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RESEARCH ARTICLE

Morphology of the arthrodial membrane gland in a Neotropical harvester (Arachnida: Opiliones)

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ABSTRACT. We describe a gland in the arthrodial membrane of the coxa-trochanter articulation in the fourth pair of legs in the Neotropical harvester *Mischonyx squalidus* Bertkau, 1880. Externally the glandular area has a rough appearance with pores on its surface, with folds of the arthrodial membrane. Internally, its secretory cells have spherical secretory vesicles, smooth endoplasmic reticulum, mitochondria and ducts that exit from the cells and cross the arthrodial membrane. Histochemical tests indicate the presence of proteins and neutral glycoproteins. The function of the gland might be to produce lubricating products that allow better movement of the coxa-trochanter region.

KEY WORDS. Elastic membrane, intersegmental membrane, lubrication, harvestman.

INTRODUCTION

The body of arthropods is composed of two main types of cuticles, one more rigid and one softer (Hepburn and Chandler 1976, Andersen et al. 1995, Moussian 2013). The often more rigid one is largely composed of exocuticle (i.e., highly tanned) and forms the main plates (sclerites) and limbs of the skeleton. The softer cuticle makes up the flexible joints (intersegmental membrane) between the rigid parts and is composed almost entirely of hydrated endocuticle and epicuticle (Vincent 2002). An exocuticle is absent (Dennel and Malek 1954). Intersegmental membranes may be part of the joints of chela, head-thorax and legs among other body regions (Govindarajan and Rajulu 1974, Tychsen and Vincent 1976, Andersen 1999). These structures have elastic properties that allow appendages to move properly (Hepburn and Chandler 1976) and the protein resilin is probably responsible for flexibility (Bennet-Clark and Lucey 1967, Govindarajan and Rajulu 1974, Neff et al. 2000, Gorb 2002). Histological studies in insects show that a specific intersegmental membrane called arthrodial membrane (= cuticle membrane – Gorb 1996) revealed exocrine glands (Billen 2009, Nijs and Billen 2015).

Studies with beetles and cockroaches have shown that they release lubricating substances in regions of the body that experience friction (femoro-tibial, head-prothorax, oc-

cipital region) (Naiden and Gorb 2021). These regions have pores (~0.5–10 µm in diameter) through which the lubricant comes out, usually in elongated cylindrical shapes, similar to toothpaste output. The authors suggest that the function of lubricants is to minimize friction and wear in the areas around these glands (Naiden et al. 2021, Naiden and Gorb 2021). Despite these recent discoveries, little is known about the internal morphology of membranes that make intense contact with other surfaces or themselves.

There is not a lot of information on arthrodial membranes in arachnids (see Shultz 2000, Sensening and Shultz 2003, 2004, Silva et al. 2021). With the aid of scanning microscopic images (SEM), Willemart et al. (2007) found pores in the external region of the arthrodial membrane (plate pore) of the fourth pair of leg of two harvester (Opiliones) species, suggesting the presence of glandular structures (Arachnida: Opiliones). However, no previous studies have described the ultrastructure of the arthrodial membrane in this group. Harvesters have approximately 6700 described species (Kury et al. 2020). They inhabit preferentially humid areas, where they shelter in caves, under rocks, trees and logs (Curtis and Machado 2007) and are an interesting group of arthropods to investigate the structure and possible role of glands in intersegmental membrane regions. Thus, the objective of our study was to characterize the arthrodial membrane of the harvester *Mischonyx squalidus* Bertkau, 1880 based on light and electron microscopy (both transmission and scanning). Specifically, we asked whether we would find characteristics of glandular structures.

MATERIAL AND METHODS

Study species, collection and laboratory conditions

Mischonyx squalidus appears in previous articles as *Mischonyx cuspidatus* or *Ilhaia cuspidata* (see Gueratto et al. 2021). Individuals of *M. squalidus* (n = 10) were collected manually in August 2018 and March 2019, under tree trunks at the Parque Ecológico do Tietê (-23.494587, -46.521383), municipality of São Paulo, state of São Paulo, Brazil. Only male individuals were used because of their large arthrodial membrane. The animals were brought to the laboratory where they were supplied with water and dog food ad libitum. They were collected under SISBIO/ICMBio (Sistema de Autorização e Informação em Biodiversidade/Instituto Chico Mendes de Conservação da Biodiversidade) license number 61431-1- 2018. The term "canal" instead of "duct" or "channel" follows the classic paper of Noirot and Quennedey (1974) and several others that followed (e.g., Blomquist and Bagnères 2010, Kheyri et al. 2014).

