Avima octomaculata* (Agoristenidae), male. E, *Heteromitobates discolor* (Gonyleptidae), male. F, *Stygnomma bispinatum* (Stygnommatidae), male.

Fig. 3. In all species studied for males and females (15 species), they were sexually monomorphic. The mid sensillum is always longer than the proximal one, both of these being shorter than the distal-hooded sensillum (Figs 2, 3). They are inserted in a flat articulation membrane, with no prominent socket. Although first described in two gonyleptid species as being short and conical (Willemart *et al.*, 2007), there is variation in length in Laniatores. Sensilla basiconica can be peg-like (Fig. 3A, D) or elongated (Figs 3B, 6A), but they are generally the shortest seta in this region of the tarsus (Fig.

2). The shaft appears smooth in most species, but some ridges are present in the basal portion of the shaft wall in *Synthetonychia glacialis* Forster, 1954 (Synthetonychiidae; Fig. 6A), a feature that is possibly an artefact of the drying process. No wall pores were detected on the shaft wall. A ubiquitous feature is the apical portion with an opening to the exterior by means of a longitudinal slit that results in two terminal flaps (Fig. 3A, B). The slit-segment of the shaft occupies approximately the distal third of the shaft (Fig. 3A, B). We detected no differences in shaft morphology between leg pairs I and II.

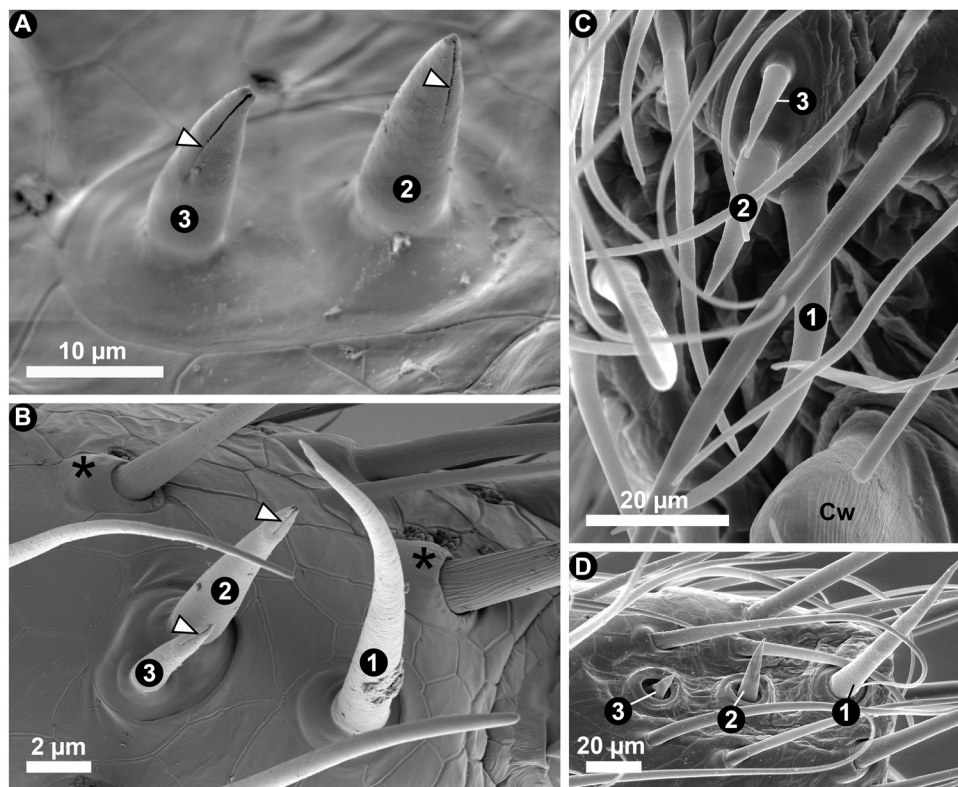


Figure 3. Laniatores. Different associations between the articulation membrane of the three sensilla on the sensory field of the distalmost tarsomeres I (C) and II (A, B, D). A, Proximal (3) and middle sensilla (2) of *Hoplobunus* sp. (Stygnopsidae), female. Distal sensillum not shown. B, *Larifuga* cf. *capensis* (Trieononychiidae), male. Proximal (3) and middle (2) sensilla have fused articulation membranes that are isolated from the articulation membrane of the distal sensillum (1). C, *Camarana flavipalpis* (Cryptogobiidae), female. Note that the three sensilla are associated in the same articulation membrane. D, *Sodreana sodreana* (Gonyleptidae), male. The three sensilla have individualized articulation membranes, with no external association. Asterisk: sensillum chaeticum, socket; Cw: claw; white arrowhead: slit opening; 1: hooded sensillum; 2: middle sensillum basiconicum; 3: proximal sensillum basiconicum.

In most species, the two sensilla basiconica are inserted in the same articulation membrane, with no cuticular-polygon lines in between the two shafts (Fig. 3A, B; Supporting Information, File S2). In some species they can be inserted individually, with cuticular-polygon lines in between them (Fig. 3D). We also observed species in which the two sensilla basiconica and the hooded sensillum shared the same articulation membrane (Fig. 3C). Therefore, three conformations occur in Laniatores: (1) the proximal and mid shafts with articulation membranes fused, separated from the distal shaft; (2) the three sensilla with isolated articulation membranes; and (3) all three shafts with articulation membranes fused. Except one species of the family Agoristenidae (*Avima octomaculata* Roewer, 1963) and one Nomoclastidae species (*Poassa limbata* Roewer, 1943), the articulation membrane configuration is generally the same in legs I and II. In these two species, leg II has the three shafts separated (2), but leg I has two sensilla basiconica

clustered (1; in Agoristenidae) or sensilla basiconica and hooded sensillum clustered (3; in Nomoclastidae). In *Sandokan truncatus* (Sandokanidae) several putative sensilla basiconica occur on the tarsi, but they do not show the typical slit opening and are sparsely distributed on the dorsal surface of the tarsomere (not shown; see Gainett *et al.*, 2018).

Dyspnoi

The three species of *Dyspnoi* studied show three sensilla aligned along the sagittal axis in a corresponding position as Laniatores, on the distalmost tarsomeres of legs I and II. The three sensilla differ from the surrounding sensilla chaetica and trichomes by having a flat articulation membrane (Figs 7, 8). The proximal two sensilla are short and conical, being also termed sensilla basiconica. The distal sensillum has a very similar morphology to the hooded sensillum in Laniatores and, therefore, we refer to it using the same name.

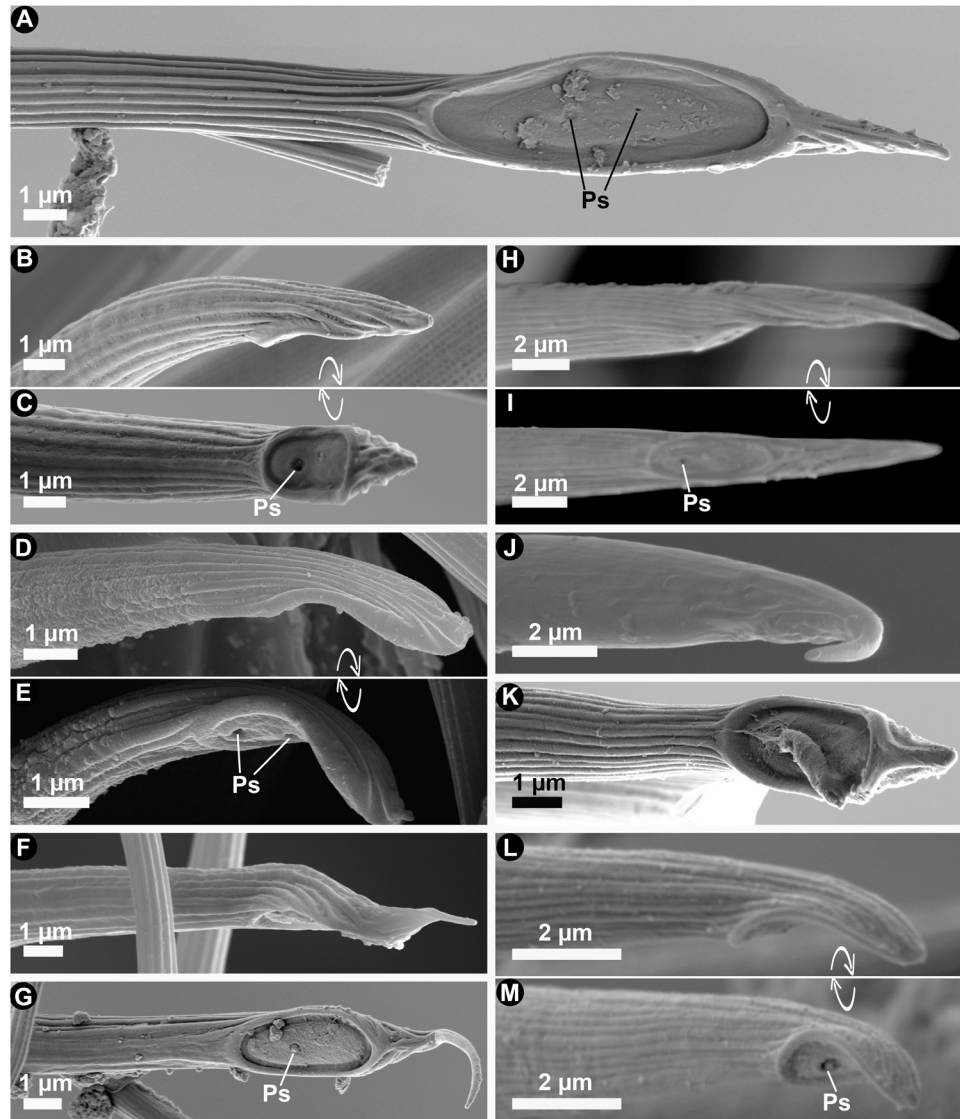


Figure 4. Laniatores. Tip of the distal sensillum (hooded sensillum) on the sensory field in selected families, leg pairs I (A–C, F, G, J) and II (D, E, H, I, K–M). A, *Erebomaster flavescens flavescens* (Travuniidae), undetermined sex. B, C, *Larifuga* cf. *capensis* (Triaenonychidae), male. D, E, *Metibalonius* sp. (Podoctidae), male. F, *Pseudoepedanus doiensis* (Epedanidae), male. G, *Tithaeus* sp. (Tithaeidae), male. H, I, *Hoplobunus* sp. (Stygnopsidae), female. J, *Phareicranaus hermosa* (Gonyleptidae, Cranainae), male. K, *Stygnomma bispinatum* (Stygnommatidae), male. L, M, *Martibianta virginsulana* (Biantidae), female. Circular arrows indicate different views of the same unit. Ps: pore-like structure.

The hooded sensillum is longer than both sensilla basiconica (Figs 7A, C, 8A, D). The shaft has longitudinal ridges and gradually tapers into a fine tip (Figs 7C, 8A). The apical portion has two pore-like structures in a region without ridges, but shows no subterminal swelling (see Ps in Figs 7D, 8D). The distal pore-like structure is smaller than the proximal (Fig. 7D). The side opposite to the pore-like structures is similar to the rest of the shaft, with ridges.

