The sensory equipment of a sandokanid: An extreme case of tarsal reduction in harvestmen (Arachnida, Opiliones, Laniatores)

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1 | INTRODUCTION

Detailed knowledge of sensory structures has the potential to provide insights into the natural history and evolution of animals. The sensory structures of arachnids are usually concentrated on the pedipalps (the tritocerebral appendages) or on the distal podomere (tarsus) of the anterior walking legs, the latter being the case for armored harvestmen (Opiliones, Laniatores). Therefore, modifications of the tarsus could have direct impacts on the sensory equipment of these animals. Using scanning electron microscopy, we investigated the sensory equipment in an extreme case of reduction in tarsal articles in the harvestman Sandokan truncatus (Sandokanidae), which bears a single tarsomere in all legs, and the potential consequences of this reduction. Additionally, we review the literature on the natural history of the family Sandokanidae. Tarsomeres of all legs are equipped with gustatory sensilla, mechanoreceptors, and a pore organ, but wall-pored olfactory sensilla are restricted to tarsi I and II. Tarsi II present a higher density of olfactory sensilla and also putative campaniform sensilla (strain detectors), which indicates a special sensory function of this pair of legs. Other podomeres are covered with shelled sensilla, a probable chemoreceptor previously unreported in Opiliones. Overall, S. truncatus has types of sensilla largely comparable to harvestmen with longer and subdivided tarsi. However, S. truncatus also exhibits extra-tarsal sensory fields of sensilla basiconica (putative thermo-/hygroreceptors) in previously undescribed sites, and the unique pore organs. Our results establish a basis for further research investigating the natural history, as well as the evolutionary correlations and mechanistic causes of the tarsal reduction in this enigmatic lineage.

KEYWORDS
leaf litter, Sandokan, Sandokanidae, sensilla, sensory biology

Abstract
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1 | INTRODUCTION

Detailed knowledge of sensory structures has the potential to provide insights into the natural history of animals, and further serve as a framework for future research on the evolution of behaviors. For instance, the presence of air vibration detectors (trichobothria) on the proximal parts of the walking legs of whip spiders (Arachnida, Amblypygi) is related not only to detecting prey but also to receiving intraspecific agonistic signals in stereotypical male–male whip vibration “duels” (Santer & Hebets, 2008). Similarly, the discovery of nerves and putative sensilla on the bulb of the pedipalp of male spiders, previously considered a numb structure, has implications for directing future research on the evolution of mating strategies in this group (Lipke, Hammel, & Michalik, 2015; Sentenská, Müller, Pekár, & Uhl, 2017).
In mandibulate arthropods, the taxon that includes myriapods and panarthropods (including hexapods; Giribet, Edgecombe, & Wheeler, 2001; Hwang, Friedrich, Tautz, Park, & Kim, 2001), the antennae are specialized sensory appendages with a concentration of sensory structures (Altner & Prillinger, 1980; Zacharuk 1985; Grünew & Ache, 1988; Chapman, Simpson, & Douglas, 2013; Müller & Sombke, 2015). Other important sensory fields of the body are the legs, appendages that are typically used for locomotion. In some hexapods, the legs are equipped with mechanoreceptors, such as campaniform sensilla and chordotonal organs that are important for proprioception, as well as contact chemoreceptors (Altner & Prillinger, 1980; Chapman et al., 2013). In scutigeromorph centipedes (Chilopoda), the last pair of legs is not used for walking and instead assumes a major sensory role, exhibiting numerous sensory structures on hundreds of articles (annuli) (Kenning, Müller, & Sombke, 2017).

Sensory structures are not uniformly distributed on the legs of arthropods. In hexapods, for example, contact chemoreceptors occur mostly on the distalmost podomere, the tarsus (Chapman et al., 2013). In mandibulate arthropods, arachnids do not possess antennae, and their sensory structures occur mostly on the pedipalps (tritocerebral appendages) and/or legs (Foelix, 1985). In contrast, the tarsus and the distal part of the tibia of the antenniform legs of amblypygids concentrate the majority of types of sensilla on more than 100 tibial and tarsal articles (Santer & Hebets, 2011).

Arachnids are the most diverse group of Chelicerata, the other major branch in Arthropoda (sister group to Mandibulata) (e.g., Rota-Stabeli et al., 2011; Giribet & Edgecombe, 2012). In contrast with mandibulate arthropods, arachnids do not possess antennae, and their sensory structures occur mostly on the pedipalps (tritocerebral appendages) and/or legs (Foelix, 1985). In comparison with mandibulates, the legs of arachnids seem to be relatively more important sensory areas, harboring sensilla that detect volatiles, air currents, and changes in temperature and humidity (Foelix, 1985; Talarico, Palacios-Vargas, Silva, & Alberti, 2006; Santer & Hebets, 2011; Foelix & Schabronath, 1983; Willemart, Farine, & Gnaspini, 2009). Some arachnids, such as Amblypygi, Uropygii, Ricinulei, and Opiliones, also have legs I or II especially elongated and committed to sensory functions (Beccaloni, 2009; Santer & Hebets, 2011).

Harvestmen (order Opiliones) are readily recognizable by their usually long legs, particularly the elongated second leg pair of Phalangida (Pinto-da-Rocha, Machado, & Giribet, 2007). Both legs I and II possess the majority of sensory structures (Willemart & Gnaspini, 2004; Willemart et al., 2009) and usually higher numbers of tarsomeres in comparison with legs III and IV. The pedipalps are important sensory appendages in species of Palpatores, but there is no evidence of a sensory function of the pedipalps in Laniatores (Willemart et al., 2009), the suborder that includes more than two-thirds of Opiliones species (Giribet & Sharma, 2015), as they tend to have a raptorial function in prey capture. Instead, the anterior legs of laniatorean harvestmen, particularly their tarsi, have the highest density and diversity of sensilla (Willemart & Gnaspini, 2004; Willemart et al., 2017a, 2017b). Therefore, modifications in the number of tarsomeres, especially on the first two pairs of legs, could have a direct impact on the overall sensory equipment of laniatorean harvestmen.

