

Morphological coupling of the distal organ in the Peruvian walking stick (*Oreophoetes peruana*): Structural and functional aspects

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Abstract

In insects, the detection of mechanical stimuli from body movements, airborne sound, substrate vibration, medium flow, or gravity by mechanosensory organs plays an important role. These mechanosensory organs can have complex morphologies with numerous sensilla, and the functional morphology with specific attachments of the sensory neurons to surrounding tissues and structures determines the stimulation. In stick insects, the subgenual organ complex in the tibia of all legs is an elaborate system of two chordotonal organs, which respond to substrate vibrations, and associated tibial campaniform sensilla, which respond to cuticular strain. One chordotonal organ, the distal organ, is characterized by a linear set of sensilla. This distal organ has not been studied for its physiological characteristics in detail, but the attachment or mechanical coupling is functionally important. Here we characterize two aspects of attachment or mechanical coupling of the distal organ: At the dorsal side, the organ is connected to the inner side of the dorsal cuticle by connective tissue, which is shown to also contain the axons of campaniform sensilla. At the proximal end, a fine membrane runs to the adjacent chordotonal organ, the subgenual organ. This membrane spans the tibia in transverse direction. It does not contain neuronal elements, but as a connection between the subgenual and the distal organ, it may influence the mechanosensory activity of these organs. Such a connection is not present in other insects such as locusts or cockroaches and could affect the sensory function in stick insects (e.g., in vibration detection by the subgenual organ) or even couple the two organs, resulting in similar mechanical responses.

KEYWORDS

chordotonal organ, functional morphology, mechanoreception, physiology, vibration

1 | INTRODUCTION

Insect mechanoreceptor systems are highly developed with different types of sensilla (Keil, 1997; Shields, 2008). These are functional units with one or few sensory neurons, and additional cell types. The

sensilla can occur on the cuticle (e.g., Garza et al., 2021; Haberkorn et al., 2019; Ichikawa et al., 2014; Nowińska et al., 2020; Pflüger et al., 1981; Yang et al., 2017; Zhao et al., 2021) or form complex internal organs (Field & Matheson, 1998; Kavlie & Albert, 2013; Nation, 2008).

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For the activation of the sensilla, the contact to the medium or the coupling to other parts of the body transferring stimuli like movements, vibration or sound is crucial, leading to specialized orientations of sensilla or attachments of the sensory organs (Bässler, 1977; Schnorbus, 1971; Strauß & Stritih-Peljhan, 2022). Especially for the internal chordotonal organs, the structural connections to other elements of the insect body are important, for example, for proprioception or detection of substrate vibrations. Such connections can be achieved by attachment cells associated with the sensory neurons, connective tissue, or ligaments (Field & Matheson, 1998; Nishino, 2003). These structures are important for the functional characteristics of the mechanosensory system (Barth, 2019; Strauß & Stritih-Peljhan, 2022).

In orthopteroid insects, the subgenual organ (SGO) complex is an elaborate mechanosensory system placed in the proximal tibia of all legs (Figure 1A). In most insects, the SGO is present as the main vibration receptor organ (Čokl & Virant-Doberlet, 2003; Field &

Matheson, 1998; Shaw, 1994). In orthopteroid insects, additional chordotonal organs occur next to the SGO (Strauß, Stritih-Peljhan, et al., 2021). In stick insects, a unique organization of the sensory complex is present, consisting of two chordotonal organs, the SGO and the distal organ (DO) (Strauß, 2020; Strauß & Lakes-Harlan, 2013) (Figure 1B). These organs have multiple attachment points to the inner leg cuticle by connective tissue and also fine tissue strands (Figure 1B). These tissues, as well as the finer strands, may provide attachments to keep the organs in position but could also transfer vibration stimuli to the sensilla of the SGO and DO. The SGO is connected to the cuticle at the posterior side by a relatively strong tissue strand, while the DO is connected at its proximal end to the dorsal leg cuticle by a connective tissue strand (Jägers-Röhr, 1968; Strauß, 2021; Strauß, Moritz, & Rühr, 2021) (Figure 1B). The latter strand is also associated with a group of campaniform sensilla (CS) placed in the cuticle (Strauß, Moritz, & Rühr, 2021). The CS are specialized mechanoreceptors that detect cuticular strain (Zill