The sensilla basiconica are thin pegs, being the shortest sensilla in the tarsomere (Figs 7A, B,

8A–C). The proximal sensillum in *Anelasmacephalus* sp. (Trogulidae) is shorter than the mid sensillum, both being shorter than the distal-hooded sensillum (Fig. 8A), but in the other species we could not access their relative length. The shaft is bent forward in its mid portion, in an angle of almost 90° (Figs 7B, 8A–C). Apically, it bears a subterminal pore with a short slit, which faces down in *Nemastoma bimaculatum* Fabricius, 1775 (Nemastomatidae) (Fig. 7A, B) or up in *Anelasmacephalus* sp. (Fig. 8A–C). The slit apparently does not divide the shaft into two flaps as

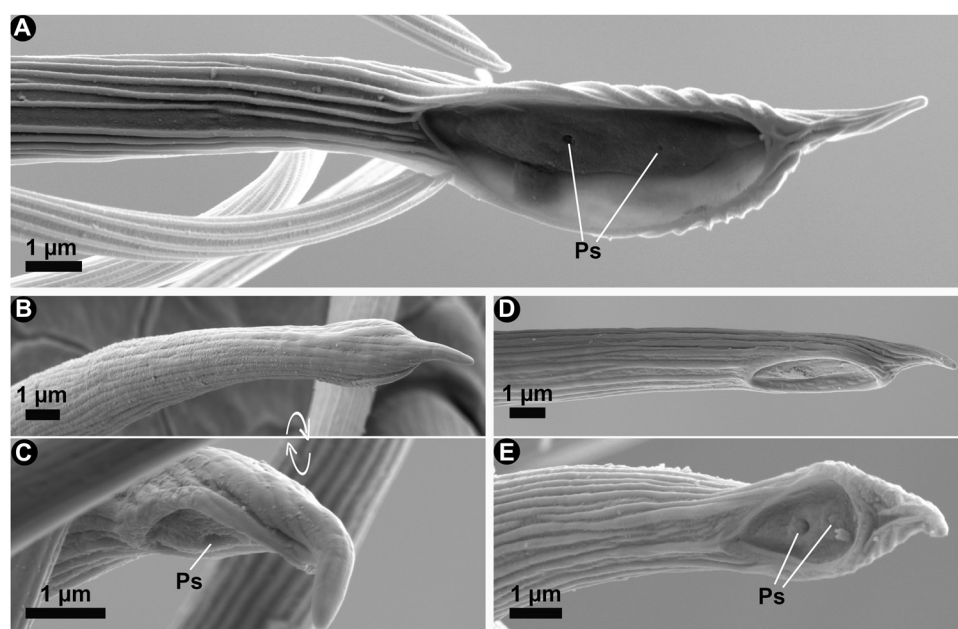


Figure 5. Laniatores. Tip of the distalmost sensillum of the triad (hooded sensillum) in selected families of Laniatores, leg pairs I (A–D) and II (E). A, cf. *Pyramidops* sp. (Pyramidopidae), undetermined sex. B, C, *Montalenia* sp. (Assamidae), male. D, E, *Baculigerus milenae* (Escadabiidae), male. Circular arrows indicate different views of the same unit. Ps: pore-like structure.

in Laniatores. We observed no articulation membrane fusion between the three sensilla and three shafts are more interspaced than in Laniatores (Figs 7A, 8A).

Eupnoi

Tarsi I and II of Eupnoi species also have three sensilla with short conic shafts and flat articulation membranes in the same corresponding position as Laniatores, which we refer to as sensilla basiconica. These structures differ from the ones in Laniatores in their disposition and morphology of the shaft opening. In the unidentified phalangiid species (Eupnoi, Phalangiidae), both legs I and II have a triad of sensilla basiconica in the distal third of the tarsomere (Fig. 9A–D). In its proximal third, the dorsal surface of the last tarsomere also has two and four additional isolated sensilla basiconica on legs I and II, respectively (Fig. 9A). In *Astrobus grallator* (Sclerosomatidae), a similar triad of sensilla basiconica occurs in the distal third of legs I, but legs II have only isolated sensilla basiconica in a dorsal position, totalling seven. Legs I also have two isolated sensilla basiconica (Fig. 10A, B) in the proximal third of the tarsomere and thus the total number in the tarsomere is five sensilla. The apical portion of the sensilla basiconica of the unidentified phalangiid has a pore-like structure (Fig. 9B–D), while in *A. grallator* there is a pore opening with a slit below it (Fig. 10C). This second condition is similar to the morphology observed in *Anelasmacephalus* sp. (Dyspnoi, Fig. 8C). Even though the three shafts

in the triad are similar in shape, the three shafts are progressively longer in the unidentified species (Phalangiidae), with the most distal being the longest and thickest (Fig. 9C, D). In *A. grallator* the three shafts have a similar length (Fig. 10C).

Cyphophthalmi

The distal third of the last tarsomeres of legs I and II of the cyphophthalmid species studied show a structure termed the subapical process (Fig. 11). Juberthie (1979, 1988, 2000) first described the subapical process, which occurs isolated on the distal third of tarsomeres I and II only, of males and females of some Cyphophthalmi species. Willemart & Giribet (2010) later found this structure to be widespread in Cyphophthalmi. Our investigation of two Cyphophthalmi species, belonging to one family each (Troglosironidae and Pettalidae), confirms the occurrence of this structure in the corresponding position of the hooded sensillum and sensilla basiconica found in the other suborders. In the species here investigated, this single sensillum occurs among other types of sensilla (solenidia, sensilla chaetica), but no short and conic sensilla, such as sensillum basiconicum, is present in the tarsomere (Fig. 11A). The shaft is wide at the base (~6.5 µm) and tapers to a fine rounded tip (Fig. 11C), possessing a high density of longitudinal ridges (Fig. 11B, inset). The apical portion has no subterminal swelling and no pore-like structures.

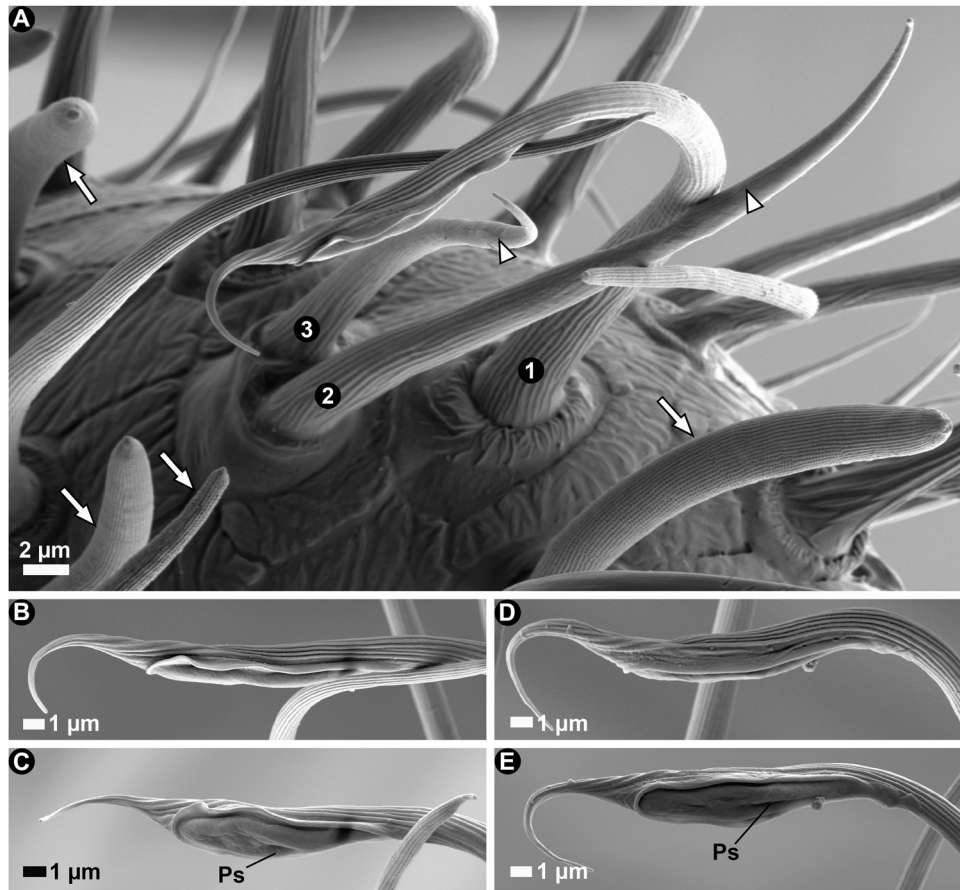


Figure 6. Laniatores. Triad on the sensory field of *Synthetonychia glacialis* (Sythetonychiidae) (male). A, Overview of the three sensilla (1–3), on the distal third of the distalmost tarsomere I, dorsal region. B–E, comparison of the ‘hood’ of the distal sensillum between legs I (B, C) and II (D, E). Ps: pore-like structure; white arrow: wall-pored sensilla chaetica; white arrowhead: slit opening; 1: hooded sensillum; 2: middle sensillum basiconicum; 3: proximal sensillum basiconicum.

CHARACTERS AND CODING

In the previous section, we described the comparative incidence of sensilla with external morphological similarities in the shaft and articulation membrane, with a restricted distribution to the distal third of the distalmost tarsomeres of legs I and II of representatives of all suborders of Opiliones. In order to understand the variation in Phalangida (Eupnoi+Dyspnoi+Laniatores), we established morphological characters and hypothesized, based on position and morphological similarity, that the subapical process of Cyphophthalmi is homologous to the hooded sensillum of Phalangida.

This conserved sensory field shows variation in the association of the articulation membrane between the shafts, composition of sensillar types and shaft morphology. In order to trace how this sensory field has been modified across Opiliones evolution, we propose the following characters and character states:

- Character 1: Sensory cluster, number of sensilla. States: (1a) one, (1b) three.
- Character 2: Sensory cluster, articulation membrane configuration. States: (2a) distal, mid and proximal sensilla not fused (notation: ‘1 2 3’); (2b) distal sensillum isolated, mid and proximal sensilla fused (notation: ‘1 (2 3)’); (2c) distal, mid and proximal sensilla fused (notation: ‘(1 2 3)’).
- Character 3: Hooded sensilla, hood morphology. States: (3a) terminal swelling (spoon-shaped), (3b) sub-terminal swelling (‘death’s hood’), (3c) no swelling (regular).
- Character 4: Mid and proximal sensilla, apical opening: (4a) pore-like, (4b) complete slit.