Sandokanidae (formerly Oncopodidae; see Özüdiken & Kury, 2007) is a family of cryptic leaf-litter inhabiting armored harvestmen endemic to southeast Asia, China, and Nepal (Schwendinger, 2007b; Sharma & Giribet, 2009). Despite being relatively rare and inconspicuous, sandokanids are a curious lineage of highly sclerotized harvestmen that have a complete fusion of the prosomal tergites and all opisthosomal tergites (scutum completum) and a numeric reduction of tarsomeres (Schwendinger, 2007b; Sharma & Giribet, 2009). The ancestral number of tarsomeres in Sandokanidae is inferred to be one tarsomere on legs I and II, whereas the derived genera Gnomulus and Biantoncopus exhibit increases in this count for its corresponding legs (maximum likelihood tree topology of Sharma & Giribet, 2009). By comparison, some small species of other Laniatores families inhabiting leaf-litter (e.g., Zalmoxidae, Petrobunidae, Podoctidae) have a minimum of two tarsomeres per leg (and typically more in leg II), while some of the largest species of Laniatores (e.g., Gonyleptidae: Goriasoma venustum) may have up to 31 tarsomeres (Shultz & Pinto-da-Rocha, 2007; DaSilva & Gnaspini, 2009). There are currently 70 described sandokanid species in six genera, which have been extensively revised taxonomically: Sandokan (formerly Oncopus), Gnomulus, Caenoncopus, Palaeoncopus, Biantoncopus, and Martensiellus (Martens & Schwendinger, 1998; Schwendinger, 1992, 2006, 2007a, 2007b; Schwendinger & Martens, 1999, 2002, 2004, 2006; Sharma & Giribet, 2009). More recently, attention has been paid to their phylogenetic position and biogeography, given the autapomorphic nature of their external morphology and genitalia, and their importance for the biogeography of the Malay Archipelago (Schwendinger & Martens, 2002b; Sharma & Giribet, 2009; Fernández, Sharma, Tourinho & Giribet, 2017). However, not a single study has exclusively addressed aspects of their sensory biology (but see Gainett, Sharma, Pinto-da-Rocha, Giribet & Willemart, 2014) or natural history, and the possible impacts of tarsal reduction on their sensory equipment.

Herein, we characterize for the first time the sensory structures on the chelicera, pedipalp and all leg pairs of a sandokanid species using scanning electron microscopy. For this, we chose S. truncatus (Figure 1), which belongs to the genus showing the highest degree of tarsal reduction in Laniatores: a single tarsomere on each leg. In addition, we also review the scarce information about the natural history of the family Sandokanidae. Our results establish a basis for further research investigating the natural history, as well as the evolutionary correlations and mechanistic causes of the tarsal reduction in this enigmatic lineage.

2 MATERIAL AND METHODS

Two adult specimens of S. truncatus Thorell, 1891 were collected in Singapore, Bukit Timah Nature Reserve, Jungle Fall Valley, at 1°20′53.3″N 103°46′35.4″E (MCZ 131271, ex. MHNG SV-03/01; leg., P. Schwendinger; male) and 1°21′1.66″N 103°47′0.97″E (UFMG
16231; collector: A. Anker; female). The same male specimen used in a previous phylogenetic study (Sharma & Giribet 2009; MCZ 131271, ex. DNA101099) was selected for scanning electron microscopic examination. Appendages of this specimen were dissected with a razor blade and cleansed in three rounds of ultrasound cleaning; immersed in distilled water; then in a 1:10 solution of distilled water and detergent (Alconox®); and immersed in distilled water only. Appendages were critical point-dried (Tousimis 931 GL), mounted with carbon bi-adhesive tabs (Electron Microscopy Science, Hatfield, PA) on aluminum stubs and sputter-coated with Pt-Pd targets (EMS 300T D Dual Head Sputter-Coater). Images were taken on two field emission scanning electron microscopes (Zeiss Ultra-Plus FESEM and Zeiss Supra FESEM; Center for Nanoscale Systems, Harvard University), and subsequently had brightness and contrast levels adjusted in Photoshop CC 2015 (tools used: “Brightness”, “Contrast” and “Levels”). Measurements were taken using the Fiji plugin for ImageJ software (Schindelin et al., 2012). Measurements of sensilla are given in approximate values, given that micrographs have not always been taken with the exact same angle. The images of live sandokanids to which we refer in the discussion can be found in the following links: Nicky Bay, https://goo.gl/UdbiXd; Kurt G., https://goo.gl/Sa8w5H; Melvyn Yeo, https://goo.gl/Lc6urc. For descriptions of tegumental structures, we followed the terminology of Willemart et al. (2009) and Gainett et al. (2017a).