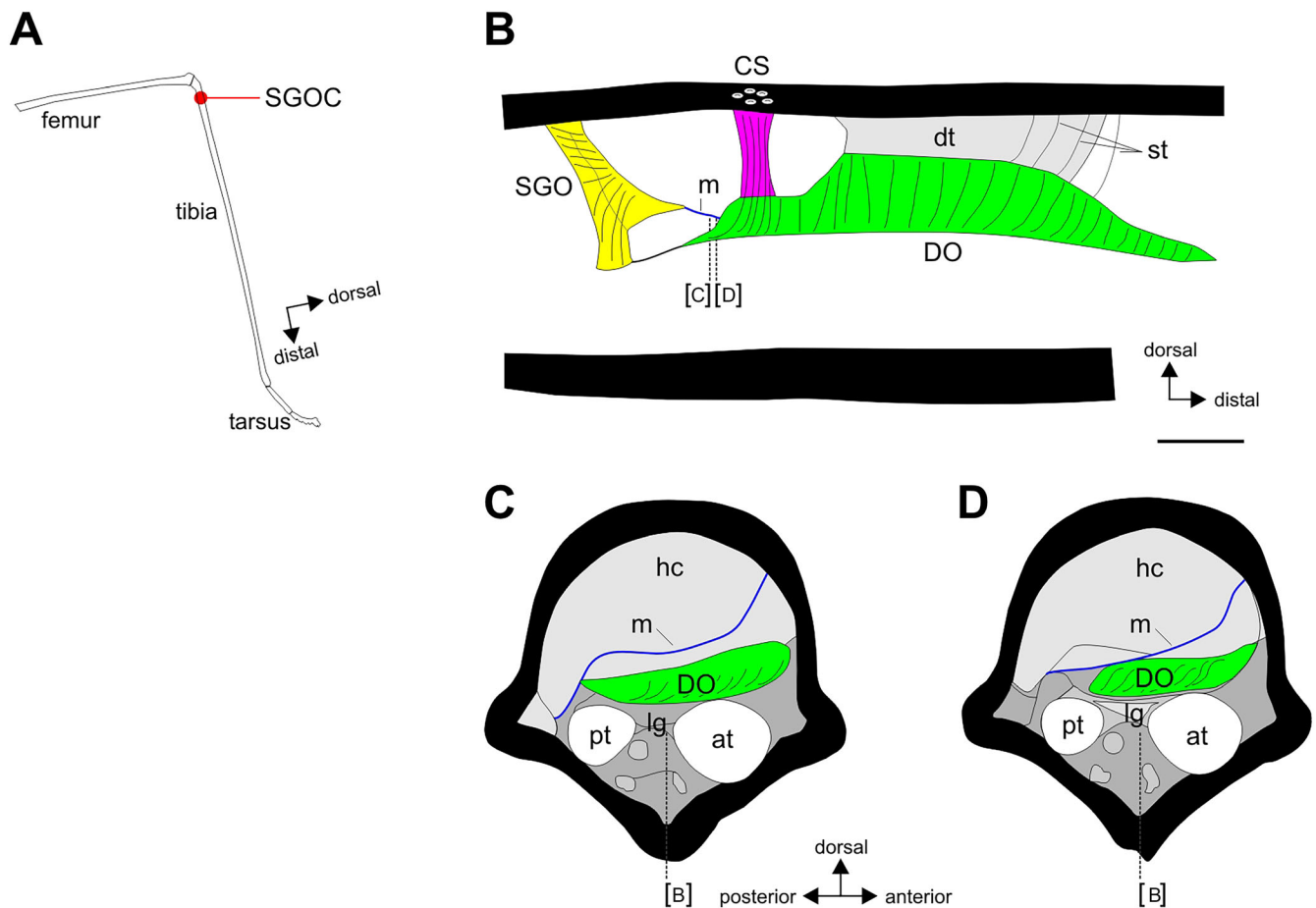


FIGURE 1 Schematics of the subgenual organ complex in stick insects. (A) Position of the subgenual organ complex (SGOC) in the proximal tibia. (B) Partial sagittal section (shown as hatched lines in C and D) of the tibia, showing attachment structures of sensory organs in the subgenual organ complex. The membrane (m; blue) between subgenual organ (SGO, yellow) and distal organ (DO, green) and the connective tissue strand (magenta) towards the campaniform sensilla (CS; indicates as white caps) are shown. Further connections to the leg cuticle are by fine strands (st) and diffuse tissue (dt) at the dorsal side of the tibia. (C, D) Transverse sections (shown as hatched lines in B) of the tibia showing the position of the membrane at the proximal DO. Anatomy of the subgenual organ complex adapted from *Sipylodea sipylus* (Strauß, Moritz, & Rühr, 2021). Scale = 200 μ m. at, anterior trachea; CS, campaniform sensilla; DO, distal organs; dt, diffuse tissue; hc, hemolymph channel; lg, ligament; m, membrane; pt, posterior trachea; SGO, subgenual organ; SGOC, subgenual organ complex; st, strands

et al., 1999; Zill & Moran, 1981), and they can occur as single sensilla or in groups (Harris et al., 2020; Keil, 1997). Previously, a fine membrane was documented between the SGO and DO in stick insects, forming a connection between the two chordotonal organs (Figure 1B–D). This membrane is placed between the anterior and posterior sides of the tibia (Strauß, Moritz, & Rühr, 2021) and runs between both organs in the horizontal longitudinal level (Ball & Field, 1981) of the tibia (Figure 1C). It contacts the DO on the dorsal side and eventually merges with the DO surface (Figure 1D).

The functional morphology and particularly the attachments of sensory organs are important to analyze because they provide the basis of mechanosensory functions in the SGO complex. Axonal tracing allows staining of sensory organs and location of other tissues around the neuronal components in cleared preparations. The structures are analyzed here in *Oreophoetes peruana* (SAUSSURE 1868) as a representative species of the Occidophasmata, the New World stick insects (Simon et al., 2019), and this study adds to previous work on these structures in species of the Oriophasmata, the Old World stick insects, such as *Carausius morosus* and *Sipylodea sipylus* (Strauß, Moritz, & Rühr, 2021). In *O. peruana*, the neuroanatomy of the sensory organs (SGO and DO) resembles that of other stick insects (Strauß, 2022), but the connecting tissues were previously not documented. Here, the connection of the DO towards the dorsal CS and the connection between SGO and DO are investigated, and data are gathered to assess the similarity or possible divergence of sensory structures in different Phasmatodea. In addition, the elements could indicate a role in stabilization by connective tissue, or a functional coupling to mechanical stimuli reaching the organs. The DO of stick insects with its linear set of sensilla is particularly interesting for its role in mechanoreception, because the linear organization is a feature shared with ensiferan hearing organs (Strauß, Moritz, & Rühr, 2021). A better understanding of the DO functional morphology and the organ's attachment structures will support the analysis of its physiological function.

2 | METHODS

2.1 | Animals

Individuals of *Oreophoetes peruana* were raised in a lab colony maintained at the Institute for Animal Physiology, Justus Liebig University Gießen. The colony was maintained at $\sim 21^{\circ}\text{C}$ room temperature, fed with fern (*Nephrolepis*) leaves, and sprayed every 1–2 days with water.

The legs from nine individuals of *O. peruana* were analyzed (five female, four male animals). All studied animals were adult. In this sample, 48 legs were studied.

2.2 | Neuroanatomy

In the legs, the sensory organs and their nerves were stained by retrograde axonal tracing using 5% cobalt chloride ($\text{CoCl}_2 \times 6 \text{H}_2\text{O}$; Merck, Darmstadt, Germany) solution in distilled water (see Pitman

et al., 1972). The tracing procedure follows the description given in Strauß (2022). Before the dissection, animals were briefly cold-anesthetized at 4°C for 10 min, and the legs were cut off at the femur with scissors. They were fixed in a glass dish (covered with Sylgard; Sylgard 184, Suter Kunststoffe AG, Fraubrunnen, Switzerland) with insect pins, and the preparation was carried out under *Carausius* saline (Bässler, 1977). The cuticle was cut open in the femur with a piece of a blade (Feather FA-10, 0.1 mm, Feather, Osaka, Japan), and the leg nerve was exposed with forceps (Dumont #5, Fine Science Tools, Heidelberg, Germany). The nerve was cut with iridectomy scissors. The cut end was placed in a glass capillary filled with 5% cobalt solution and the legs were then incubated at 4°C for 48 h. The cobalt staining was precipitated in a 1% solution of ammonium sulfide (Alpha Aesar, Karlsruhe, Germany) in *Carausius* saline for 15 min. The leg preparations were next rinsed in *Carausius* saline, fixed 60 min in chilled paraformaldehyde solution (4%; Sigma-Aldrich, St. Louis, MO, United States, in phosphate buffer, 0.04 mol/L Na_2HPO_4 , 0.00574 mol/L $\text{NaH}_2\text{PO}_4 \times 2 \text{H}_2\text{O}$; pH = 7.4), and dehydrated in a graded ethanol series (Carl Roth, Karlsruhe, Germany). For clearing, the legs were incubated and stored in methyl salicylate (Merck, Darmstadt, Germany).