External ultrastructure

To characterize the external morphology of the arthrodial membrane located in the coxa-trochanter joint of the leg IV of *M. squalidus*, the animals (n = 5) were first euthanized in a freezer at 4 °C, then fixed in Bouin's solutions. The animals were submitted to three-step ultrasonic cleaning: (1) stirring with distilled water; (2) incubation with 1:10 detergent solution (Alconox); and (3) stirring with distilled water again. Then, the regions of interest were cut with micro scissors and dehydrated in graded series of 50% to 100% ethanol. Samples were then critical point dried and mounted on aluminum stubs with carbon adhesives on both sides. Finally, the samples were sputter coated with gold and photographed with scanning electron microscope (Quanta 250, FEI Company, Netherlands).

Histological anatomy

To reconstruct the internal morphology of the arthrodial membrane of *M. squalidus*, we anesthetized five individuals and fixed them in Karnovsky's (1965) or Bouin's solution for three days. Subsequently, we cut the arthrodial membrane area with a micro scissor and embedded the samples in Leica Historesin. The samples were sectioned with a Microm HM 340 microtome 3 µm-thick. The histological sections were stained with hematoxylin and eosin. To characterize the chemical composition of the arthrodial membrane, we applied the following histochemical staining methods: bromophenol blue (for proteins), alcian blue pH 2.5 and periodic acid-Schiff (PAS) (for acid and basic mucopolysaccharides, respectively) and Sudan black B (for lipids). The samples were analyzed using light microscope (Leica) and photographed with a camera (Olympus) mounted on to the microscope.

Internal ultrastructure

To reconstruct the internal ultrastructure of the arthrodial membrane of *M. squalidus*, the samples were dissected in cold $NaH_2PO_4x2H_2O$ buffer and then fixed in a mixture of 2.5% Glutaraldehyde and 2% Paraformaldehyde in 0.1 M buffer $NaH_2PO_4x2H_2O$ (according to Karnovsky 1965) and kept at 4 °C. The samples were washed in Siena PBS buffer, fixed in 2% osmium tetroxide for three hours and washed again in Siena PBS buffer. For resin embedding (Spurr 1969) the samples were serially dehydrated from 50% to 100% pure ethanol gradually infiltrated in resin with alcohol/resin series 2/1, 1/1, 1/3 and pure resin. We made cross sections with 50 nm thickness using an ultramicrotome (Microm HM 340). The samples were stained with uranyl

acetate and lead citrate and analyzed (10 kV) in a Jeol JEM-100 CX22 Transmission Electron Microscope (TEM).

RESULTS

Externally, the arthrodial membrane of *M. squalidus* is located in the coxa-trochanter region of the leg IV (Fig. 1A) has smooth and textured regions of cuticle, which have several folds ("Fo", Fig. 1B). Furthermore, there are pores of approximately 2 µm diameter dispersed on both surfaces (white arrows, Fig. 1B–D).

The arthrodial membrane (AM) is between the coxa (CX) and trochanter (TR), regions filled with muscles (m) responsible for leg movements, and it stains more clearly with hematoxylin in the histological sections (Fig. 2A, B). The AM is clearly thinner than regions with cuticle only (Fig. 2B), and extends the whole circumference of the trochanter although it is visible only on the dorsal region in the figure (Fig. 2B). Folds composed of a stratified fibrous matrix can be observed (Fig. 3A–C). The epidermal region is composed of several secretory cells with a prismatic shape, found exclusively in arthrodial membrane regions (Fig. 3). Each cell has a nucleus arranged in the center and the cytoplasm is filled with granules of varying shapes (Fig. 3C). Irregular cuticular canals can be observed within the cells and arthrodial membrane (Fig. 3B, C).