2.1 | Note on terminology

We use the term sensilla chaetica following the harvestman literature (e.g., Willemart et al., 2009) to denote long robust setae (“bristles”) articulating in sockets, despite differences in the thickness of the tip (blunt or sharp). The wall-pored sensilla here described are referred to as sensilla chaetica following Gainett et al. (2017b), who have found wall-pored subtypes of sensilla chaetica in Laniatores (sensu Willemart et al., 2009). Allantoid setae with blunt tips and wall pores (see Wall-pored sensilla) have been commonly termed solenidia in the arachnid literature, including papers on Opiliones of the suborder Cyphophthalmi, Eupnoi, and Dyspnoi (van der Hammen, 1985; reviewed in Willemart et al., 2009; Willemart & Giribet, 2010). Finally, we use the term sensilla basiconica to denote the sensilla with correspondence in position and general morphology with the sensilla basiconica first reported in harvestmen by Willemart et al. (2007). Neither inference of function nor homology with other arthropod structures is intended here with the use of latinized sensillar terminology.

3 | RESULTS

3.1 | General morphology of appendages

Most podomeres of S. truncatus lack long setae and spines. Instead, almost all articles appear mostly covered with button-like structures, which resemble small tubercles (Figures 1 and 3) (see Shelled sensilla below). We observed typical hair-like sensilla only in specific regions of chelicerae, pedipalps, and legs, most notably restricted to the tarsus of legs I–IV (Figures 4–9). The tarsus of all legs of S. truncatus consists of a single robust article, which varies in shape among legs.

Tarsi I and II have an overall globular shape with a single claw (Figure 2a–d). Tarsi III and IV resemble each other in having an elliptical lateral outline, which is considerably different from the globular tarsi I and II (Figure 2e,f).

The density and diversity of sensory structures is comparable between tarsi III and IV but these differ from tarsi I and II. The highest diversity of sensillar types is found on tarsi I and II, with some sensitillar types restricted to these podomerese.

3.2 | Sensory structures

3.2.1 | Tip-pored pegs (Figure 4)

We observed these sensilla only on the distal portions of chelicerae and pedipalps (Figure 3, dashed boxes). On the chelicera, they occur on the proximal portions of the fixed and movable fingers, with a higher density on the mesal surface (Figures 3b and 4a). On the pedipalps, they are restricted to the distal half of the tibia (Figures 3c and 4b). Each sensillum occurs inside a circular sunken pit, which renders only the apical portion of the shaft exposed at the surface level of the surrounding integument (Figure 4, white arrows). The shaft rises from a membranous cuticle, which forms a circular rim around the base of the shaft (Figure 4d). The distal shaft has a small apical pore (Figure 4e).

3.2.2 | Shelled sensilla (Figure 5)

These sensilla occur on all podomeres, except for tarsi I–IV, and are also absent on the pedipalpal claw. Each sensillum consists of a small

FIGURE 1 Image of a live specimen of S. truncatus (UFMG 16231), dorso-lateral view. I–IV indicate legs I–IV, respectively; Pp: pedipalp; Ch: chelicera. Scale bar is approximate [Color figure can be viewed at wileyonlinelibrary.com]
peg inserted in a depression, with most of the shaft surrounded by a conspicuous cuticular layer (Figure 5). This cuticular layer, henceforth referred to as a “shell,” is ornamented with longitudinal ridges that orient toward the exposed shaft of the peg, but not all of them converge at the tip (Figure 5a, b, d). The distal part of the shaft is curved upwards and presents a clear tip pore with well-delimited margins (Figure 5b–d). The shell may be short and ovate (Figure 5a, b), or occur as a longer variant, extending underneath on a smaller shell (Figure 5d). The integument at the insertion of the sensilla can be rugose, as the adjacent cuticle (Figure 5a–b, d), or finely wrinkled (Figure 5c).

**FIGURE 2** Scanning electron micrographs of tarsi of all pairs of legs of a male *S. truncatus* (MCZ 131271). (a) Right tarsus I, frontal view. (b) Left, tarsus I, lateral view. (c) Right tarsus II, frontal view. (d) Left, tarsus II, lateral view. (e) Left, tarsus III, lateral view. (f) Left tarsus IV, lateral view. Cl: claw; Hs: hooded sensillum; Mtt: metatarsus; Ts: tarsus. Scale bars: 100 μm.
3.2.3 | Sensilla chaetica with or without tip pores (Figure 6)

We found long sensilla chaetica on the ventral mesal surface of the basal cheliceral article, on the distal part of the tibia of the pedipalps (close to the claw) and on all surfaces of tarsi I–IV. These sensilla have robust shafts (length: ~70–130 μm) with shallow sockets, which are in some cases slightly sunken into the integument (Figure 6). Sensilla chaetica on chelicerae and pedipalps have shallow longitudinal grooves (Figure 6a, b), while those on the tarsi have well-marked deeper grooves that merge along the shaft (Figure 6c–f). On the pedipalps, the shafts appear to end in a blunt tip (Figure 6b), but we cannot rule out that they were broken or worn. On the chelicerae and leg tarsi the shaft tapers toward a fine tip (Figure 6a, c–e). Putative tip pores have been observed only in some sensilla chaetica of the tarsi (Figure 6e). Broken sensilla chaetica of this type on the tarsi reveal a massive cuticular wall surrounding a narrow lumen, which continues to the tip (Figure 6d, h). Apart from the tarsi of the walking legs, sensilla chaetica with a tip pore or without pores occur only on the ventral surface of the distalmost region of the metatarsus of the legs (Figure 6g). In this region, they are shorter and more robust (Figure 6g).

3.2.4 | Wall-pored sensilla (Figure 7)

Wall-pored sensilla are restricted to tarsi I and II, clearly in higher density on tarsi II (Figure 7a, b; dashed circles). We identified two different morphologies, referred to herein as type 1 and type 2 wall-pored sensilla.