For the terminology of sensory organs in the SGO complex of stick insects, see Strauß and Lakes-Harlan (2013). The nomenclature for nerve branches in *O. peruana* follows Strauß (2022). The terminology for groups of CS in the tibia follows Zill et al. (2011), for *C. morosus*.

2.3 | Microscopy and documentation

From the tibia, the cuticle was removed with a piece of a blade to isolate the fixed tissues within the leg. They were viewed in methyl salicylate with an Olympus BH-2 microscope (Olympus, Shinjuku, Japan). Documentation was carried out with a Leica DFC 7000 T camera (1920×1440 pixel) at the microscope using the Leica Application Software V4.9 (Leica Microsystems CMS GmbH, Wetzlar, Germany). A series of photographs were combined for stacked photographs by the program Combine ZP. These were further processed by adjusting brightness and contrast when necessary. Figure panels were assembled and labeled in CorelDraw 11 (Corel, Ottawa, Canada).

3 | RESULTS

The SGO complex in *O. peruana* consisted of SGO and DO (Figure 2A,B; see also Strauß, 2022). At the level of the proximal DO, the distal 6B CS were placed dorsally on the tibia (Figure 2B). These CS were innervated by a distinct nerve branch, termed T23 (Figure 2C). This overall organization of sensory elements was identical in all leg pairs (Strauß, 2022). Axonal staining revealed the CS axons in ventral orientation (Figure 2C). The axon of the proximal 6B CS joined nerve branches from the SGO (Figure 2B).

Light microscopy of the cleared tracing preparations also showed the tissues associated with SGO and CS (Figures 2 and 3). The nerve

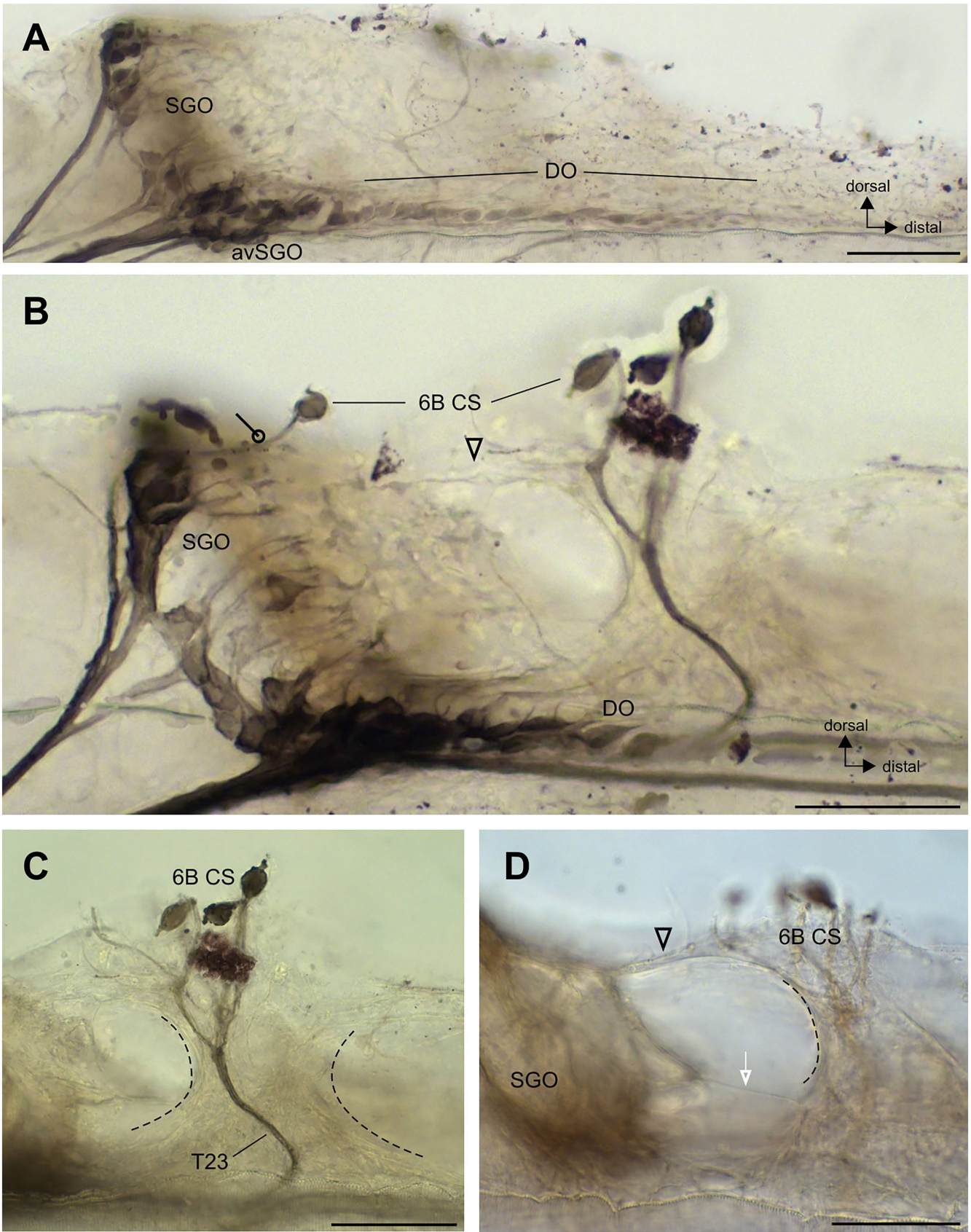


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FIGURE 2 The subgenual organ complex and campaniform sensilla in *Oreophoetes peruana*, stained by axonal tracing. (A) Lateral view of the subgenual organ complex with the subgenual organ (SGO) and distal organ (DO) and staining of the sensory neurons (midleg). The anteroventral subgenual organ is a dense group of SGO sensilla. (B) The SGO and campaniform sensilla (CS) in group 6B (foreleg). Axon of the proximal 6B CS indicated by rounded arrow. (C) Detail of the distal 6B campaniform sensilla with their axons in nerve branch T23, surrounded by tissue (outlined by hatched lines). (D) The tissue associated with the SGO connects to the distal 6B campaniform sensilla (outlined by hatched line) dorsally by a strand (empty arrowhead) and to the distal organ (DO) ventrally (arrow) (hindleg). Orientation as indicated in A and B. Scales = 100 μm . avSGO, anteroventral subgenual organ; CS, campaniform sensilla; DO, distal organ; SGO, subgenual organ

branch T23 containing axons of the distal 6B CS were surrounded by connective tissue at the proximal DO level (Figure 2C). This shows that the CS axons are included in the dorsal attachment of the DO. Notably, this tissue was thicker in diameter than the nerve branch (Figure 2C). In addition, a tissue strand was running between the dorsal side of the SGO and the tissue containing the 6B CS axons (Figures 2D and 3B).