Supporting Information, File S2 contains the list of features organized by characters and character states for all species studied. States

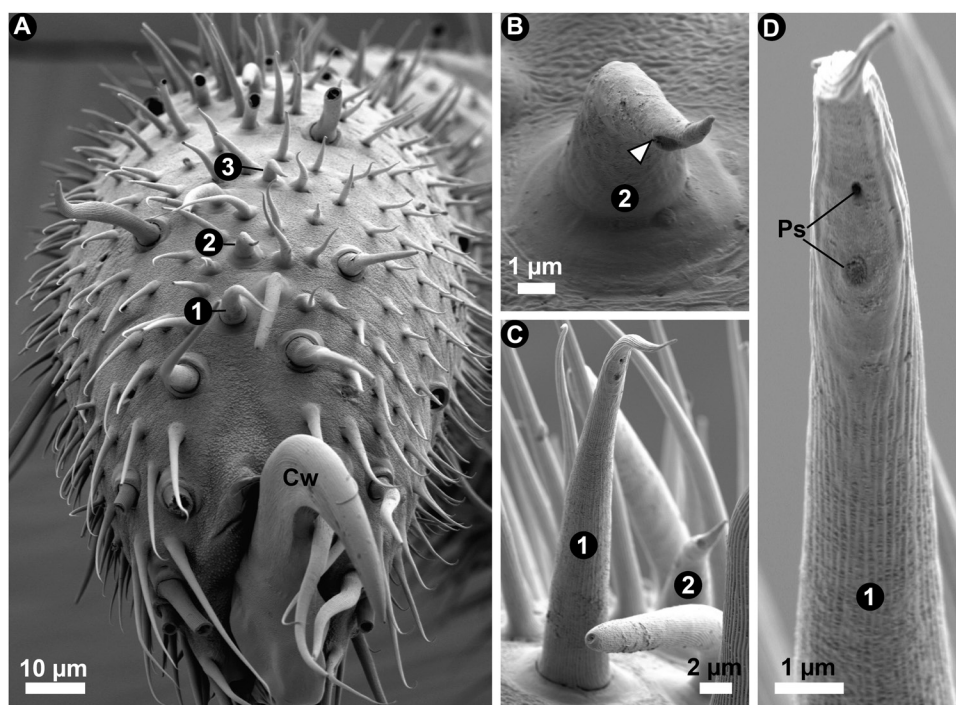


Figure 7. Dyspnoi. Distalmost tarsomere of leg pair II of *Nemastoma bimaculatum* (Nemastomatidae), undetermined sex. A, frontal view of the tarsomere. B, Detail of the middle sensillum in 'A' (2). C, the distal sensillum marked in 'A' (1), frontal view. D, detail of the tip of the distal sensillum in 'C'. Cw: claw; Ps: pore-like structure; white arrowhead: slit opening; 1: hooded sensillum; 2: middle sensillum basiconicum; 3: proximal sensillum basiconicum.

listed are representative of the morphology of leg pairs I and II, unless otherwise stated in the few cases of serial polymorphisms observed. In *Avima octomaculata* Agoristenidae (Laniatores), *Poassa limbata* (Laniatores, Nomoclastidae) and *Astrobunus grillator* (Eupnoi, Sclerosomatidae) legs I and II have different character states (for character 2). Apart from that, species in which we could access the character in both legs always showed the same character state and morphology (27 species). In order to ensure comparability, we chose to code leg pair I for our analysis. For eight species in which legs I could not be accessed properly for character 2, the sensilla on leg pair II was used as proxy for coding leg pair I (species marked with asterisk in [Supporting Information, File S2](#)).

ANCESTRAL STATE RECONSTRUCTION

Variation in the number of sensilla composing the sensory field (distal-third regions of the distalmost tarsomeres of legs I and II) (character 1) has two equally parsimonious optimizations (cost = 2; PTP < 0.05; mean: 2.97; median: 3): either having one sensillum (1a, white) is the ancestral state of

Opiliones, which has increased to three sensilla (1b, black) in Phalangida (Eupnoi+Dyspnoi+Laniatores); or having three sensilla is the ancestral state of Opiliones (1b, black), which has become secondarily reduced to one sensillum (1a, white) in Cyphophthalmi ([Fig. 12](#)). Reversion from three (1b, black) to one sensillum (1a, white) is unambiguously recovered in the laniatorean family Sandokanidae (*Sandokan truncatus*) ([Fig. 12](#)).

Association between the articulation membranes of the three sensilla (character 2) is inapplicable for Cyphophthalmi species and ambiguous for the most recent common ancestor of Phalangida and Palpatores ([Fig. 13](#)). The ancestral state in Laniatores is recovered as '1 (2 3)'. Several transformations occur in the equally parsimonious reconstructions (cost = 9; PTP < 0.05; mean: 18.69; median: 19), with ambiguous ancestral states in most cases. The character state, all sensilla fused (2c, black), occurs independently in some families of Laniatores (Podoctidae, Stygnommatidae, Biantidae and Agoristenidae) and is recovered as the ancestral character state for the Gonyleptoidea clade that excludes Stygnopsidae, Agoristenidae and Stygnidae ([Fig. 13](#)). Three separated sensilla (2a, white) were

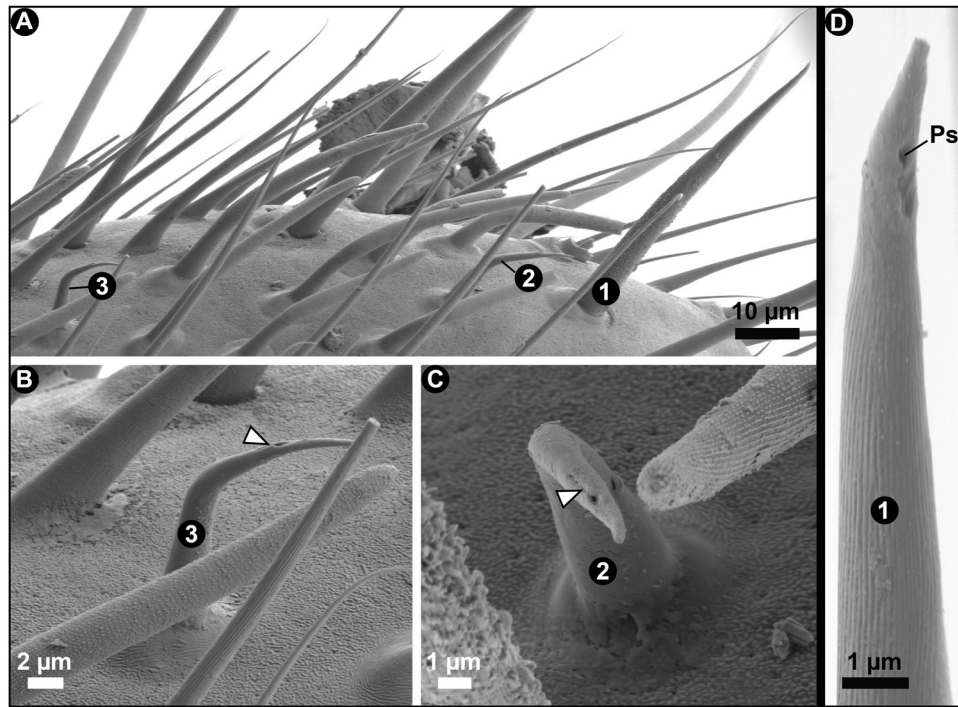


Figure 8. Dyspnoi. *Anelasmacephalus* sp., undetermined sex. A, dorsal region of the distal third of the distalmost tarsomere II, showing the three sensilla with flat articulation membranes (see text for explanation), lateral view. B, proximal sensillum (3) of the three marked in 'A', lateral view. C, middle sensillum (2) on the same region of leg I, frontal-superior view. D, detail of the tip of the distal sensillum (1) in 'A', lateral view. Ps: pore-like structure; white arrowhead: slit opening; 1: hooded sensillum; 2: middle sensillum basiconicum; 3: proximal sensillum basiconicum.

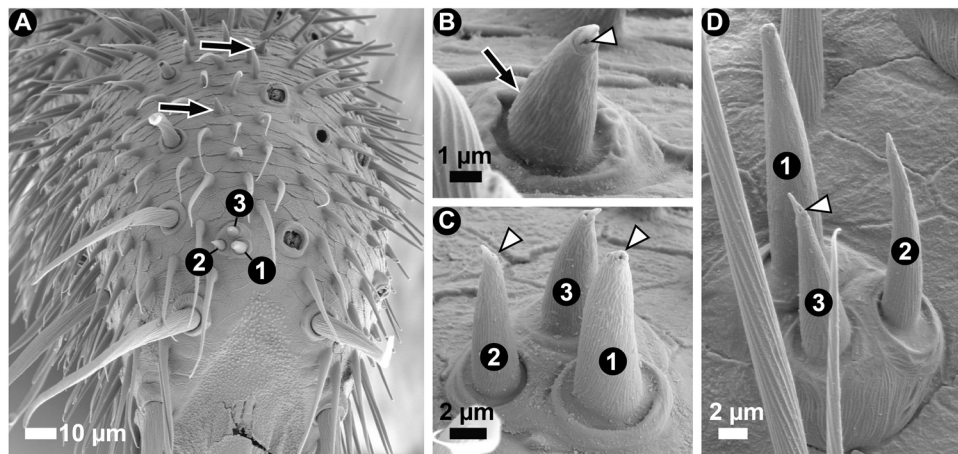


Figure 9. Eupnoi. Unidentified species (Phalangiidae), female. A, frontal view of the last tarsomere I. B, detail of an isolated sensillum basiconicum proximal to the triad. C, detail of the triad (1–3) on the distal third of the tarsomere I shown in 'A'. Note the all three sensilla share the same articulation membrane. D, detail of the same region on leg II, showing the triad (1–3). Note that the three sensilla have increasing length of the shaft, from proximal to distal (3 to 1). Black arrow: proximal sensilla basiconica; white arrowhead: apical opening; 1: hooded sensillum; 2: middle sensillum basiconicum; 3: proximal sensillum basiconicum.

acquired independently in Dyspnoi and a clade inside the laniatorean superfamily Gonyleptoidea (Metasarcidae+Cometidae+Gonyleptidae) (Fig. 13).

Character 3 concerns the tip of the distal sensillum with hood morphology, which is a condition that is not directly applicable in Cyphophthalmi and Eupnoi,

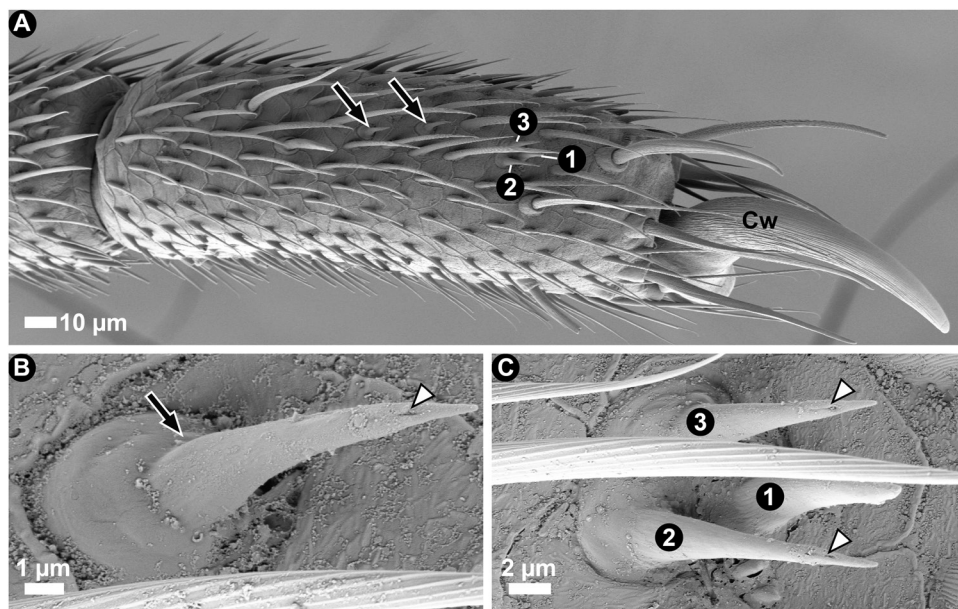


Figure 10. Eupnoi. *Astrobunus grillator* (Sclerosomatidae), undetermined sex. A, lateral view of the last tarsomere I, indicating three sensilla (1–3) clustered on the distal-third region, and two isolated sensilla basiconica on the middle of the tarsomere. B, detail of an isolated sensillum basiconicum on the last tarsomere I, dorsal region. C, detail of the three sensilla clustered on 'A'. Black arrow: proximal sensilla basiconica; Cw: claw; white arrowhead: apical opening; 1: hooded sensillum; 2: middle sensillum basiconicum; 3: proximal sensillum basiconicum.

since the distalmost sensillum in Cyphophthalmi apparently has no pore and in Eupnoi the pore is not subterminal. Therefore, coding encompasses the comparable hood morphology observed in Dyspnoi and Laniatores only. Character 3 has undergone several changes in the phylogeny and is ambiguously optimized (cost = 8; PTP < 0.05; mean: 11.91; median: 12). In the clade Grassatores, five transformation events are unambiguous (Fig. 14). Terminal swelling (3a, white) has independently evolved from subterminal swelling (3b, green) in the laniatorean families Podoctidae, Biantidae and Guasiniidae. No swelling (3c, black) has independently evolved two times: in the family Stygnopsidae (Gonyleptoidea) and in a clade inside Gonyleptoidea including the families Metasarcidae, Cosmetidae and two Gonyleptidae (Fig. 14).