3.2.5 | Type 1 wall-pored sensilla

These are sensilla with shafts measuring about 20 μm in length. Each sensillum is inserted in a shallow membranous cuticle (Figure 7c, d). The shaft is proximally curved at almost 90°, causing its distal part to run almost parallel to the integument (Figure 7c, d). Multiple slits (pores) occur on the shaft, from less than a micrometer above the insertion to the tip (Figure 7e, f; slit length: ~70–150 nm). The tip is blunt and has a constricted infolding, resembling a pore (Figure 7e).
3.2.6 | Type 2 wall-pored sensilla

Shafts are longer than type 1 wall-pored sensilla, measuring \( \sim 35 \mu m \) in length. They are inserted in a shallow membranous cuticle (Figure 7g). The shaft tapers toward the tip, where we observed no tip pore (Figure 7g, h). Multiple slits perforate the distal third of the shaft (slit length: \( \sim 100-250 \) nm) (Figure 7h, i). The proximal parts of the shaft have no pores, showing only longitudinal ridges (Figure 7g).

3.2.7 | Hooded sensilla (Figure 8)

A single hooded sensillum occurs in the distalmost dorsal region of tarsi I and II only. On tarsi I, it occupies a lateral position close to the claw (Figure 2a), while on tarsi II it occurs in a central depression of the cuticle, aligned with the longitudinal axis of the claw (Figure 2c). The surface of the shaft has very closely adjoined longitudinal ridges (not shown). The shaft (length: \( \sim 60 \mu m \)) is inserted at an angle of almost 90° tapering toward the tip (Figure 8a, b). The tip is curved and bears a single sub-apical pore (Figure 8a-d).

3.2.8 | Sensilla basiconica (Figures 8–9)

Sensilla basiconica were observed on tarsi I and II and on the metatarsi of all pairs of legs. On tarsi I and II, they are distributed along the dorsal surface of the tarsi (Figures 7a, b, dashed squares and 8e). The shaft is a short nonarticulated peg (length: \( \sim 12 \mu m \)), which tapers toward the tip (Figure 8e). Most of the shaft surface is smooth, but the distal third has longitudinally aligned pores, which in some portions form a slit (Figures 8e and 9c, i, j). The tip of the shaft has a pore (Figures 8e and 9c, i, j). On the dorsal surface of the metatarsi, sensilla basiconica occur in two discrete aggregations, distally and proximally. The overall morphology of sensilla basiconica in these metatarsal clusters and sensilla basiconica on tarsi I and II is very similar (Figure 8e and 9h–j).

The distal aggregation (Figure 9a–c) occurs dorsally on the metatarsi-tarsi junction of legs I–IV. The number of sensilla basiconica in the distal aggregation varies across legs. On their distal aggregation, legs I, II, III, and IV have, respectively, 14, 18, 5, and 8 sensilla basiconica, in the examined specimen. Among the sensilla in the distal aggregation, we observed some variation in the length of the shaft (\( \sim 10-45 \mu m \)) (Figure 9a).

The proximal aggregation is present on the metatarsi III and IV only, in the proximal quarter of the metatarsus (Figure 9d, e). The sensilla are located inside an oval pit with smooth integument, which differs from the elaborated microstructure of the surrounding integument of the metatarsi (Figure 9f–h). Eight sensilla are found in the
proximal aggregation of metatarsi III (Figure 9f). The proximal aggregation on leg IV is composed of five sensilla in a pit and a single adjacent sensillum basiconicum (Figure 9g). We observed some variation in shaft length and in the morphology of the terminal pore (Figure 9f, i, j). The terminal pore is either a circular terminal opening (Figure 9i) or a less uniform and sub-terminal (Figure 9j), but we cannot rule out that this difference is an artifact of sample preparation.

3.2.9 | Pore organ (Figure 10)

The proximal dorsal surface of the tarsus of all pairs of legs has a large oval pore (Figure 10). The pore measures about 50 μm by 30 μm and is partially hidden below the portion of the metatarsus that partially overlaps the tarsus (Figure 2). This pore occurs in a region of the cuticle devoid of setae. The interior of the pore appears filled with an amorphous substance of uncertain nature.

3.2.10 | Putative campaniform and slit sensilla (Figures 9d, e, 11)

Putative campaniform sensilla were observed on the dorsal and lateral surfaces of tarsi II only. They appear as flat oval membranes, each with an approximate area of 20 μm² (Figure 11a, b). Most putative campaniform sensilla have ruptured membranes, but this is likely an artifact of our cleansing and drying protocol. Single slit sensilla occur on all appendages (Figures 9d, e and 11c) and paired slits occur close to the tibia-metatarsus junction (Figure 9d, e).

3.2.11 | Trichomes (Figures 6d, f, 7a–c, 8a, 11a, c)

Trichomes are cuticular projections that occur on all surfaces of tarsi I–IV. The shaft has a wide base that abruptly tapers to a fine curved tip. They occur on most parts of the tarsus, being uniformly interspersed with sensilla. The tips of trichomes form a layer above all small hairsensilla on the tarsus (Figures 7c and 8a, b). The only sensilla with tips above the layer of trichomes are the sensilla chaetica with or without a tip pore and the hooded sensilla.