The dendrites of the SGO pointed distally in the tibia (Figure 3A). They were covered by rounded cap cells (Figure 3A). The dendrites of SGO sensilla inserted into a tissue spanning the hemolymph channel of the tibia (Figure 3B). This tissue showed a proximal and distal layer (Figure 3B). The distal layer showed larger vacuous areas likely containing attachment cells associated with the sensory neurons (Figure 3B). The SGO tissue was rhombus-shaped and appeared pulled out in distal direction at the ventral side (Figure 3B,C). A thin membrane was visible between the distal SGO and the proximal DO (Figure 3C). It ran through the tibia from the anterior to posterior side of the SGO towards the DO (Figure 3D), with a form that was rather more like a sail than a thin strand (see Figure 1C,D). By the position, it separated the dorsal and ventral tibial space. The membrane did not contain neuronal structures like dendrites, as seen from the staining by axonal tracing (Figure 3C,D). The thin membrane between the SGO and DO was seen in legs from all thoracic segments and overall in 46 of 48 legs. No morphological differences between the leg pairs were evident. The membrane showed some variation in length: Although it was occasionally rather short before contacting DO tissues (Figure 4A), usually, it was longer and clearly distinguishable (Figures 3C and 4B). In only two preparations was the membrane not detected (Figure 4C,D). In one of these cases, the SGO and DO were in direct contact (Figure 4C), whereas in the other, no obvious membrane was formed, with the most distal tip of the SGO absent (Figure 4D). In both preparations, in the corresponding leg on the other side of the body, the membrane was clearly developed. These legs came from a female (Figure 4C) and a male (Figure 4D), respectively. The absence in very few preparations was likely due to a developmental aberration. In sum, these data support the development of the membrane between the SGO and DO in *O. peruana*, forming a connection between the SGO and DO.

4 | DISCUSSION

4.1 | Functional morphology of chordotonal organs

In this study, connecting structures of the chordotonal organs in the SGO complex of the Peruvian stick insect were studied to better

understand its functional morphology. The overall organization of the chordotonal organs (SGO and DO) in *O. peruana* is identical to other stick insects in organization and numbers of the sensilla, the shape of the SGO, the distal extension of the SGO, and the connection to the DO (Strauß, Moritz, & Rühr, 2021). The present study documents a major tissue strand at the proximal DO to the cuticle and a thin membrane between the SGO and the DO. Both elements are also found in other stick insects including *Carausius morosus* and *Sipylodea sipylus* (Strauß, Moritz, & Rühr, 2021). In *O. peruana*, these structures were clearly visible by light microscopy after removal of the cuticle, and this allowed for an analysis combined with axonal tracing.

Chordotonal organs are versatile mechanoreceptors that can respond to stretch, substrate vibration, airborne sound, or gravity (Field & Matheson, 1998; Kavlie & Albert, 2013; Matsuo & Kamikouchi, 2013). Their coupling is relevant for the mechanical stimulation of sensilla. The organs can be attached to a receptor apodeme or tendon, such as the femoral chordotonal organ (Alt & Lakes-Harlan, 2018; Bäessler, 1977; Eberhard et al., 2010; Nishino et al., 2016; Shelton et al., 1992; Theophilidis, 1986), to allow for monitoring of the movement of leg parts. In the abdominal chordotonal organs of larvae in *Drosophila*, the strain of stretched organs is mainly attributed to the cap cells (Prahlaad et al., 2017). The SGO in the proximal tibia is located in the hemolymph channel and is excited by vibration stimuli transferred through the hemolymph in the proximal direction of the leg (Kilpinen & Storm, 1997). In orthopteroid insects, the SGO attachments are particularly complex because the SGO has strands of different thickness on different sides of the organ towards the leg cuticle (Eberhard et al., 2010; Lin et al., 1994; Strauß et al., 2017). Although these attachments do not seem to serve primarily for the stimulus transfer, they determine the SGO movements in the hemolymph induced by vibrations. Hence, a connection between the SGO and DO as seen in stick insects may also be relevant for the function of the two sensory organs by influencing their mechanical response to vibrations. The slender legs common in stick insects (e.g., Bradler & Buckley, 2018) could also affect the vibration transfer in the hemolymph.

4.2 | Attachment of the DO

The connective tissue strand at the proximal DO contains the axons from the 6B CS, as shown by staining from axonal tracing. However, this tissue strand is notably broader than would be required by a sheath layer covering the CS axons (Figure 2B,C). This connective tissue could also provide structural support (attachment to the cuticle)