Finally, the type of apical opening of the mid and proximal sensilla (character 4, inapplicable for Cyphophthalmi) is ambiguously reconstructed in Phalangida (cost = 1; PTP < 0.05; mean: 4.91; median: 5) (Fig. 15), but having a pore-like opening (4a, white) is clearly ancestral for Palpatores (Eupnoi+Dyspnoi), whereas having a complete slit (4b) is unambiguously ancestral for Laniatores.

Ancestral state reconstructions under maximum likelihood using a one-parameter Markov model yielded virtually identical ancestral states for all nodes (Supporting Information, File S4).

DISCUSSION

THE TRIAD IN LANIATOIRES, DYSPNOI, EUPNOI AND CYPHOPHTHALMI

We show that hooded sensillum and a pair of sensilla basiconica on the sensory appendages are morphologically conserved across Laniatores. The triad shows four marked characteristics in Laniatores: (1) occurrence on the distal third of the distalmost tarsomeres in legs I and II, dorsally; (2) distal sensillum externally different from mid and proximal sensilla; (3) an increasing length of the sensillar shafts, from proximal to distal; and (4) distal sensillum (hooded sensillum) occurring only on this region of leg pairs I and II. We assumed a topographic/positional homology and used these four characteristics as criteria for homology of comparable structures across Opiliones. We discuss the remaining three suborders in turn.

The Dyspnoi species here investigated meet all four criteria. The distal sensillum in Dyspnoi has remarkable similarity with the external morphology of laniatorean hooded sensillum, having a hood with two pore-like openings (compare Figs 7, 8 to Figs 4, 5).

In Eupnoi, at least criteria 1, 3 and 4 are met (Figs 9, 10). We did not investigate in detail the morphology of each sensillum in the triad, so we provisionally interpret them as the same type of sensillum, leaving criterion 2 to be investigated in the future. In the Phalangidae

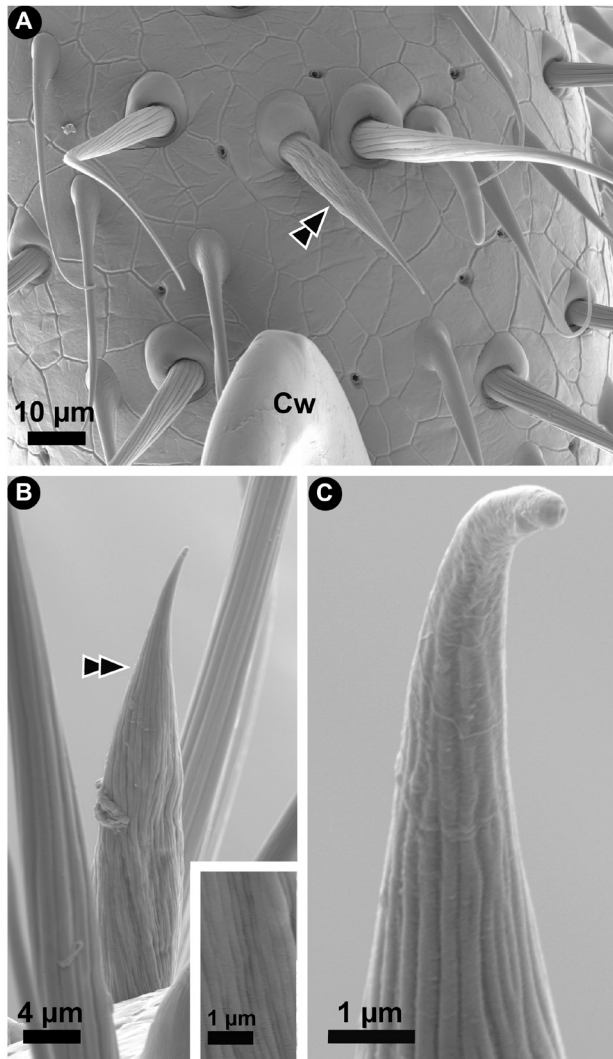


Figure 11. Cyphophthalmi. *Aoraki longitarsa* (Pettalidae), male. A, frontal view of the tarsi II, showing the subapical process. B, frontal view of the subapical process. Insight: detail of the shaft's wall, showing longitudinal ridges. C, detail of the tip of the subapical process in 'b'. Cw: claw; double arrowhead: subapical process.

species, the three shafts have an increasing length, which follows criteria 3 of Laniatores (Figs 9, 10). Even though sensilla basiconica occur in other body regions in Eupnoi species (Willemart *et al.*, 2009; Wijnhoven, 2013), the triad (or 'trident', *sensu* Wijnhoven, 2013) occurs only on legs I and II (unidentified Phalangidae; Fig. 9) or only on legs I (*Astrobinus grillator*, Sclerosomatidae; Fig. 10), meeting our criterion 4.

Cyphophthalmi show no sensilla basiconica (see also: Willemart & Giribet, 2010) and have a subapical process, a bottle-shaped sensillum on the distal third of the tarsi of pair of legs I and II only (Fig. 11; Juberthie, 1979, 1988, 2000; Willemart & Giribet, 2010). Since

sensilla basiconica do not occur in this group, criteria 2 and 3 are inapplicable. Nevertheless, the positional criterion (1) and the characteristic occurrence on the sensory appendages (2) support our hypothesis that the subapical process is homologous to what is observed in the corresponding position of other suborders. Since no sensilla basiconica occur, we assume, under positional homology, that the subapical process corresponds to the distal sensillum of the triad in Eupnoi, Dyspnoi and Laniatores.

Given this positional/topographic congruence and the list of similarities, we find it reasonable to trace a correspondence between these structures and consider it to be a conserved sensory field in the sensory appendages of all Opiliones, an idea further supported by the available ultrastructural information on a laniatorean species (Gainett *et al.*, 2017b; also see below).

THE MORPHOLOGY OF THE TRIAD INFORMS DIFFERENT PHYLOGENETIC LEVELS

Using a supertree phylogeny of Opiliones, we conducted an ancestral state reconstruction to test the homology of these structures and to investigate how specific character states have been modified during harvestman diversification. We recognize the variation observed as belonging to four discrete characters, pertaining to number of sensilla (character 1), association of articulation membranes (character 2), shape of the hood (character 3) and shape of the apical portion of mid and proximal sensilla (character 4). These characters display different degrees of homoplasy, but all exhibit high phylogenetic structure, as inferred from the low values of the permutation tail probability test ($PTP < 0.05$). Below, we address the contribution of each character at different levels of the Opiliones phylogeny, with emphasis on Laniatores.

Ordinal and supra-ordinal level

Modification of the number of sensilla in the cluster is an event that occurred early in Opiliones, but given the difficulty in comparing the character states with the divergent morphology of outgroup orders, the ancestral state of Opiliones is untested for this character. At shallow taxonomic levels, this character is mostly invariable, as observed in Laniatores. Despite this ambiguity, the disposition of this character state mirrors the early split of Cyphophthalmi and Phalangida, with either a reduction to a single shaft in Cyphophthalmi, or transition to three shafts in Phalangida. The same reasoning applies to the variation in the apical opening of the mid and proximal sensilla in Phalangida: pore-like opening and complete slit opening are, respectively, characteristics of

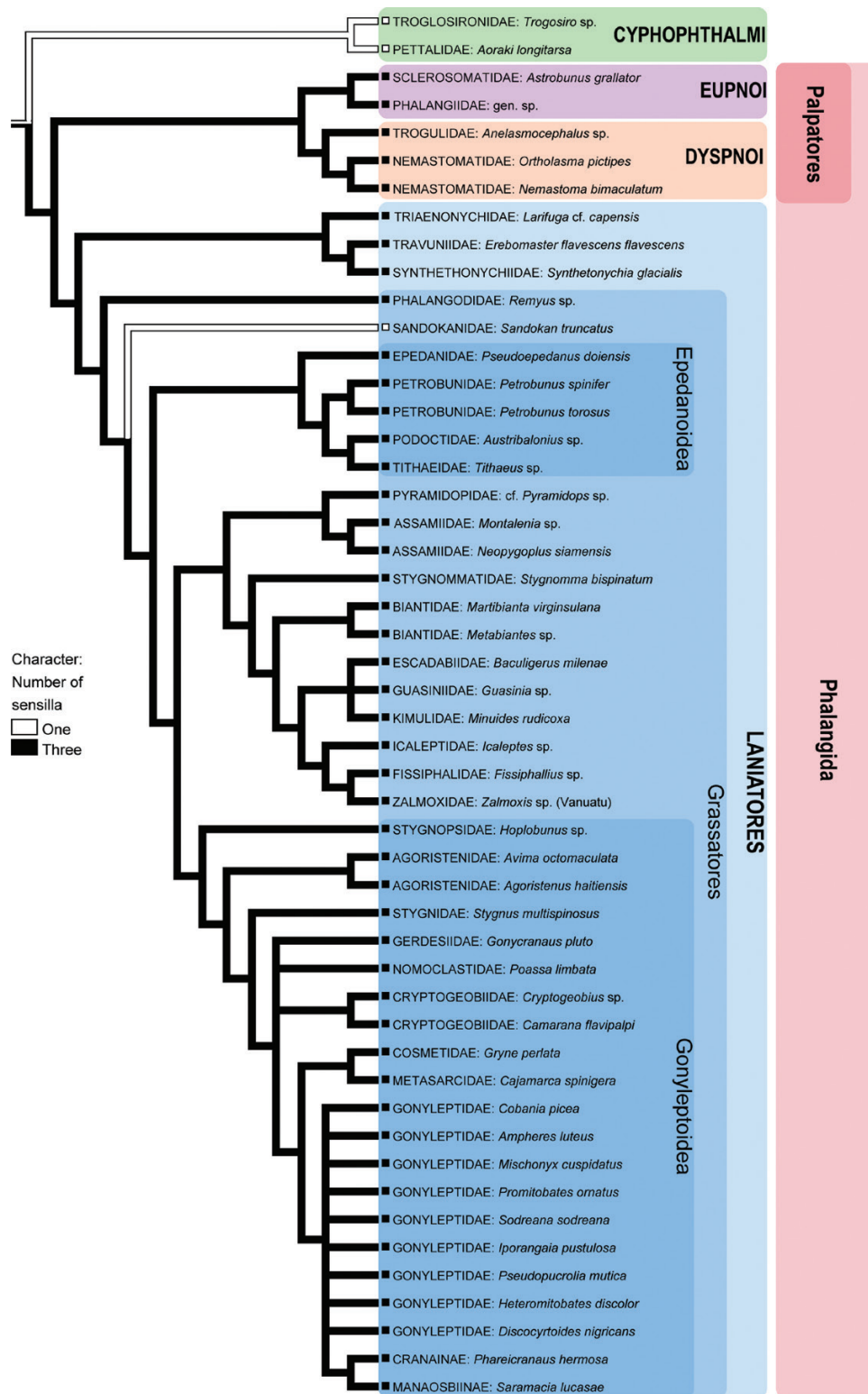


Figure 12. Ancestral state reconstruction of 'character (1) number of sensilla' on a compiled phylogeny of Opiliones after Giribet & Sharma (2015) and Fernández *et al.* (2017). White: (1a) one. Black: (1b) three. Branches with more than one colour represent equally parsimonious reconstructions.