4 | DISCUSSION

4.1 | Function of sensory structures

The two small tip-pored sensilla that we report from S. truncatus, that is, the tip-pored pegs and the shelled sensilla, have not been previously reported in other harvestmen. The presence of a tip pore in both types of sensilla suggests a chemoreceptive function, probably gustation (Altner & Prillinger, 1980; Foelix, 1985). The gustatory sensilla of arachnids...
usually have long prominent shafts, but in some cases they may also be short. For instance, the scorpion pectines house a group of short pegs with a terminal slit, which can detect chemicals in the substrate (Foelix & Muller-Vorholt, 1983; Gaffin & Brownell, 2001; Wolf, 2008). Pegs similar to sandokanid tip-pored pegs have also been described to occur in the distal region of the pedipalp in several scorpion species (constellation array: Fet, Brewer, Soleglad & Neff, 2006). Such pegs have also been suggested to be chemoreceptors. In nonchelicerate arthropods, short conic tip-pored pegs on chilopod antennae (Myriapoda) and on the maxillary and labial palps of insect larvae have been proposed to be gustatory receptors (Keil, 1996; Sombke, Rosenberg, Hilken, Westermann & Ernst, 2011; Eilers, Talarico, Hansson, Hilker & Reinecke, 2012). In Opiliones, the only report of a structure similar to the outer layer (“shell”) seen in shelled sensilla are the modified setae on the ventral side of the opisthosoma of males of some Gnomulus species. These previously described setae are covered by a crust of crystalized
FIGURE 7  Scanning electron micrographs of sensilla chaetica with wall-pores on right tarsi I and II of a male S. truncatus (MCZ 131271). (a) Frontal view of dorsal surface of tarsus I, above the claw. (b) Frontal view of dorsal surface of tarsus II, above the claw. See Figure 2a, c for positional orientation. (c) Type 1 wall-pored sensillum (white arrow) on tarsus I. (d) Detail of a type 1 wall-pored sensillum on tarsus I. Black arrow indicates terminal infold. (e) Apical region of a type 1 wall-pored sensillum on tarsus II. Black arrow indicates terminal infold. (f) Basal region of a type 1 wall-pored sensillum on tarsus II. (g) Type 2 wall-pored sensillum (white arrow) on tarsus II. Protuberance on the mid shaft is an artifact of sample preparation. The distalmost part of the shaft is broken. (h) Apical region of a type 2 wall-pored sensillum on tarsus I. (i) Detail of the mid shaft of a broken type 2 wall-pored sensillum on tarsus II. Dashed squares: sensilla basiconica; dashed circles: sensilla chaetica with wall-pores. Am: articulation membrane; Hs: hooded sensillum; Po: pore; Tr: trichome.
secretion (Schwendinger & Martens, 1999, 2002). The cuticular shell in S. truncatus, however, presents a highly-ornamented surface, which is not what would be expected if it was formed by a crystalized secretion. Similar ridged cuticular structures are found on the limbs of members of the family Ogoveidae, but these do not seem be associated with sensilla (see Giribet & Prieto, 2003).

Sensilla chaetica with a tip pore or without pores occur in all groups of Opiliones (Guffey, Townsend & Felgenhauer, 2000; Willemart et al., 2009; Willemart & Giribet, 2010) and morphological evidence supports a chemoreceptive and/or mechanoreceptive function for these sensilla, as commonly seen in other long tip-pored sensilla of hexapods and arachnids (Foelix & Chu-Wang, 1973; Keil & Steinbrecht, 1984; Foelix, 1985; Keil, 1997). Tip-pores are not always observed in these large setae (Willemart & Gnaspini, 2004; Willemart et al., 2007, 2009; Gainett et al., 2017a; this study), which may indicate that some of them are only mechanoreceptors, as previously suggested (Willemart et al., 2009).

The two types of wall-pored sensilla observed (types 1 and 2) have morphological features typical of olfactory sensilla, as observed in several hexapods and some arachnids (Schneider & Steinbrecht, 1968; Keil & Steinbrecht, 1984; Zacharuk, 1985; Steinbrecht, 1996; Tichy & Barth, 1992). The external morphology of both types is also similar to what has been described for the “sensilla chaetica with wall pores” of other laniatorean species: type 1 wall-pored sensillum presents an infold at the tip and a short allantoid shaft with pores along almost its entire length; and type 2 wall-pored sensillum has a tapering shaft with pores only in the distal third of the shaft. The characteristics of the two types here identified suggest a correspondence with the sensilla chaetica with wall pores type 1 and 2 described by Gainett et al. (2017a). A remarkable difference, when compared with other Laniatores, is the slit shape of the pores, in contrast with the circular shape of the pores observed in other species (Gainett et al., 2017a). The type 1 wall-pored sensilla here reported are also similar to wall-pored sensilla reported in non-laniatorean harvestmen, referred to as solenidia. Solenidia are short setae with blunt tips (allantoid) and wall pores, which have been reported in the suborders Cyphophthalmi, Eupnoi, and Dyspnoi (reviewed in Willemart et al., 2009; Willemart & Giribet, 2010).
Hooded sensilla have recently been reported on the distalmost tarsomere I and II of *Heteromitobates discolor* (Laniatores, Gonyleptidae) (Gainett et al., 2017b). Histological evidence shows that this sensillum is innervated by two bundles of dendrites and that the wall of the shaft has an unusual ultrastructure. It has been suggested that they are probably thermo-/hygroreceptors.