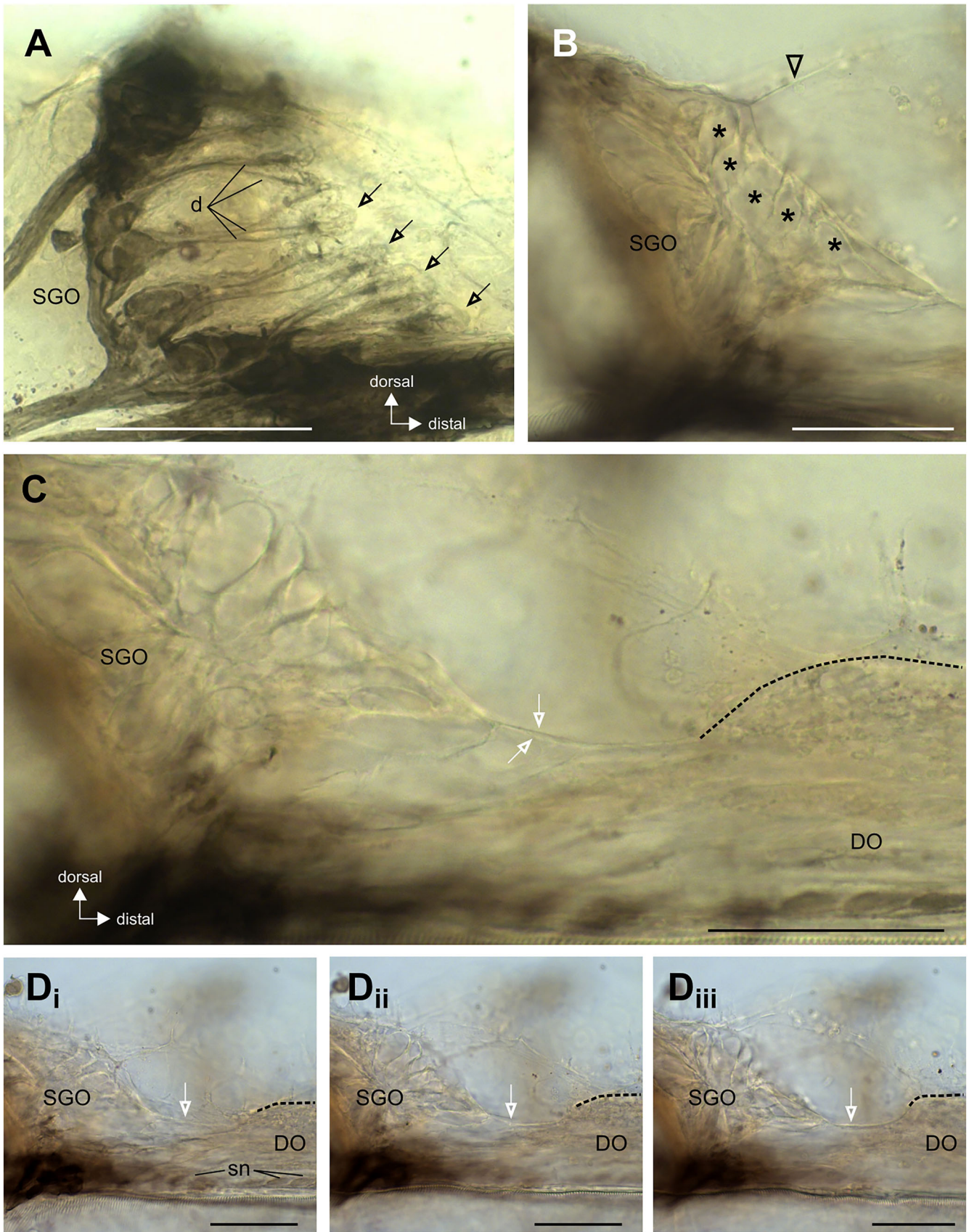


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FIGURE 3 The connection of subgenual organ and distal organ in *Oreophoetes peruana*. (A) The subgenual organ (SGO) has dendrites (d) pointing in distal direction, covered by rounded cap cells (empty arrows). (B) The subgenual organ has more dense tissue dorsally and more vacuous tissue ventrally (asterisks). Empty arrowhead indicates dorsal tissue strand to the 6B campaniform sensilla. (C) The ventral end of the SGO shows an extension by the thin membrane (arrows) towards the distal organ (DO) (dorsal part outlined by hatched line). (D) The membrane (arrow) between the SGO is present at the level of the (D_i) anterior, (D_{ii}) central and (D_{iii}) posterior DO. The DO is dorsally outlined by the dotted line. All photographs from foreleg preparations. Orientation as indicated in A. Scales = 100 µm. d, dendrites; DO, distal organ; SGO, subgenual organ; sn, sensory neuron

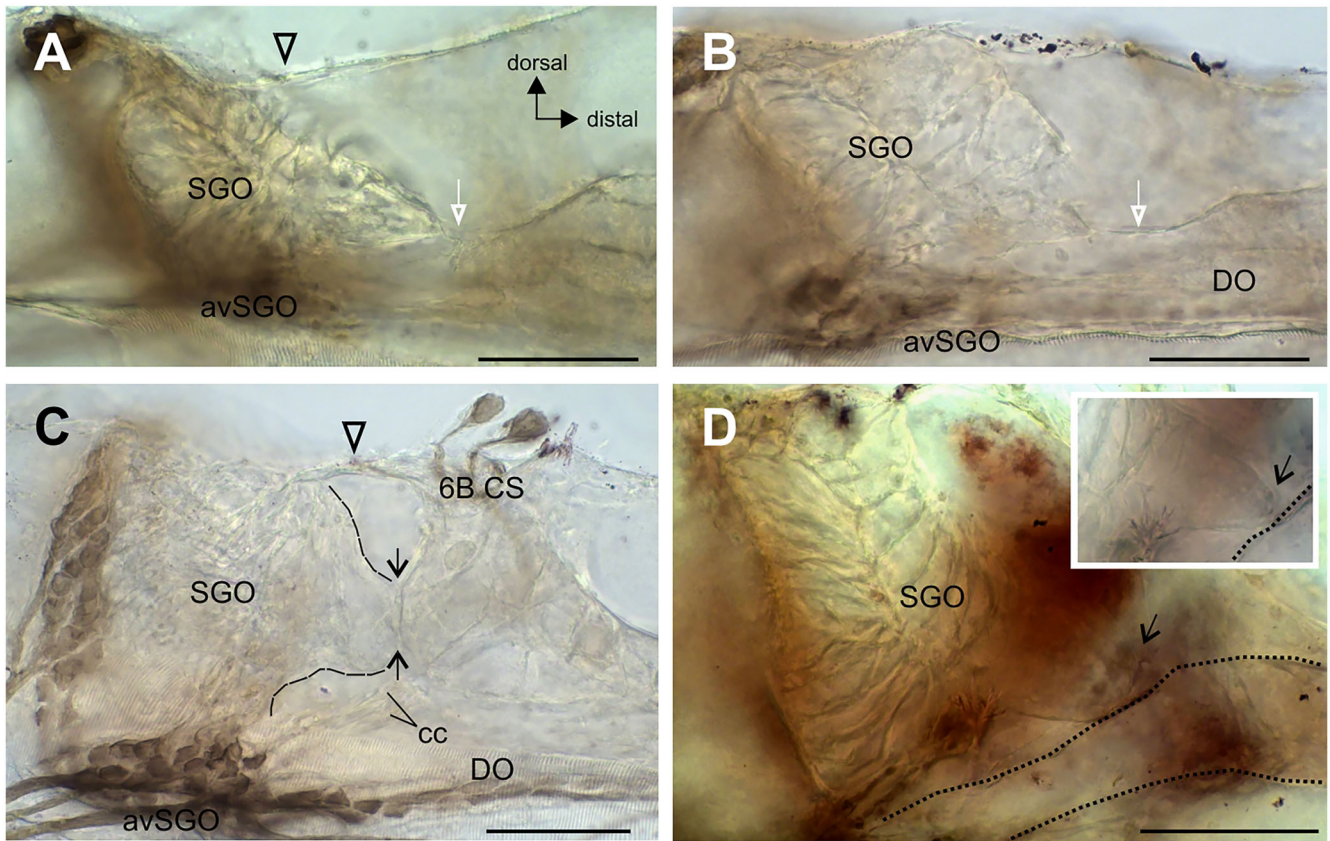


FIGURE 4 The link between subgenual organ (SGO) and distal organ (DO) in *Oreophoetes peruana*. (A) A relatively short connection between the SGO and DO (indicated by arrow). (B) A relatively long connection between the SGO and DO (indicated by arrow). (C, D) The connection was absent from two preparations, where the distal end of the SGO tissue (outlined by hatched lines) (C) is in contact with the DO (arrows) or (D) does not form a membrane. Dotted line in D outlines a tibial trachea. D inset: Detail of the SGO which does not form the thin tip at the distal end. Arrows in D indicate the distal end of the SGO. All photographs from midleg preparations. Orientation as indicated in A. Scales = 100 µm. avSGO, anteroventral subgenual organ; cc, cap cells; CS, campaniform sensilla; DO, distal organ; SGO, subgenual organ

for the DO at the proximal end. Notably, a similar attachment is lacking at the level of the distal DO.