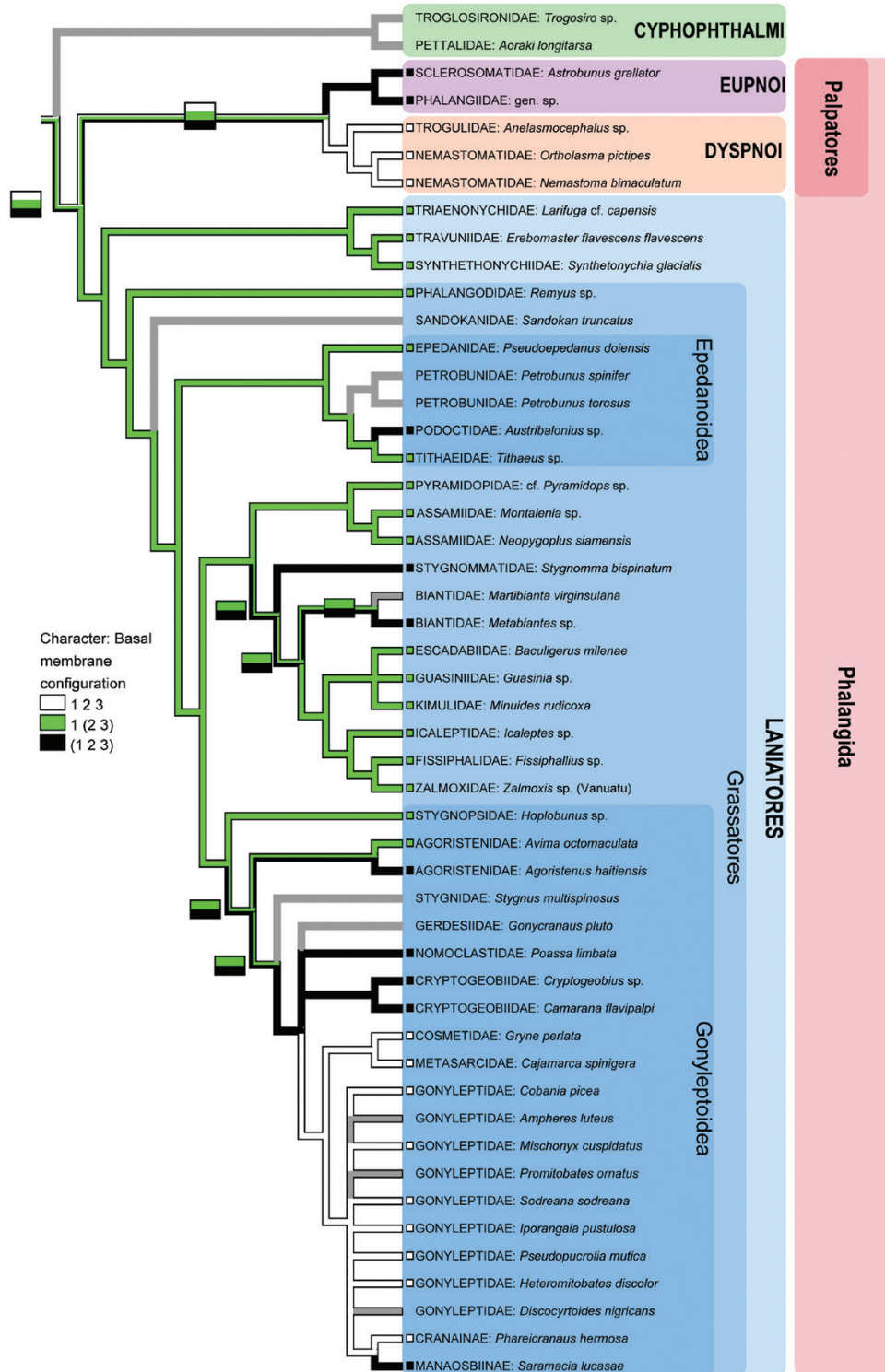


Figure 13. Ancestral state reconstruction of ‘character (2) articulation membrane association’ on the same topology as in Figure 12. White: (2a) distal, middle and proximal sensilla not fused. Green: (2b) distal sensillum isolated, middle and proximal sensilla fused. Black: (2c) distal, mid and proximal sensilla fused. Branches with more than one colour represent equally parsimonious reconstructions. This character is not applicable to the terminals in Cyphophthalmi, and coding is unclear for Sandokanidae (Laniatores). Other terminals with grey branches are missing data.

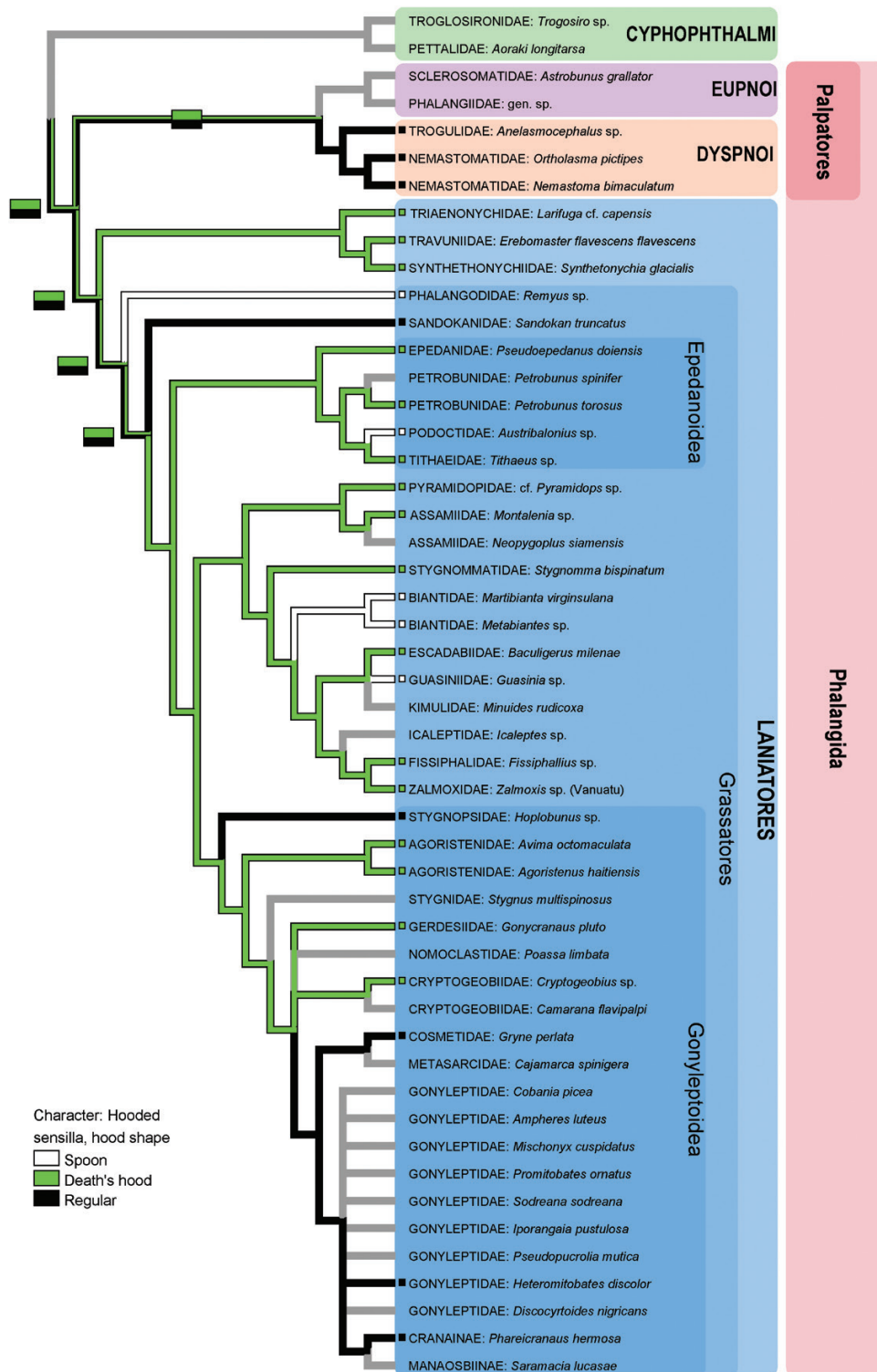
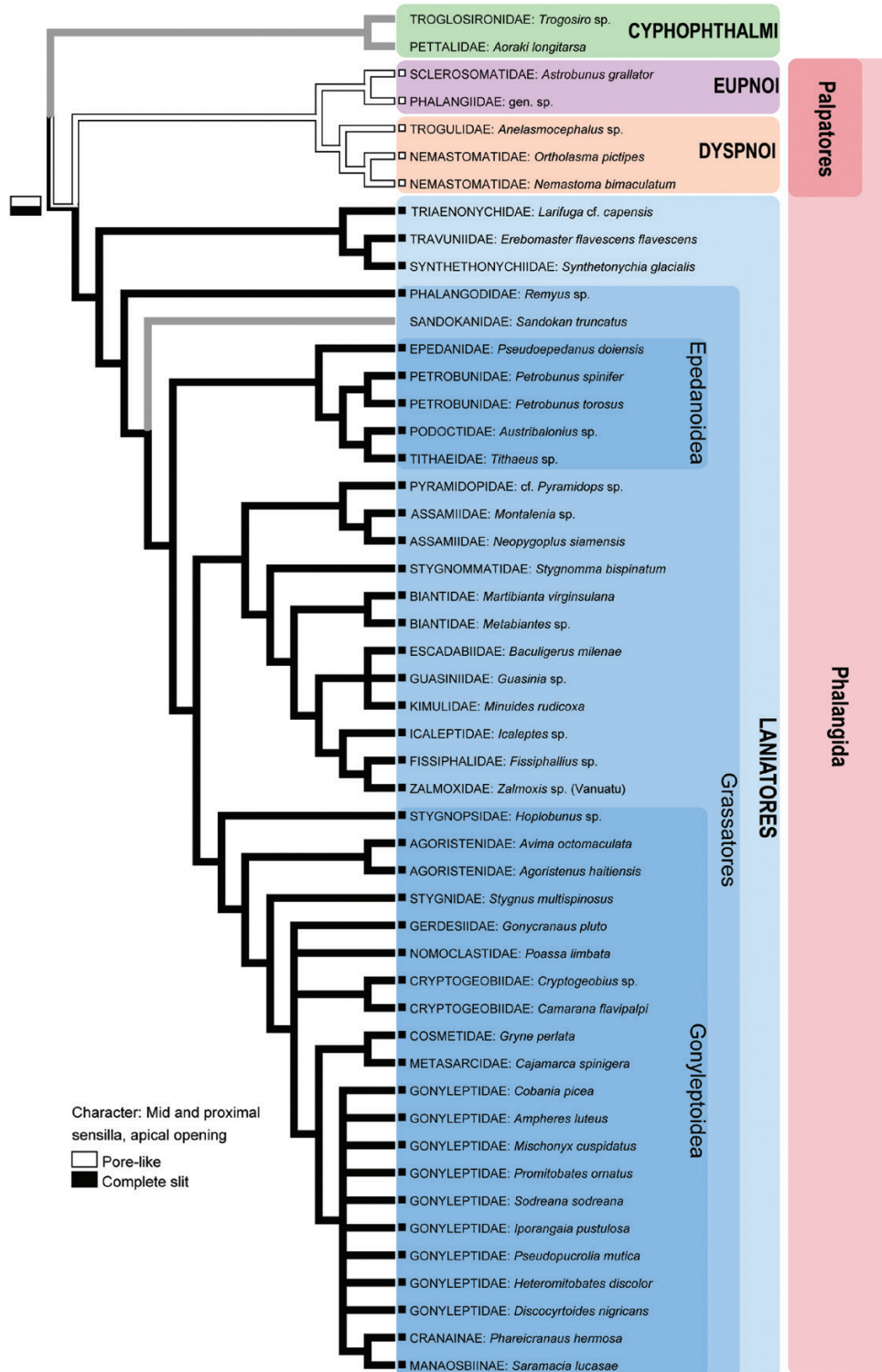