**FIGURE 9** Scanning electron micrographs of sensilla basiconica on metatarsus I–IV of a male *S. truncatus* (MCZ 131271). (a) Distal aggregation of sensilla basiconica (dashed circle) on the metatarsus II. (b, c) Detail of the shaft and tip, respectively, of a sensillum basiconicum of the distal metatarsal aggregation. (d, e) Proximal aggregation of sensilla basiconica (dashed circles) on metatarsus III and IV, respectively. The left edge of each slit sensillum is indicated by a black arrowhead. (f, g) Detail of the proximal aggregation of sensilla basiconica on metatarsus III and IV, respectively. (h) Two sensilla basiconica in the proximal aggregation of sensilla basiconica on the metatarsus III. (i) Detail of the tip of the upper sensilla showed in (h). (j) Detail of a different sensillum basiconicum in the same aggregation on the metatarsus III, with an irregular terminal pore. Black arrowheads are indicating slit sensillum; Po: pore; Sb: sensillum basiconicum; Ss: shelled sensillum.
Sensilla basiconica were first reported by Willemart et al. (2007) on the distalmost tarsomere and on the metatarsus–tarsus junction of all pairs of legs in Laniatores. Their typical morphology includes a conical shaft with a terminal slit, which divides the distal third of the shaft in two halves (Willemart et al., 2009; Gainett et al., 2017b). Even though terminal openings in shafts are generally indicative of chemoreceptive function (Altner & Prillinger, 1980; Foelix, 1985), these sensilla in Opiliones have also been recently proposed to be specialized hygro-/thermoreceptors based on histological evidence (Gainett et al., 2017b). The putative sensilla basiconica of *S. truncatus* have a subapical series of micropores and a well-delimited terminal pore, which are atypical characteristics of sensilla basiconica in other Laniatores (Gainett et al., 2017b; Gainett et al., in prep). Their occurrence on tarsi I and II, as well as in the metatarsus–tarsus junction, however, is evidence of homology with the sensilla basiconica with a continuous slit present in other Laniatores.

A distal aggregation of sensilla basiconica on the distalmost metatarsus–tarsus junction of all legs is a highly conserved structure in Laniatores, usually occurring in the vicinity of a pair of slit sensilla (metatarsal paired slits; Willemart et al., 2007; Gainett et al., 2014). *Sandokan truncatus* differs from the typical laniatorean pattern in lacking metatarsal paired slits and by possessing 8–14 sensilla basiconica, in contrast with usually 2–5, as seen in other families (Gainett et al., 2014).

We also found an aggregation of sensilla basiconica inside a pit on the metatarsi of legs III and IV only. This structure has never been reported in other harvestmen. Considering that it is composed of several sensilla basiconica, it is possibly an additional site of hygro-/thermoreception and/or chemoreception in legs III and IV, as inferred from the putative function of sensilla basiconica in other species (Gainett et al., 2017b).

Extensive morphological and physiological studies on the slit sensilla of spiders have demonstrated their function as strain detectors (Barth, 2004). They occur in all investigated arachnid orders (Barth & Stagl, 1976) and are thought to be the arachnid counterpart of campaniform sensilla in insects (McIver, 1975), which are round membranous sensilla occurring almost exclusively in hexapods (Barth & Blickhan, 1984; McIver, 1975; Keil, 1997; Chapman et al., 2013). In arachnids, putative campaniform sensilla have only been reported in harvestmen of the suborder Eupnoi (reviewed in Willemart et al., 2009), which are distantly related to Sandokanidae. The putative campaniform sensilla we report in *S. truncatus* have been observed exclusively on the dorsal surface of the tarsus II, while single slit sensilla occur on all other appendages as well (Table 1). Assessing if these structures in Opiliones are indeed campaniform sensilla will require future histological investigation.

A large pore occurs on the dorsal surface of the tarsi of all legs of *S. truncatus*. A similar pore structure has first been illustrated with scanning electron microscopy in the sandokanid *Martensiellus* sp., and reported for some other sandokanid genera (Schwendinger, 2006). The....
occurrence of this pore is regarded as an autapomorphy of the family Sandokanidae, as no similar structure has been reported in other harvestmen (Schwendinger, 2006). Overall shape, dimensions and presence of a putative secretion are almost identical between S. truncatus and Martensiellus sp. Similarly, a deep pore in the distal dorsal surface of the tarsus has also been reported in Araneae (e.g., Blumenthal, 1935; Foelix & Chu-Wang, 1973), Ricinulei (Talarico, Palacios-Vargas, Silva & Alberti, 2005; Talarico, Palacios-Vargas & Alberti, 2008), Scorpiones (Foelix & Schabronath, 1983) and Amblypygi (Foelix, Chu-Wang & Beck, 1975; Igelmund, 1987; Santer & Hebets, 2011), and usually bears hygro-/thermoreceptors or olfactory sensilla. The external morphology of the pore organ of sandokanids is particularly similar to the pore organ of ricinuleids (Talarico et al., 2005). Therefore, the pore organ in species of Sandokanidae probably has a sensory function, but investigating the internal morphology is necessary to test this hypothesis.