4.3 | Membrane between SGO and DO

The membrane between the ventral side of the SGO and the DO is present in all leg pairs in *O. peruana*. This connection towards the dorsal DO is seen in lateral view (Figure 3C,D). The data from *O. peruana*, combined with previous data from *Carausius morosus*, *Ramulus artemis*, and *Sipylodea sipylus* (Strauß, Moritz, & Rühr, 2021), show that this membrane is present in both major clades of stick insects, the Oripasmata and Occidophasmata. The thin membrane contains no

obvious neuronal elements as seen from the preparations following axonal tracing. Viewed in the lateral perspective, the membrane appears tensed (see also Strauß, Moritz, & Rühr, 2021). It may thus provide a functionally relevant coupling between the SGO and DO. Although the attachments of sensory organs to the surrounding structures such as joints or leg cuticle are relevant for the activation of the sensory organs (Strauß, Stritih-Peljhan, et al., 2021), a connection between adjacent sensory organs is less known.

The SGO complex of the Neoptera is unusual by containing more sensory organs close to the SGO. In orthopteroid insects, the SGO and DO are both well developed with a relatively high number of sensilla. In *O. peruana*, these organs contain sensilla similar to other stick insects (SGO, ~45 sensilla; DO, ~20 sensilla; Strauß, 2022). In

comparison, other insect groups have only very few sensilla in the SGO (one sensory neuron in Mecoptera, Debaisieux, 1938; two sensory neurons in Hemiptera, Alt & Lakes-Harlan, 2018; Michel et al., 1983; Nishino et al., 2016; and three neurons in Neuroptera Chrysopidae, Devetak & Pabst, 1994) and in Dermaptera (Friedrich, 1929). Furthermore, the connection between SGO and DO is unique in a comparative perspective. In insect taxa related to stick insects, these organs can be separated by a membrane (locust, Lin et al., 1995; heelwalkers, Eberhard et al., 2010). In the cockroach, the situation is more complex, with proximal DO sensilla at the same level as the SGO, whereas the DO is covered by a membrane originating distal to the SGO (Schnorbus, 1971). The homologous intermediate organ (IO) in Ensifera (the long-horned grasshoppers) is also covered by a tectorial membrane (Jeram et al., 1995; Lin et al., 1994). Generally, the SGO in these insects does not show the strong distal extension on the ventral side as found in stick insects (Figure 3B–D).

4.4 | The functional morphology of the SGO complex in stick insects

The SGO complex in stick insects shows a unique organization consisting of the SGO and the DO, and the DO is unique in its linear organization of sensilla (Figure 2A). The sensory function of the DO in stick insects is so far not established, but summed recordings including responses from the SGO and DO showed responses to vibration stimuli (Strauß & Lakes-Harlan, 2017). The DO likely also responds to vibration stimuli transferred through the hemolymph or over the cuticle, where close contact to a leg trachea occurs in the most distal part of the organ (Strauß, Moritz, & Rühr, 2021). The membrane between SGO and DO is also described only in stick insects. In the tibia, it transverses the tibia (Figure 3D). Microcomputed tomography showed in three species that the membrane contacts the DO at the proximal end below its most dorsal extension (Strauß, Moritz, & Rühr, 2021; Figure 1B) and is thus not placed as a covering membrane over the DO.

A connection between the SGO and DO by the membrane raises the interesting possibility that the two organs are not strictly independent from each other but structurally coupled, which could affect the responses to mechanical stimulations. So far, the physiological activation and function of the DO is not sufficiently understood, though different connections to the cuticle and the position in the hemolymph are consistent with a role in detecting substrate vibrations (Strauß, Moritz, & Rühr, 2021). Friedrich (1929) described membranes at the SGO and DO in *C. morosus*, including an insertion membrane for the accessory cells from SGO sensilla. This membrane was suggested to add tension to the SGO (Friedrich, 1929). However, based on the descriptions, this insertion membrane did not meet the position of the membrane described here and did not contact the DO. Rather, the membrane extends distally from this insertion membrane which was found in direct contact with the SGO sensilla.

Given the position of the SGO and DO in the hemolymph channel, what functional implications and relevance could the membrane have? For the connective tissues of internal sensory organs, the

material properties are not well analyzed (Strauß & Stritih-Peljhan, 2022). However, some general effects of the connection between the two organs could be suggested. The DO coupled to the distal SGO might act like a mass attached to the SGO, if it was suspended rather freely in the hemolymph, and would probably contribute to tension acting on the SGO. Whether this would limit the displacements of the SGO tissue and thus affect sensory thresholds would depend on the suspension of the DO by other tissues. In addition to the proximal DO attachment, the DO strands linking to the cuticle appear rather light compared with the SGO attachment points (Strauß, Moritz, & Rühr, 2021). The connection might also affect displacements of the proximal DO in response to hemolymph vibrations similar to the SGO. By this, a part of the DO sensilla may be excited similarly to the SGO by stimuli transmitted via the hemolymph. For the distal part of the DO, which also has smaller neurons, the resonance properties may differ. Notably, both SGO and DO have dendrites pointing in the distal direction of the leg, which suggests responses to forces acting along the tibia's main axis. For such biomechanical aspects, physical modeling could analyze the effects of coupling. This would also allow insights in the adaptive aspect of influences on the SGO function by the action of the second sensory organ.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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REFERENCES

- Alt, J. A., & Lakes-Harlan, R. (2018). Sensing of substrate vibrations in the adult cicada *Okanagana rimosa* (Hemiptera: Cicadidae). *Journal of Insect Science*, 18, 16. <https://doi.org/10.1093/jisesa/iey029>
- Ball, E. E., & Field, L. H. (1981). Structure of the auditory system of the Weta *Hemideina crassidens* (Blanchard, 1851) (Orthoptera, Ensifera, Gryllacridoidea, Stenopelmatidae). 1. Morphology and histology. *Cell and Tissue Research*, 217, 321–343. <https://doi.org/10.1007/BF00233584>
- Barth, F. G. (2019). Mechanics to pre-process information for the fine tuning of mechanoreceptors. *Journal of Comparative Physiology a*, 205, 661–686. <https://doi.org/10.1007/s00359-019-01355-z>
- Bässler, U. (1977). Sense organs in the femur of the stick insect and their relevance to the control of position of the femur-tibia-joint. *Journal of Comparative Physiology*, 121(1), 99–113. <https://doi.org/10.1007/BF00614183>
- Bradler, S., & Buckley, T. R. (2018). Biodiversity of Phasmatodea. In R. G. Foottit & P. H. Adler (Eds.). *Insect biodiversity: Science and society* (Vol. II) (pp. 281–313). Wiley-Blackwell. <https://doi.org/10.1002/9781118945582.ch11>
- Čokl, A., & Virant-Doberlet, M. (2003). Communication with substrate-borne signals in small plant-dwelling insects. *Annual Review of Entomology*, 48, 29–50. <https://doi.org/10.1146/annurev.ento.48.091801.112605>