Figure 14. Ancestral state reconstruction of ‘character (3) hooded sensilla, hood morphology’ on the same topology as in Figure 13. White: (3a) terminal swelling (spoon-shaped). Green: (3b) sub-terminal swelling (‘death’s hood’). Black: (3c) no swelling (regular). Branches with more than one colour represent equally parsimonious reconstructions. This character is not applicable to the terminals in Cyphophthalmi and Eupnoi. Other terminals with grey branches are missing data.



Palpatores and Laniatores. Ambiguity in the ancestral state of this character in Phalangida could possibly be resolved in future surveys with a denser sampling of Eupnoi and Dyspnoi. Specifically, studying the triad in a member of Acropsopilionidae could help refine the character states in the basal split of Dyspnoi, whereas studying the triad in Caddidae would be key to understand the evolution of this character in Eupnoi.

Superfamilial level

Gonyleptoidea is the largest superfamily of Laniatores, including the second most diverse harvestman family, Gonyleptidae (Caetano & Machado, 2013; Pinto-da-Rocha *et al.*, 2014). Relationships in Gonyleptoidea have been addressed extensively using molecular markers (Sharma & Giribet, 2011; Pinto-da-Rocha *et al.*, 2014; Fernández *et al.*, 2017). Considerable changes in the topology have been made since the first cladistic hypothesis by Kury (1993), such as the erection of the families Cryptogeobiidae (Kury, 2014), Gerdesiidae (Bragagnolo *et al.*, 2015) and Metasarcidae (Benedetti, 2012; Pinto-da-Rocha *et al.*, 2014).

A condition with all three sensilla separated (2a, white) is synapomorphic for some families in the superfamily Gonyleptoidea, namely, Gonyleptidae (including Manaosbiinae and Cranainae), Comestidae and Metasarcidae [Microsetata *sensu* Kury (2014)]. This group has been recovered in molecular phylogenetic analyses of Gonyleptoidea with high support and nodal stability (Pinto-da-Rocha *et al.*, 2014) and morphologically it is supported by the presence of a conspicuous covering of microsetae on the penis (Kury, 2014). A relationship between these families is substantiated by the shared condition of a hooded sensillum without swelling (character 3, character state 3c, black), which is recovered as an unambiguous synapomorphy of this clade.

Nonetheless, a true Nomoclastidae was not included in the analysis of Pinto-da-Rocha (2014) and other phylogenetic studies have either not included Metasarcidae (Giribet *et al.*, 2010; Sharma & Giribet, 2011) or excluded both families (Sharma & Giribet, 2014; Fernández *et al.*, 2017). However, an unpublished phylogenomic analysis sampling Microsetata and Nomoclastidae recovers the family Nomoclastidae as sister group to Gonyleptidae and both families as sister to the clade Cosmetidae+Metasarcidae (R. Pinto-da Rocha, G. Giribet, pers. comm.), thus rendering Microsetata paraphyletic. To accommodate this forthcoming tree topology, a separate family of analyses was conducted herein with the same character states and terminals, but with an updated phylogenetic hypothesis. Under this new hypothesis, the ancestral state of the clade Cosmetidae+Metasarcidae+Nomoclastidae+Gonyleptidae becomes

ambiguously optimized, given the character state of *Poassa limbata* (Nomoclastidae) (2c, ' (1 2 3) ') (Supporting Information, File S3). It is worth noting that the species of Nomoclastidae sampled here is one of the few families that present serial dimorphism for character 2: leg I has all three sensilla fused, whereas leg II has the condition present in the other three families (namely, with all three sensilla separated). Therefore, sampling additional nomoclastids for character 2 could potentially elucidate the diagnosis of the suprafamilial relationships of these four families.

Familial level

In Laniatores, the loss of two sensilla basiconica associated with the hooded sensillum (character 1, character state 1a, white) is a condition exclusively found in *Sandokan truncatus* (Sandokanidae) (Fig. 12). The placement of this family in the Laniatores phylogeny remained elusive in recent attempts to reconstruct its history (Schwendinger, 2007; Sharma & Giribet, 2009, 2011; Giribet *et al.*, 2010), but a transcriptome-based approach has recovered it as a basally branching Grassatores (Fig. 1; Fernández *et al.*, 2017). Sandokanids have several morphological autapomorphies, such as the complete fusion of the carapace and opisthosomal tergites (scutum completum, a condition also present in cyphophthalmids) and reduced or undivided tarsomeres (Schwendinger, 2007; Sharma & Giribet, 2009; Gainett *et al.*, 2018). This is also the only Laniatores family lacking the metatarsal paired slits, a synapomorphic slit sense organ for the suborder (Gainett *et al.*, 2014). It should be noted that even though a triad is not observed in sandokanids, they have putative sensilla basiconica on tarsi I and II and in other regions of the body, as in other Laniatores. The tip of these sensilla show a series of vertical pore openings instead of the complete slit typical of Laniatores (Gainett *et al.*, 2014, 2018).

Three sensilla sharing the same articulation membrane (character 2, character state 2c, black) have been independently acquired in the laniatorean species studied of the families Podoctidae, Stygnommatidae, Biantidae and Cryptogeobiidae, although ambiguous optimization does not resolve whether the character states in Stygnommatidae and Biantidae are homologous. The association of the three shafts in Cryptogeobiidae is interesting, because this group was previously considered as part of the subfamily Tricommatinae in the family Gonyleptidae (Pinto-da-Rocha & Giribet, 2007; Kury, 2014). Therefore, the autapomorphic condition in Cryptogeobiidae is in accordance with the recent erection of this family as a group outside Gonyleptidae (Kury, 2014; Pinto-da-Rocha *et al.*, 2014); with the later family having all three shafts separated (character state 2a).

The shape of the hood of the hooded sensillum as a terminal swelling (3a, white) was independently acquired in Podoctidae, Biantidae, Guasiniidae and Phalangodidae. Testing if these character states are diagnostic of these families will require denser sampling at the familial level and is beyond the scope of this work.

Other sources of characters

The only laniatorean species in which we found different character states between legs I and II (character 2) are *Avima octomaculata* (Agoristenidae) and *Poassa limbata* (Nomoclastidae). Thus, it is possible that having a serial dimorphism in the degree of fusion of the three shafts (character 2) is a characteristic restricted to these families. Similarly, serial dimorphism has also been observed in the tarsal organ of spiders, in which a dimorphism in the shape of the sensilla of the tarsal organs of anterior and posterior legs has been suggested as a synapomorphy of the family Oonopidae (Platnick *et al.*, 2012). A similar, but independent condition occurs in the Eupnoi *Astrobonus grallator* (Sclerosomatidae), in which legs I have the three sensilla clustered and legs II have isolated ones (Fig. 10). Interestingly, Wijnhoven (2013) reported three clustered sensilla (or 'trident') on the distalmost region of the last tarsomere I and last segment of the pedipalps of *Dicranopalpus ramosus* (Phalangiidae). Therefore, it would be interesting to investigate if the same condition occurs in *A. grallator* and if it has any taxonomic value.

In summary, this sensory field on the sensory appendages is widespread in harvestmen and constitutes a promising source of information to be explored at several levels of phylogenetic relationships.

A WIDESPREAD TARSAL ORGAN

Relatively few studies have been conducted on the sensory structures of harvestmen in comparison with most arachnid orders (Foelix, 1985; Willemart *et al.*, 2009). Most of what is known about the function of specific sensillar types in harvestmen comes from studies in the suborder Laniatores, including data on the function of sensilla basiconica and the hooded sensillum (Willemart *et al.*, 2009; Gainett *et al.*, 2017b). Therefore, we base our discussion on the triad observed in this suborder. We show that the pair of sensilla basiconica and the hooded sensillum are phylogenetically conserved in Laniatores and with corresponding structures in Eupnoi, Dyspnoi and Cyphophthalmi. This conserved association indicates that they might be functionally linked, possibly operating as a sensory unit or organ. In *Heteromitobates discolor* (Laniatores, Gonyleptidae), the dendrites innervating the three shafts are

concentrically arranged (Gainett *et al.*, 2017b). The three shafts are inserted in a relatively thinner area of the dorsal cuticle and their inner parts occupy a considerable space of the distal third of the terminal tarsomeres of legs I and II, which is unusual when compared with the remaining tarsal sensilla. Moreover, in several species the three shafts share the same articulation membrane. Therefore, the widespread phylogenetic conservatism and morphological evidence of association suggest that they may function as a joint sensory organ at the tip of the sensory legs.