4.2 | Natural history data in Sandokanidae

Because natural history is essential for understanding sensory biology, we review below the natural history data available for Sandokanidae. The sandokanid literature is scarce, and much of the existing publications focus almost exclusively on systematics and taxonomy. No behavioral or natural history paper has ever been published on this family, and only a few scattered mentions of the sensory structures exist. Bristowe (1976) referred to the tarsi of Sandokan feae (as Oncopus feae) as "a thick rounded pale pad or pre-tarsus" that "presumably serves a sensory function and bears a few short bristles and hairs." Anecdotal observations appear in the taxonomic literature and internet photo repositories, which we compile below. Wolff et al. (2016) mention "A photo of a species of Sandokanidae eating a snail was also taken," by Melvyn Yeo. Flicker (www.flickr.com) webpage photo repository by Melvin Yeo features two Gnomulus sp. using chelicerae and pedipalps to hold at least two species of snails. A juvenile (possibly of a Gnomulus species) was also photographed holding a snail shell with chelicera and pedipalps (Kurt G.; Flickr repository). Even though harvestmen in general are known to feed on gastropods opportunistically, specialized predation on snails occurs only in a few harvestmen in the families Ischyropsalididae and Trogulidae (both Dyspnoi; Acosta & Machado, 2007). The generally robust chelicerae of sandokanids could potentially be used to break shells, as in the specialized gastropod feeder Ischyropsalis hellwigi (Ischyropsalididae) (Martens, 1965), but direct observations are needed to fully understand this behavior. Pictures in Flickr repository also suggest stereotypical thanatotic posture and support the presence of UV-fluorescence by a species of Sandokan (possibly S. truncatus) (NickyBay; Melvyn Yeo). Throughout the extensive series of revisionary work by P. Schwendinger and J. Martens, they consistently reported collecting sandokanids mostly from sifted leaf litter, but at least the species Gnomulus sundaicus can also be found on low hanging tree branches (Schwendinger, 1992). Several specimens of an undescribed Gnomulus sp. in southern Thailand were found under the bark of dead trees (P. Schwendinger, pers. comm.). Interestingly, at least two species (G. bedoharvengorum and G. pulvillatus) have also been collected in limestone caves, which suggests that they may use caves at least as temporary refuges (Schwendinger & Martens, 1999, 2006). Schwendinger (1992) reported the presence of phoretic mites on G. sundaicus and G. lannaianus. Several sandokanid specimens have thalli of Laboulbeniales attached to their limbs (P. Schwendinger, pers. comm.). Interestingly, at least two species (G. bedoharvengorum and G. pulvillatus) have also been collected in limestone caves, which suggests that they may use caves at least as temporary refuges (Schwendinger & Martens, 1999, 2006). Schwendinger (1992) reported the presence of phoretic mites on G. sundaicus and G. lannaianus. Several sandokanid specimens have thalli of Laboulbeniales attached to their limbs (P. Schwendinger, pers. comm.). These parasitic fungi were recently reported for the first time to occur on Opiliiones (Santamaria, Enghoff, Gruber & Reboleira, 2017). Schwendinger and Martens (2004) mentioned personal observations of S. Huber about S. truncatus mating "in an upright position with the ventral
sidest of both partners facing each other.” A pair of Gnomulus sp. was also photographed in the same position by Kurt G. (Flicker repository).

Schwendinger and Martens (2004) also report that G. truncatus “are very sensitive to light and air movement, and either freeze or run for cover upon the slightest disturbance.” An interesting feature is that Gnomulus exsudans consistently shows paired spots of denaturized secretions (by preservation in alcohol) on the lateral margins of the prosoma (Schwendinger & Martens, 2002). This observation indicates that the emission of defensive chemical secretion in this species involves displacement along the margins of the body and the formation of droplets, a mechanism that increases the evaporative surface (mechanism 2, sensu Acosta, Poretti & Mascarelli, 1993; Hara & Gnaspini 2003).

Formation of droplets of secretion and displacement along the lateral margins of the body have been observed in Gnomulus latoperculum (Sharma & Giribet, 2003). Accordingly, S. truncatus has also been observed to displace enteric fluid (and likely defensive secretion) along the lateral margin of the body and over the prosoma when disturbed (A. Anker pers. comm.; Supporting Information Figure S1). Males of Gnomulus crassipes (A. Anker pers. comm.; Supporting Information Figure S1) have also been photographed in the same position by Kurt G. (Flicker repository). Schwendinger and Martens (2004) also report that S. truncatus is used in a sensory context in Laniatores (Willemart et al., 2009). Here, we show that the raptorial pedipalps of S. truncatus not only lack spines (see also Wolff et al., 2016), but are mostly covered with shelled and tip-pored pegs. The density of shelled sensilla on the pedipalp of S. truncatus is comparable with the density of shelled sensilla on the metatarsi of all legs, which appears higher than the density of sensilla normally present on the pedipalps of Laniatores (Willemart et al., 2009). Given these observations, we expect a relatively higher sensory importance of the pedipalp in this species when compared to other Laniatores.

Sandokan truncatus have wall-pored sensilla on tarsi I and II, and a similar composition of types of sensilla between this leg pairs. This supports the idea that both leg pairs are important sensory appendages, as previously suggested (Willemart et al., 2009; Gainett et al., 2017a). However, the density of sensilla chaetica with wall- pores and of sensilla basiconica on the dorsal surface of tarsi II is clearly higher than in tarsi I. This observation suggests that in this species legs II may be more important for olfactory reception than leg pair I. The exclusive presence of campaniform sensilla on the dorsal surface of tarsi II also suggests that legs II have a more important sensory function.

Sandokan truncatus shows a surprisingly high diversity of types of sensory structures on their single tarsomers and on other parts of the appendages (Table 1). Despite having a reduced number of tarsal articles, S. truncatus has types of sensilla mostly comparable to other Laniatores with longer and subdivided tarsi (Gainett et al., 2017a, 2017b). Apart from sensillar types already reported on the tarsi of laniatorean harvestmen (Willemart et al., 2009; Gainett et al., 2017a, 2017b), S. truncatus is also equipped with tarsal pore organs, two metatarsal sensory fields of sensilla basiconica and putative campaniform sensilla on tarsi II. In addition, pedipalps and chelicerae appear to have

table 1 Distribution of the 12 types of cuticular structures studied, on the pedipalps, chelicerae and legs I–IV of a male Sandokan truncatus, and their inferred sensory functions. “X” indicates presence