- Debaisieux, P. (1938). Organes scolopidiaux des pattes d'insectes II. *La Célule*, 47, 77–202.
- Devetak, D., & Pabst, M. A. (1994). Structure of the subgenual organ in the green lacewing, *Chrysoperla carnea*. *Tissue and Cell*, 26, 249–257. [https://doi.org/10.1016/0040-8166\(94\)90100-7](https://doi.org/10.1016/0040-8166(94)90100-7)
- Eberhard, M. J. B., Lang, D., Metscher, B., Pass, G., Picker, M. D., & Wolf, H. (2010). Structure and sensory physiology of the leg scolopial organs in Mantophasmatodea and their role in vibrational communication. *Arthropod Structure & Development*, 39(4), 230–241. <https://doi.org/10.1016/j.asd.2010.02.002>
- Field, L. H., & Matheson, T. (1998). Chordotonal organs of insects. *Advances in Insect Physiology*, 27, 1–228. [https://doi.org/10.1016/S0065-2806\(08\)60013-2](https://doi.org/10.1016/S0065-2806(08)60013-2)
- Friedrich, H. (1929). Vergleichende Untersuchungen über die tibialen Scolopalorgane einiger Orthopteren. *Zeitschrift für Wissenschaftliche Zoologie*, 134, 84–148.
- Garza, C., Ramos, D., & Cook, J. L. (2021). Comparative morphology of antennae in the family Pleidae (Hemiptera: Heteroptera). *Zoomorphology*, 140, 243–256. <https://doi.org/10.1007/s00435-021-00522-8>
- Haberkorn, A., Gruhn, M., Zill, S. N., & Büschges, A. (2019). Identification of the origin of force feedback signals influencing motor neurons of the thoraco-coxal joint in an insect. *Journal of Comparative Physiology a*, 205, 253–270. <https://doi.org/10.1007/s00359-019-01334-4>
- Harris, C. M., Dinges, G. F., Haberkorn, A., Gebehart, C., Büschges, A., & Zill, S. N. (2020). Gradients in mechanotransduction of force and body weight in insects. *Arthropod Structure & Development*, 58, 100970. <https://doi.org/10.1016/j.asd.2020.100970>
- Ichikawa, T., Toh, Y., Ohkubo, K., & Nishino, H. (2014). Microscopic analysis of mechanosensory system monitoring the dynamic claw actions in the tenebrionid beetle *Zophobas atratus*. *Zoomorphology*, 133, 273–284. <https://doi.org/10.1007/s00435-014-0225-8>
- Jägers-Röhr, E. (1968). Untersuchungen zur Morphologie und Entwicklung der Scolopodialorgane bei der Stabschrecke *Carausius morosus* Br. *Biologisches Zentralblatt*, 87, 393–409.
- Jeram, S., Rössler, W., Čokl, A., & Kalmring, K. (1995). Structure of atympnate tibial organs in legs of the cave-living Ensifera, *Troglophilus neglectus* (Gryllacridoidea, Raphidophoridae). *Journal of Morphology*, 223, 109–118. <https://doi.org/10.1002/jmor.1052230110>
- Kavlie, R. G., & Albert, J. T. (2013). Chordotonal organs. *Current Biology*, 23, R334–R335. <https://doi.org/10.1016/j.cub.2013.03.048>
- Keil, T. A. (1997). Functional morphology of insect mechanoreceptors. *Microscopy Research and Technique*, 39, 506–531. [https://doi.org/10.1002/\(SICI\)1097-0029\(19971215\)39:6<506::AID-JEMT5>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1097-0029(19971215)39:6<506::AID-JEMT5>3.0.CO;2-B)
- Kilpinen, O., & Storm, J. (1997). Biophysics of the subgenual organ of the honeybee, *Apis mellifera*. *Journal of Comparative Physiology a*, 181, 309–318. <https://doi.org/10.1007/s003590050117>
- Lin, Y., Rössler, W., & Kalmring, K. (1994). Complex tibial organs in the fore-, mid- and hindlegs of the bushcrickets *Gampsocleis gratiosa* (Tettigoniidae): Comparison of the morphology of the organs. *Journal of Morphology*, 221, 191–198. <https://doi.org/10.1002/jmor.1052210208>
- Lin, Y., Rössler, W., & Kalmring, K. (1995). Morphology of the tibial organs of Acrididae: Comparison of subgenual and distal organs in fore-, mid-, and hindlegs of *Schistocerca gregaria* (Acrididae, Catantopinae) and *Locusta migratoria* (Acrididae, Oedipodinae). *Journal of Morphology*, 226, 351–360. <https://doi.org/10.1002/jmor.1052260310>
- Matsuo, E., & Kamikouchi, A. (2013). Neuronal encoding of sound, gravity, and wind in the fruit fly. *Journal of Comparative Physiology a*, 199, 253–262. <https://doi.org/10.1007/s00359-013-0806-x>
- Michel, K., Amon, T., & Čokl, A. (1983). The morphology of the leg scolopial organs in *Nezara viridula* (L.) (Heteroptera, Pentatomidae). *Revue Canadienne de Biologie Experimentale*, 42, 139–150.
- Nation, J. L. (2008). Chordotonal sensory organs. In J. L. Capinera (Ed.), *Encyclopedia of entomology* (2nd ed.) (pp. 863–865). Springer.
- Nishino, H. (2003). Somatotopic mapping of chordotonal organ neurons in a primitive ensiferan, the New Zealand tree weta *Hemideina femorata*: I. femoral chordotonal organ. *Journal of Comparative Neurology*, 464, 312–326. <https://doi.org/10.1002/cne.10779>
- Nishino, H., Mukai, H., & Takanashi, T. (2016). Chordotonal organs in hemipteran insects: Unique peripheral structures but conserved central organization revealed by comparative neuroanatomy. *Cell and Tissue Research*, 366, 549–572. <https://doi.org/10.1007/s00441-016-2480-0>
- Nowińska, A., Chen, P.-p., & Brožek, J. (2020). Comparative study of antennal sensilla of Corixidae and Micronectidae (Hemiptera: Heteroptera: Nepomorpha: Corixoidea). *Insects*, 11, 734. <https://doi.org/10.3390/insects11110734>
- Pflüger, H.-J., Bräunig, P., & Hustert, R. (1981). Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. *Cell and Tissue Research*, 216, 79–96. <https://doi.org/10.1007/BF00234546>
- Pitman, R. M., Tweedle, C. D., & Cohen, M. J. (1972). Branching of central neurons: Intracellular cobalt injection for light and electron microscopy. *Science*, 176, 412–414. <https://doi.org/10.1126/science.176.4033.412>
- Prahlad, A., Spalthoff, C., Kong, D., Großhans, J., Göpfert, M. C., & Schmidt, C. F. (2017). Mechanical properties of a *Drosophila* larval chordotonal organ. *Biophysical Journal*, 113, 2796–2804. <https://doi.org/10.1016/j.bpj.2017.08.061>
- Schnorbus, H. (1971). Die subgenualen Sinnesorgane von *Periplaneta americana*: Histologie und Vibrationsschwellen. *Zeitschrift für Vergleichende Physiologie*, 71, 14–48. <https://doi.org/10.1007/BF03395969>
- Shaw, S. R. (1994). Re-evaluation of the absolute threshold and response mode of the most sensitive known “vibration” detector, the cockroach's subgenual organ: A cochlea-likedisplacement threshold and a direct response to sound. *Journal of Neurobiology*, 25, 1167–1185. <https://doi.org/10.1002/neu.480250911>
- Shelton, P. M. J., Stephen, R. O., Scott, J. J. A., & Tindall, A. R. (1992). The apodeme complex of the femoral chordotonal organ in the metathoracic leg of the locust *Schistocerca gregaria*. *Journal of Experimental Biology*, 163, 345–358. <https://doi.org/10.1242/jeb.163.1.345>
- Shields, V. D. C. (2008). Ultrastructure of insect sensilla. In J. L. Capinera (Ed.), *Encyclopedia of entomology* (2nd ed.) (pp. 4009–4023). Springer. https://doi.org/10.1007/978-1-4020-6359-6_2295
- Simon, S., Letsch, H., Bank, S., Buckley, T. R., Donath, A., Liu, S., Machida, R., Meusemann, K., Misof, B., Podsiadlowski, L., Zhou, X., Wipfler, B., & Bradler, S. (2019). Old World and New World Phasmatoidea: Phylogenomics resolve the evolutionary history of stick and leaf insects. *Frontiers in Ecology and Evolution*, 7, 345. <https://doi.org/10.3389/fevo.2019.00345>
- Strauß, J. (2020). Neuronal innervation of the subgenual organ complex and the tibial campaniform sensilla in the stick insect midleg. *Insects*, 11, 40. <https://doi.org/10.3390/insects11010040>
- Strauß, J. (2021). The tracheal system in the stick insect prothorax and prothoracic legs: Homologies to Orthoptera and relations to mechanosensory functions. *Arthropod Structure & Development*, 63, 101074. <https://doi.org/10.1016/j.asd.2021.101074>
- Strauß, J. (2022). Comparative neuroanatomy of the mechanosensory subgenual organ complex in the Peruvian stick insect, *Oreophoetes peruana*. *Brain, Behavior and Evolution*, in press. <https://doi.org/10.1159/000525323>
- Strauß, J., & Lakes-Harlan, R. (2013). Sensory neuroanatomy of stick insects highlights the evolutionary diversity of the orthopteroid subgenual organ complex. *Journal of Comparative Neurology*, 521, 3791–3803. <https://doi.org/10.1002/cne.23378>
- Strauß, J., & Lakes-Harlan, R. (2017). Vibrational sensitivity of the subgenual organ complex in female *Sipyloidea sipyilus* stick insects in different experimental paradigms of stimulus direction, leg attachment, and ablation of a connective tibial sense organ. *Comparative Biochemistry and Physiology a*, 203, 100–108. <https://doi.org/10.1016/j.cbpa.2016.09.002>