Ultrastructural data (*H. discolor*) supports a hygro-/thermoreceptive function for sensilla basiconica, although the function of the hooded sensillum remains unclear (hygro-/thermoreceptive and/or chemoreceptive), due to its unique ultrastructural characteristics, such as a double innervation of the shaft (suggesting a fusion of two sensilla into one shaft), two pore-like structures at the tip of the shaft and unusual meshed structure of the shaft wall (Gainett *et al.*, 2017b). The complete slit opening of the sensilla basiconica has been argued to be related to the transduction mechanism involved in hygroreception (Gainett *et al.*, 2017b). This feature is conserved in all Laniatores species studied, but is different in Eupnoi and Dyspnoi. Expanding knowledge of the distribution of this triad to the whole suborder Laniatores provides a unique opportunity for generalizing these functional inferences for a large portion (two-thirds) of Opiliones species. Given the variations of the external morphology of the three sensilla in Eupnoi and Dyspnoi, and the occurrence of a single sensillum in Cyphophthalmi, it remains imperative to investigate ultrastructure in these suborders, both to test the homology scheme proposed here and to investigate if they are functionally similar to what has been observed in Laniatores.

A phylogenetically conserved cluster of sensilla on the distal tarsomeres of the legs have also been observed in other arachnid orders. These so called 'tarsal organs' have been reported in Araneae, Amblypygi, Scorpiones, Parasitiformes (Haller's organ) and Ricinulei (pore organ) (Blumenthal, 1935; Foelix & Axtell, 1972; Foelix & Chu-Wang, 1973; Foelix *et al.*, 1975; Foelix & Schabronath, 1983; Anton & Tichy, 1994; Tichy & Loftus, 1996; Talarico *et al.*, 2005) (Table 1). In Parasitiformes, the Haller's organ sensilla contain olfactory and hygro-/thermoreceptors (Foelix & Axtell, 1972; Hess & Vlimant, 1983; Hess & Loftus, 1984). In the spider *Cupiennius salei* Keyserling, 1877, tip-pored sensilla of the tarsal organ respond to humidity, temperature and chemical stimuli (Foelix & Chu-Wang, 1973; Ehn & Tichy, 1994). The tarsal organs of Ricinulei, Amblypygi and Scorpiones have never been studied with electrophysiology and their exact function is unclear, although ultrastructural data suggest at least an olfactory function in Ricinulei (Talarico *et al.*,

Table 1. Comparison of morphology, distribution and function between the reported tarsal organs in the arachnid orders Araneae, Amblypygi, Scorpiones, Parasitiformes, Ricinulei and Opiliones

	Araneae	Amblypygi	Scorpiones	Parasitiformes	Ricinulei	Opiliones
Appendages	Pedipalp; Legs I-IV	Legs I	Legs I-IV	Legs I	Legs I & II	Legs I & II
Position	Tarsus, close to the claw, dorsal	Tarsus, close to the claw, dorsal	Tarsus, close to the claw, dorsal	Tarsus, close to the claw, dorsal	Tarsus, close to the claw, dorsal	Tarsus, close to the claw, dorsal
Cuticular invagination?	Most cases in capsule, but may be exposed	No capsule	No capsule	Anterior capsule and posterior seta cluster	Capsule	Seta cluster
External appearance of the cuticular apparatus	Pore/small peg/button or setae	Button	Button/pore	Setae	Setae	Setae
Function	Thermo/hygro/chemo	?	?	Thermo/hygro/chemo	Chemo/Thermo/hygro?	Thermo/hygro/Chemo?
References	Blumenthal, 1935; Foelix & Chu-Wang, 1973; Dumpert, 1978; Tichy & Barth, 1992; Ehn & Tichy, 1994; Anton & Tichy, 1994; Tichy & Loftus, 1996	Foelix <i>et al.</i> , 1975; Igelmund, 1987; Santer & Hebets, 2011	Foelix & Schabronath, 1983	Foelix & Axtell, 1972	Talarico <i>et al.</i> , 2005	Willemart <i>et al.</i> , 2007, 2009; Gainett <i>et al.</i> , 2017a; this study

2005). Some of these tarsal organs have been suggested as homologous structures due to their common position (dorsal region, distalmost tarsomere, close to the claw; Foelix *et al.*, 1975; Foelix & Schabronath, 1983; Talarico *et al.*, 2005). The various tarsal organs in arachnids occur on different leg pairs, have different shapes and may occur exposed or inside invaginated portions of cuticle (capsules). It is unclear whether tarsal organs in arachnids, including the triad here reported in Opiliones, are homologous structures that have undergone diversification in each order, or if they represent cases of convergence. Still, it is fascinating that several arachnid orders show conserved multifunctional association of sensilla on the distalmost part of the legs, which include the only confirmed or putative hygro-/thermoreceptors reported for Arachnida. Relatively little is known about hygro- and thermoreceptors in arachnids, apart from the studies with the tarsal organ of Araneae (Blumenthal, 1935; Anton & Tichy, 1994; Ehn & Tichy, 1994; Tichy & Loftus, 1996) and the Haller's organ in Parasitiformes (e.g. Foelix & Axtell, 1972). Studying the previously known tarsal organs and the new structures here revealed on the sensory appendages of harvestmen may shed some light on the patterns of hygro- and thermoreceptor occurrence in arachnids and reveal if these sensillar associations on distal parts of the leg

are functional convergences or derived from a common ancestry.

CONCLUSION

We show that a pair of sensilla basiconica and one hooded sensillum, putatively hygro-/thermoreceptive sensilla, are a phylogenetically widespread sensory field on the sensory appendages of laniatorean species, with comparable structures in all other suborders of Opiliones. These structures show variation in several levels of Opiliones phylogeny and constitute a promising source of diagnostic synapomorphies for the group. Further investigating the ultrastructure and physiological responses of these structures in different harvestman suborders is imperative for supporting their use as diagnostic characters, understanding their importance for the biology of Opiliones and testing hypotheses of structural homology.

ACKNOWLEDGEMENTS

This work was supported by FAPESP grant 2013/23189-1 and 2014/07671-0 to Guilherme

Gainett, FAPESP grant 2010/00915-0 and 2015/01518-9 to RHW, FAPESP grant 2013/50297-0 to RPR, National Science Foundation (NSF) grant DOB 1343578, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and NASA. Guilherme Gainett acknowledges Enio Mattos, Phyllip Lenktaits (IB-USP), Adam Graham and Carolyn Marks (CNS-Harvard) for assistance with microscopy, Pedro Dias for assistance with Mesquite, members of the Laboratory of Sensory Biology and Behaviour of Arthropods (LESCA), Alípio Benedetti, Cristiano Sampaio, Jimmy Cabra and all members of Laboratório de Aracnólogos Legais (LAL) for helpful discussions. Alípio Benedetti kindly reviewed an earlier version of this manuscript. Three anonymous reviewers provided comments that improved an earlier version of this paper.

REFERENCES

- Anton S, Tichy H. 1994.** Hygro- and thermoreceptors in tip-pore sensilla of the tarsal organ of the spider *Cupiennius salei*: innervation and central projection. *Cell and Tissue Research* **278**: 399–407.
- Benedetti A. 2012.** *Revisão e análise cladística da subfamília Metasarcinae Kury, 1994 (Opiliones; Laniatores; Gonyleptidae)*. Unpublished MSc Thesis, Instituto de Biociências, Universidade de São Paulo.
- Blumenthal H. 1935.** Untersuchungen über das ‘Tarsalorgan’ der Spinnen. *Zeitschrift für Morphologie und Ökologie der Tiere* **29**: 667–719.
- Botero-Trujillo R, Flórez ED. 2011.** A revisionary approach of Colombian *Ananteris* (Scorpiones, Buthidae): two new species, a new synonymy, and notes on the value of trichobothria and hemispermaphore for the taxonomy of the group. *Zootaxa* **2904**: 1–44.
- Bragagnolo C, Hara MR, Pinto-da-Rocha R. 2015.** A new family of Gonyleptoidea from South America (Opiliones, Laniatores). *Zoological Journal of the Linnean Society* **173**: 296–319.
- Brazeau MD. 2011.** Problematic character coding methods in morphology and their effects. *Biological Journal of the Linnean Society* **104**: 489–498.
- Brozek J, Zettel H. 2014.** A comparison of the external morphology and functions of labial tip sensilla. *European Journal of Entomology* **111**: 275–297.
- Caetano DS, Machado G. 2013.** The ecological tale of Gonyleptidae (Arachnida, Opiliones) evolution: phylogeny of a Neotropical lineage of armoured harvestmen using ecological, behavioural and chemical characters. *Cladistics* **29**: 589–609.
- Derkarabetian S, Starrett J, Tsurusaki N, Ubick D, Castillo S, Hedin M. 2018.** A stable phylogenomic classification of Travunioidea (Arachnida, Opiliones, Laniatores) based on sequence capture of ultraconserved elements. *ZooKeys* **760**: 1–36.
- Dumpert K. 1978.** Spider odor receptor: Electrophysiological proof. *Experientia* **34**: 754–756.
- Ehn R, Tichy H. 1994.** Hygro- and thermoreceptive tarsal organ in the spider *Cupiennius salei*. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* **174**: 345–350.
- Faith DP, Cranston OS. 1991.** Could a cladogram this short have arisen by chance alone? On permutation tests for cladistic structure. *Cladistics* **7**: 1–28.
- Fernández R, Sharma P, Tourinho AL, Giribet G. 2017.** The Opiliones tree of life: shedding light on harvestmen relationships through transcriptomics. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20162340.
- Foelix RF. 1985.** Mechano- and chemoreceptive sensilla. In: Barth FG, ed. *Neurobiology of arachnids*. Berlin, Heidelberg: Springer-Verlag, 118–134.
- Foelix RF, Axtell RC. 1972.** Ultrastructure of Haller’s organ in the tick *Amblyomma americanum* (L.). *Zeitschrift für Zellforschung und mikroskopische Anatomie (Vienna, Austria: 1948)* **124**: 275–292.
- Foelix RF, Chu-Wang IW. 1973.** The morphology of spider sensilla. II. Chemoreceptors. *Tissue & Cell* **5**: 461–478.
- Foelix RF, Schabronath J. 1983.** The fine structure of scorpion sensory organs. I. Tarsal sensilla. *Bulletin of the British Arachnological Society* **6**: 53–67.
- Foelix RF, Chu-Wang IW, Beck L. 1975.** Fine structure of tarsal sensory organs in the whip spider *Admetus pumilio* (Amblypygi, Arachnida). *Tissue & Cell* **7**: 331–346.
- Gainett G, Sharma PP, Pinto-da-Rocha R, Giribet G, Willemart RH. 2014.** Walk it off: predictive power of appendicular characters toward inference of higher-level relationships in Laniatores (Arachnida: Opiliones). *Cladistics* **30**: 120–138.
- Gainett G, Michalik P, Müller CH, Giribet G, Talarico G, Willemart RH. 2017a.** Ultrastructure of chemoreceptive tarsal sensilla in an armored harvestman and evidence of olfaction across Laniatores (Arachnida, Opiliones). *Arthropod Structure & Development* **46**: 178–195.
- Gainett G, Michalik P, Müller CHG, Giribet G, Talarico G, Willemart RH. 2017b.** Putative thermo-/hygroreceptive tarsal sensilla on the sensory legs of an armored harvestman (Arachnida, Opiliones). *Zoologischer Anzeiger – A Journal of Comparative Zoology* **270**: 81–97.
- Gainett G, Sharma PP, Giribet G, Willemart RH. 2018.** The sensory equipment of a sandokanid: an extreme case of tarsal reduction in harvestmen (Arachnida, Opiliones, Laniatores). *Journal of Morphology* **279**: 1206–1223.
- Garwood RJ, Sharma PP, Dunlop JA, Giribet G. 2014.** A Paleozoic stem group to mite harvestmen revealed through integration of phylogenetics and development. *Current Biology* **24**: 1017–1023.
- Giribet G, Sharma PP. 2015.** Evolutionary biology of harvestmen (Arachnida, Opiliones). *Annual Review of Entomology* **60**: 157–175.
- Giribet G, Vogt L, González AP, Sharma P, Kury AB. 2010.** A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores. *Cladistics* **26**: 408–437.