The histochemical analysis showed that the arthrodial membrane reacts only with Bromophenol Blue and PAS, indicating the presence of proteins (Fig. 4A) and neutral glycoproteins (Fig. 4B). It was not possible to identify the reaction of Bromophenol Blue and PAS in the prismatic cells because some cells were damaged at some point during histological procedures. Through the histochemically treated images, it was also possible to observe irregular cuticular

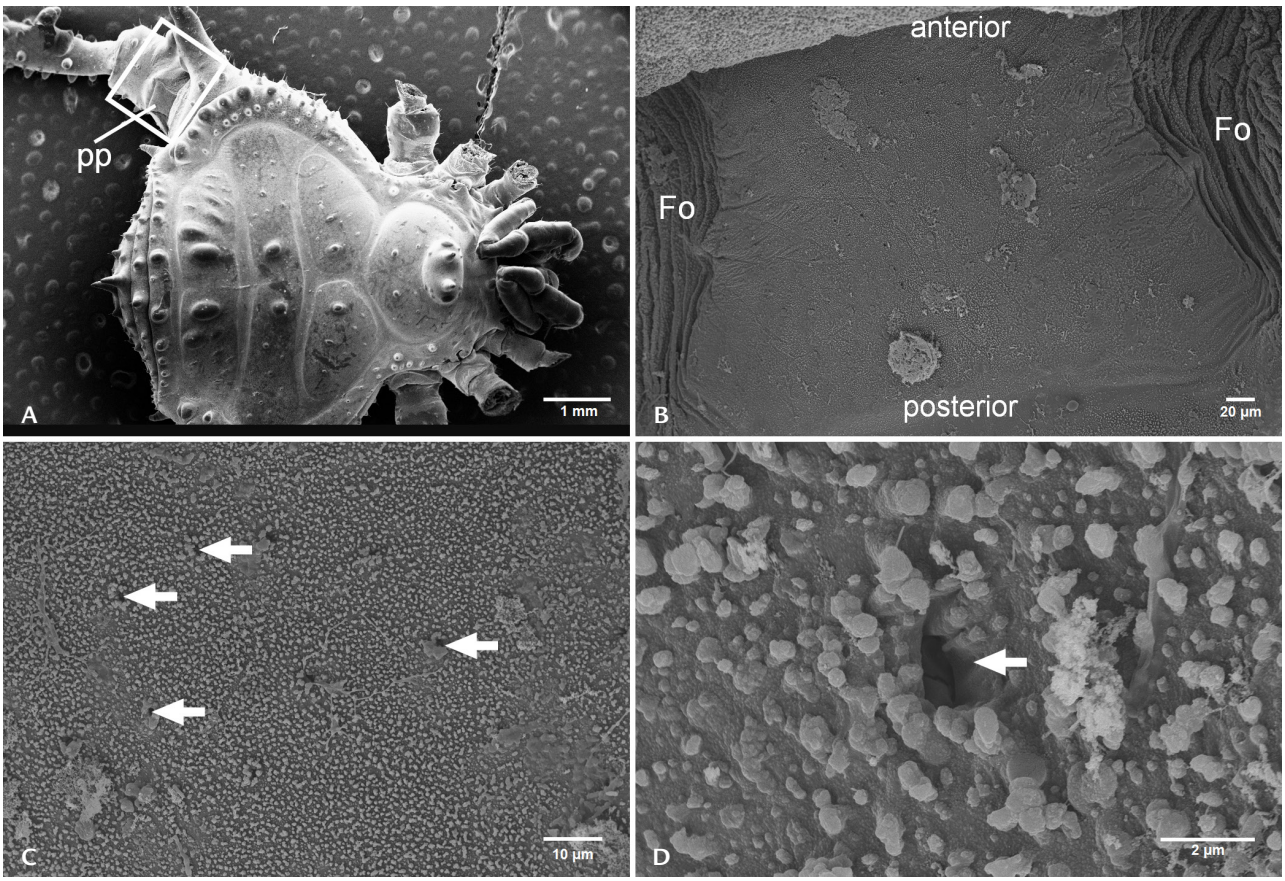

Figure 1. External morphology of a male harvester *Mischonyx squalidus*: (A) dorsal view. The anterior region is on the right, legs I, II and III were removed. The square shows the arthrodial membrane in the leg IV and the pore plate (pp); (B) regions with folds (Fo) and without folds; (B–D) show increasing zoom of the pore plate, a region without folds. Arrows show pores.