<table>
<thead>
<tr>
<th>Inferred function</th>
<th>Chelicera</th>
<th>Pedipalp</th>
<th>Leg I</th>
<th>Leg II</th>
<th>Leg III</th>
<th>Leg IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip-pored pegs</td>
<td>Chemoreceptive?</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Shelled sensilla</td>
<td>Chemoreceptive?</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sensilla chaetica with or without a tip pore</td>
<td>Gustatory/mechanoreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sensilla chaetica with wall- pores, type 1</td>
<td>Olfactory</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sensilla chaetica with wall- pores, type 2</td>
<td>Olfactory</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Hooded sensilla</td>
<td>Chemoreceptive; thermo-/hygroreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Tarsal sensilla basiconica</td>
<td>Thermo-/hygroreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Proximal metatarsal sensilla basiconica</td>
<td>Thermo-/hygroreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Distal metatarsal sensilla basiconica</td>
<td>Thermo-/hygroreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Putative campaniform sensilla</td>
<td>Mechanoreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Slit sensilla</td>
<td>Mechanoreceptive</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Trichomes</td>
<td>Non-sensory</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

4.3 Sensory biology of Sandokan truncatus (Table 1)

The pedipalps in Opiliones are used to capture prey but also have a sensory function in the suborders Eupnoi and Dyspnoi (Willemart et al., 2009 and references therein; Willemart & Hebets, 2012; Wolff et al., 2016). On the other hand, Laniatores are normally equipped with raptorial pedipalps with characteristic spines (stiff bristles with large sockets), and only few sensilla chaetica with tip pores or without pores (Pinto-da-Rocha et al., 2007; Willemart et al., 2009; Wolff et al., 2016; Townsend & Enzmann, 2018). There is no report of pedipalps being used in a sensory context in Laniatores (Willemart et al., 2009). Apart from sensillar types already reported on the tarsi of Laniatores with longer and subdivided tarsi (Gainett et al., 2017a), the density of sensilla chaetica with wall- pores and of sensilla basiconica on the dorsal surface of tarsi II is clearly higher than in tarsi I. This observation suggests that in this species legs II may be more important for olfactory reception than leg pair I. The exclusive presence of campaniform sensilla on the dorsal surface of tarsi II also suggests that legs II have a more important sensory function.
more types of sensilla than previously reported for other Laniatores, although this may be due to the lack of extensive studies on these appendages in the suborder.

The number of tarsomeres in harvestmen is highly variable and generally correlates with the mode of life. Some species inhabiting vegetation have higher numbers of tarsomeres in comparison with closely related species inhabiting leaf-litter, and it has been suggested that higher numbers of tarsomeres possibly are useful for moving around in the vegetation (Curtis & Machado, 2007). Accordingly, reduction of legs in insects is usually associated with specialized modes of life. For instance, some butterflies (Nymphalidae) have a reduced first pair of legs that is used during courtship, and tarsal reduction occurs in some lineages that are adapted to parasitic life and burrowing (Chapman et al., 2013). The reduced number of tarsomeres in S. truncatus may, therefore, be related to a life in leaf-litter, as suggested from the reported habitats where most sandokanid species have been collected. Similar correlation is also observed in other harvestmen, such as troguliids (Dyspnoi, Trogulidae) and nemastomatids (Dyspnoi Nemastomatidae) (Curtis & Machado, 2007). This is also the case in the most conspicuous leaf-litter dwelling Opiliones, the members of the suborder Cyphophthalmi, which also have a single tarsomere on all legs (with the exception of males of some species that have two tarsomeres on leg IV, this being a sexually dimorphic character; Boyer & Giribet, 2007).

The majority of sensilla on the appendages of S. truncatus are sheltered by other structures, either occurring inside pores and pits (tip-tipped pegs; proximal metamersal organ; pore organ) or being under a canopy of trichome tips (wall-pored sensilla; sensilla basiconica) or cuticular projections (shelled sensilla). Shorter and protected sensilla may be less subject to mechanical damage and contamination (Steinbrecht, 1996). Most strikingly, all leg articles proximal to the tarsus in S. truncatus are almost exclusively equipped with the small shelled sensilla. Similar shelled setae, called navicular setae, are present in some species of ricinuleids (Platnick & Shadab, 1977; Platnick, 1988). These navicular setae are large scale-like structures occurring on the legs and on the body trunk of these species, but it is unknown whether they have a sensory or protective function.

An intriguing possibility is that the habitus of S. truncatus is actually a way to reduce nonsclerotized surfaces. Laniatorean harvestmen are called “armored harvestmen” (Sharma & Giribet, 2011) for a reason: their thick integument (as one can easily perceive by handling them) offers protection against predators (Souza & Willemart, 2011; Dias & Willemart, 2013; Segovia, Del-Claro & Willemart, 2015). The soft and vulnerable parts of a laniatorean harvestman are the articulations between podomeres, the mouth and the distal parts of the legs (Souza & Willemart, 2011). These three areas are exactly the ones chosen by the recluse spider and by a scorpion species to bite and sting armored harvestmen (Albín & Toscano-Gadea, 2015; Segovia et al., 2015). If the harvestman is well fed or full of eggs, then the intersegmental membranes between the free tergites also become vulnerable. Sandokan truncatus has the prosomal and opisthosomal tergites completely fused, reduction in the number of tarsomeres and a reduced calcaneus (the thinner distal area of the metatarsus). These three characteristics significantly reduce the vulnerable areas of the exoskeleton. Therefore, future studies should aim to identify potential predators of sandokanids to evaluate the hypothesis that the bauplan of these particularly armored harvestmen reflects adaptations to predation pressure and to a life in leaf-litter.

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AUTHOR CONTRIBUTIONS
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REFERENCES