- Giribet G, Sharma PP, Benavides LR, Boyer SL, Clouse RM, Bivort BLDE, Dimitrov D, Kawauchi GY, Murienne J, Schwendinger PJ. 2012.** Evolutionary and biogeographical history of an ancient and global group of arachnids (Arachnida: Opiliones: Cyphophthalmi) with a new taxonomic arrangement. *Biological Journal of the Linnean Society* **105**: 92–130.
- Groh S, Giribet G. 2015.** Polyphyly of Caddoidea, reinstatement of the family Acropsopilionidae in Dyspnoi, and a revised classification system of Palpatores (Arachnida, Opiliones). *Cladistics* **31**: 277–290.
- Hedin M, Starrett J, Akhter S, Schönhofen AL, Shultz JW. 2012.** Phylogenomic resolution of Paleozoic divergences in harvestmen (Arachnida, Opiliones) via analysis of next-generation transcriptome data. *PLoS ONE* **7**: e42888.
- Hess E, Loftus R. 1984.** Warm and cold receptors of two sensilla on the foreleg tarsi of the tropical bont tick *Amblyomma variegatum*. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* **155**: 187–195.
- Hess E, Vlimant M. 1983.** The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae, Metastriata). II. No pore sensilla. *Revue Suisse de Zoologie* **90**: 157–167.
- Igelmund P. 1987.** Morphology, sense organs, and regeneration of the forelegs (whips) of the whip spider *Heterophrynus elaphus* (Arachnida, Amblypygi). *Journal of Morphology* **193**: 75–89.
- Juberthie C. 1979.** Un Cyphophthalme nouveau d'une grotte de Nouvelle-Calédonie: *Troglosiro aelleni* n. gen., n. sp. (Opilion Sironinae). *Revue Suisse de Zoologie* **86**: 221–231.
- Juberthie C. 1988.** Un nouvel opilion cyphophthalme aveugle d'Australie: *Austropurcellia* gen. nov., *scoparia* n.sp. *Mémoires de Biospéologie* **15**: 133–140.
- Juberthie C. 2000.** A new blind Cyphophthalmi (Opiliones) from Queensland (Australia). *Mémoires de Biospéologie* **27**: 149–154.
- Karanovic T, Kim K. 2014.** Suitability of cuticular pores and sensilla for harpacticoid copepod species delineation and phylogenetic reconstruction. *Arthropod Structure and Development* **43**: 615–658.
- Kury AB. 1993.** *Análise filogenética de Gonyleptoidea* (Arachnida, Opiliones, Laniatores). Unpublished PhD Thesis, São Paulo: Universidade de São Paulo.
- Kury AB. 2013.** Order Opiliones Sundevall, 1833. *Zootaxa* **3703**: 27–33.
- Kury AB. 2014.** Why does the Tricommatinae position bounce so much within Laniatores? A cladistic analysis, with description of a new family of Gonyleptoidea (Opiliones, Laniatores). *Zoological Journal of the Linnean Society* **172**: 1–48.
- Kury AB, Villarreal MO. 2015.** The prickly blade mapped: establishing homologies and a chaetotaxy for macrosetae of penis ventral plate in Gonyleptoidea (Arachnida, Opiliones, Laniatores). *Zoological Journal of the Linnean Society* **174**: 1–46.
- Maddison WP, Maddison DR. 2011.** *Mesquite: a modular system for evolutionary analysis. Version 2.75.* Available at: <http://mesquiteproject.org>. Accessed on August 2016.
- Murphree CS. 1988.** Morphology of the dorsal integument of ten opilionid species (Arachnida, Opiliones). *Journal of Arachnology* **16**: 237–252.
- Pinto-da-Rocha R, Giribet G. 2007.** Taxonomy. In: Pinto-da-Rocha R, Machado G, Giribet G, eds. *Harvestmen: the biology of Opiliones*. Cambridge: Harvard University Press, 88–246.
- Pinto-da-Rocha R, Bragagnolo C, Marques FPL, Antunes Junior M. 2014.** Phylogeny of harvestmen family Gonyleptidae inferred from a multilocus approach (Arachnida: Opiliones). *Cladistics* **30**: 519–539.
- Platnick NI, Abraham N, Álvarez-Padilla F, Andriamalala D, Baehr BC, Baert L, Bonaldo AB, Brescovit AD, Chousou-Polydouri N, Dupérré N, Eichenberger B, Fannes W, Gaublomme E, Gillespie RG, Grismado CJ, Griswold CE, Harvey MS, Henrard A, Hormiga G, Izquierdo MA, Jocqué R, Kranz-Baltensperger Y, Kropf C, Ott R, Ramirez MJ, Raven RJ, Rheims CA, Ruiz GRS, Santos AJ, Saucedo A, Sierwald P, Szuts T, Ubick D, Wang XP. 2012.** Tarsal organ morphology and the phylogeny of goblin spiders (Araneae, Oonopidae), with notes on basal genera. *American Museum Novitates* **3736**: 1–52.
- Rodriguez AL, Townsend VR. 2015.** Survey of cuticular structures on leg IV of cosmetid harvestmen (Opiliones: Laniatores: Gonyleptoidea). *Journal of Arachnology* **43**: 194–206.
- Rodriguez AL, Townsend VR, Johnson MB, White TB. 2014a.** Interspecific variation in the microanatomy of cosmetid harvestmen (Arachnida, Opiliones, Laniatores). *Journal of Morphology* **20**: 1–20.
- Rodriguez AL, Townsend VR, Proud DN. 2014b.** Comparative study of the microanatomy of four species of harvestmen (Opiliones, Eupnoi). *Annals of the Entomological Society of America* **107**: 496–509.
- Santer RD, Hebets EA. 2008.** Agonistic signals received by an arthropod filiform hair allude to the prevalence of near-field sound communication. *Proceedings of the Royal Society B: Biological Sciences* **275**: 363–368.
- Schwendinger PJ. 2007.** Oncopodidae Thorell, 1876. In: Pinto-da-Rocha R, Machado G, Giribet G, eds. *Harvestmen: the biology of Opiliones*. Cambridge: Harvard University Press, 211–214.
- Sharma P, Giribet G. 2009.** Sandokanid phylogeny based on eight molecular markers—the evolution of a southeast Asian endemic family of Laniatores (Arachnida, Opiliones). *Molecular Phylogenetics and Evolution* **52**: 432–447.
- Sharma PP, Giribet G. 2011.** The evolutionary and biogeographic history of the armoured harvestmen – Laniatores phylogeny based on ten molecular markers, with the description of two new families of Opiliones (Arachnida). *Invertebrate Systematics* **25**: 106–142.
- Sharma PP, Giribet G. 2014.** A revised dated phylogeny of the arachnid order Opiliones. *Frontiers in Genetics* **5**: 255.
- Strong EE, Lipscomb D. 1999.** Character coding and inapplicable data. *Cladistics* **15**: 363–371.
- Talarico G, Palacios-Vargas JG, Silva MF, Alberti G. 2005.** First ultrastructural observations on the tarsal pore organ

- of *Pseudocellus pearsei* and *P. boneti* (Arachnida, Ricinulei). *Journal of Arachnology* **33**: 604–612.
- Tichy H, Barth FG. 1992.** Fine structure of olfactory sensilla in myriapods and arachnids. *Microscopy Research and Technique* **22**: 372–391.
- Tichy H, Loftus R. 1996.** Hygroreceptors in insects and a spider: humidity transduction models. *Naturwissenschaften* **220**: 255–263.
- Tomasiewicz B, Framenau VW. 2005.** Larval chaetotaxy in wolf spiders (Araneae, Lycosidae): systematic insights at the subfamilial level. *Journal of Arachnology* **33**: 415–425.
- Townsend VR, Bertram MS, Milne MA. 2015.** Variation in ovipositor morphology among laniatorean harvestmen (Arachnida: Opiliones). *Zoomorphology* **134**: 487–497.
- Wijnhoven H. 2013.** Sensory structures and sexual dimorphism in the harvestman *Dicranopalpus ramosus* (Arachnida: Opiliones). *Arachnologische Mitteilungen* **46**: 27–46.
- Willemart RH, Giribet G. 2010.** A scanning electron microscopic survey of the cuticle in Cyphophthalmi (Arachnida, Opiliones) with the description of novel sensory and glandular structures. *Zoomorphology* **129**: 175–183.
- Willemart RH, Gnaspini P. 2003.** Comparative density of hair sensilla on the legs of cavernicolous and epigean harvestmen (Arachnida: Opiliones). *Zoologischer Anzeiger – A Journal of Comparative Zoology* **242**: 353–365.
- Willemart RH, Chelini MC, de Andrade R, Gnaspini P. 2007.** An ethological approach to a SEM survey on sensory structures and tegumental gland openings of two Neotropical harvestmen (Arachnida, Opiliones, Gonyleptidae). *Italian Journal of Zoology* **74**: 39–54.
- Willemart RH, Farine JP, Gnaspini P. 2009.** Sensory biology of Phalangida harvestmen (Arachnida, Opiliones): a review, with new morphological data on 18 species. *Acta Zoologica* **90**: 209–227.
- Wolff JO, Schönhofen AL, Martens J, Wijnhoven H, Taylor CK, Gorb SN. 2016.** The evolution of pedipalps and glandular hairs as predatory devices in harvestmen (Arachnida, Opiliones). *Zoological Journal of the Linnean Society* **177**: 558–601.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

File S1. Table of species used in this study, with taxonomy, collecting localities and museum accession numbers.

File S2. List of characteristics organized by characters and character states for the species investigated. Species sorted by family. Character states showed represent legs I and II, unless otherwise indicated. In these cases, the character state on the left indicates leg I and on the right indicates leg II. Species marked with an asterisk have missing information on legs I, and the character state observed on legs II was used in the analysis. Numbers 1, 2 and 3 on character (2) represent, respectively, the distal, middle and proximal sensilla. Parentheses indicate association.

File S3. Ancestral state reconstruction of “Character (2) Articulation membrane association” on the phylogenetic hypothesis of Gonyleptoidea after Pinto-da-Rocha, Giribet, et al. (personal communication) White: (2a) Distal, middle and proximal sensilla not fused. Green: (2b) Distal sensillum isolated, middle and proximal fused sensilla. Black: (2c) Distal, mid and proximal sensilla fused. Branches with more than one color represent equally parsimonious reconstructions.

File S4. Pages 2–4: Ancestral state reconstructions of characters 1–4, respectively, under maximum likelihood using a one-parameter Markov model, as implemented in Mesquite ver. 3.31 (Maddison & Maddison, 2011). Reconstructions yield results identical to those under equal weights parsimony (see main text). Pages 5–9: Four charts of the null distribution of tree lengths resulting from the permutation tail probability (PTP) analysis (Faith & Cranston, 1991) for characters 1–4, respectively, using the software Mesquite ver. 3.31 (Maddison & Maddison, 2011) with 500 reshuffling cycles.