- Strauß, J., Lomas, K., & Field, L. H. (2017). The complex tibial organ of the New Zealand ground weta: Sensory adaptations for vibrational signal detection. *Scientific Reports*, 7, 2031. <https://doi.org/10.1038/s41598-017-02132-1>
- Strauß, J., Moritz, L., & Rühr, P. T. (2021). The subgenual organ complex in stick insects: Functional morphology and mechanical coupling in a complex mechanosensory organ. *Frontiers in Ecology and Evolution*, 9, 632493. <https://doi.org/10.3389/fevo.2021.632493>
- Strauß, J., & Stritih-Peljhan, N. (2022). Vibration detection in arthropods: Signal transfer, biomechanics and sensory adaptations. *Arthropod Structure & Development*, 68, 101167. <https://doi.org/10.1016/j.asd.2022.101167>
- Strauß, J., Stritih-Peljhan, N., Nieri, R., Virant-Doberlet, M., & Mazzoni, V. (2021). Communication by substrate-borne mechanical waves in insects: From basic to applied biotremology. *Advances in Insect Physiology*, 61, 189–307. <https://doi.org/10.1016/bs.aip.2021.08.002>
- Theophilidis, G. (1986). The femoral chordotonal organs of *Decticus albifrons* (Orthoptera: Tettigoniidae) – I. structure. *Comparative Biochemistry and Physiology*, 84A, 529–536. [https://doi.org/10.1016/0300-9629\(86\)90361-0](https://doi.org/10.1016/0300-9629(86)90361-0)
- Yang, Y., Ren, L., Wang, T., Xu, L., & Zong, S. (2017). Comparative morphology of sensilla on antenna, maxillary palp and labial palp of larvae of *Eucryptorrhynchus scrobiculatus* (Olivier) and *E. brandti* (Harold) (Coleoptera: Curculionidae). *Acta Zoologica*, 98, 400–411. <https://doi.org/10.1111/azo.12185>
- Zhao, H., Liang, C., Gao, P., Xie, Y.-H., Wang, Z.-J., Wu, G.-X., Tang, G.-W., Cheng, B., & Gao, X. (2021). Observation of the fine structure of antennal sensilla of the stink bug, *Eocanthecona furcellata* (Hemiptera: Pentatomidae). *Micron*, 150, 103143. <https://doi.org/10.1016/j.micron.2021.103143>
- Zill, S. N., Büschges, A., & Schmitz, J. (2011). Encoding of force increases and decreases by tibial campaniform sensilla in the stick insect, *Carausius morosus*. *Journal of Comparative Physiology a*, 197, 851–867. <https://doi.org/10.1007/s00359-011-0647-4>
- Zill, S. N., & Moran, D. T. (1981). The exoskeleton and insect proprioception. I. Responses of tibial campaniform sensilla to external and muscle-generated forces in the American cockroach, *Periplaneta americana*. *Journal of Experimental Biology*, 91, 1–24. <https://doi.org/10.1242/jeb.91.1.1>
- Zill, S. N., Ridgel, A., DiCaprio, R., & Frazier, S. (1999). Load signaling by cockroach trochanteral campaniform sensilla. *Brain Research*, 822, 271–275. [https://doi.org/10.1016/S0006-8993\(99\)01156-7](https://doi.org/10.1016/S0006-8993(99)01156-7)

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