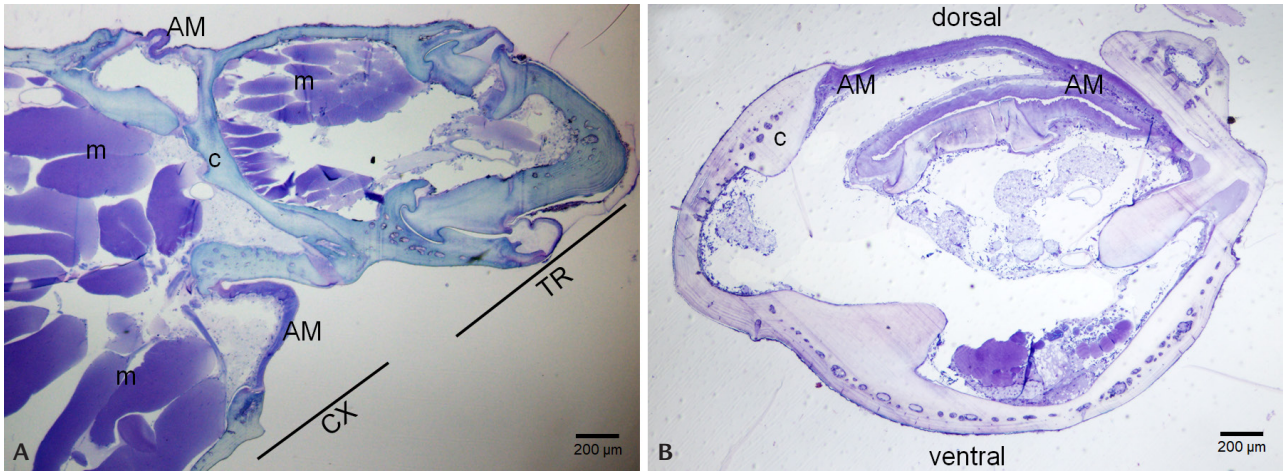

Figure 2. Sections through an arthrodial membrane of the coxa (CX) - trochanter (TR) articulation of a leg IV in a male harvester *Mischonyx squalidus*: (A) frontal longitudinal section; (B) transversal section between the coxa and the trochanter of leg IV. (AM) Arthrodial membrane, (c) cuticle (sclerite cuticle), (m) muscle.

canals penetrated in the prismatic cells and in the arthrodial membrane. (Fig. 4 A,B). We found no reservoir in this set of cells. It is hard to be exclude the possibility that the hundreds of small dark spots stained granules are not an artifact, and therefore we cannot be sure if they correspond the observed granules in TEM.

The TEM images also provide evidence that prismatic cells are full of secretion granules with variable morphology and electron density (Fig. 5A,B). The electron-dense granules have a diameter between 0.4 and 0.8 µm. Also, a smooth endoplasmic reticulum (SER) could also be observed (Fig. 5A) in the cytoplasm of cells, between the secretion granules, in addition to mitochondria (Fig. 5B).

DISCUSSION

Our study demonstrates that the arthrodial membrane of the coxa-trochanter joint of leg IV of a harvester has cuticular pores in the outer region and cuticular canals internally. The arthrodial membrane presents also proteins and glycoproteins as well as a set of prismatic cells in the epidermal region.

The external rough appearance of the arthrodial membrane outside the pore plate may be related to elastic properties of the membrane. Not only do the SEM micrographs show folded areas but the histological analysis also shows contracted or folded cuticle. Indeed, typically arthrodial membranes differs from adjacent cuticle (see Hackman 1982). We also found that the arthrodial membrane consists of, among other components, proteins and glycoproteins. The lower degree or absence of sclerotization combined with specific proteins give elastic properties to the arthrodial membranes of many arthropods (Willis 1987, Andersen 1999, Moussian 2013). A protein with elastic proteins commonly found in arthropods including harvesters is resilin, which efficiently stores elastic energy. Though resilin fluoresces blue under UV light (Michels et al. 2016), as did AM (NFS Silva, personal observations), we do not have evidence of its presence. Folds similar to the reported herein have been previously reported (Compere and Goffinet 1987), and variations of such folds are common in regions of soft cuticle in insects (Hackman and Goldberb 1987). Microscopically, the external surface of the arthrodial membrane has several cuticular pores, which suggested the presence of canals to release secreted material. Canals have been previously reported in the AM (Billen 2009, Billen and Plancken 2014, Nijs and Billen 2015) and, indeed, our histological section revealed that the prismatic cells below the arthrodial membrane have cuticular canals that run from the secretory cells to the membrane itself (Fig. 3B,C and 4A,B). The irregular nature of canals has also been observed in previous studies (Hackman and Goldberg 1987). The cuticular canals we found were probably filled with secreted material (Fig. 5B), which suggests that the glandular material must exit to the outer region of the arthrodial membrane. However, it was not possible to find a relationship between the amount of cuticular canals and the secretory cells observed. A fibrous matrix similar to the found here and cells with mitochondria

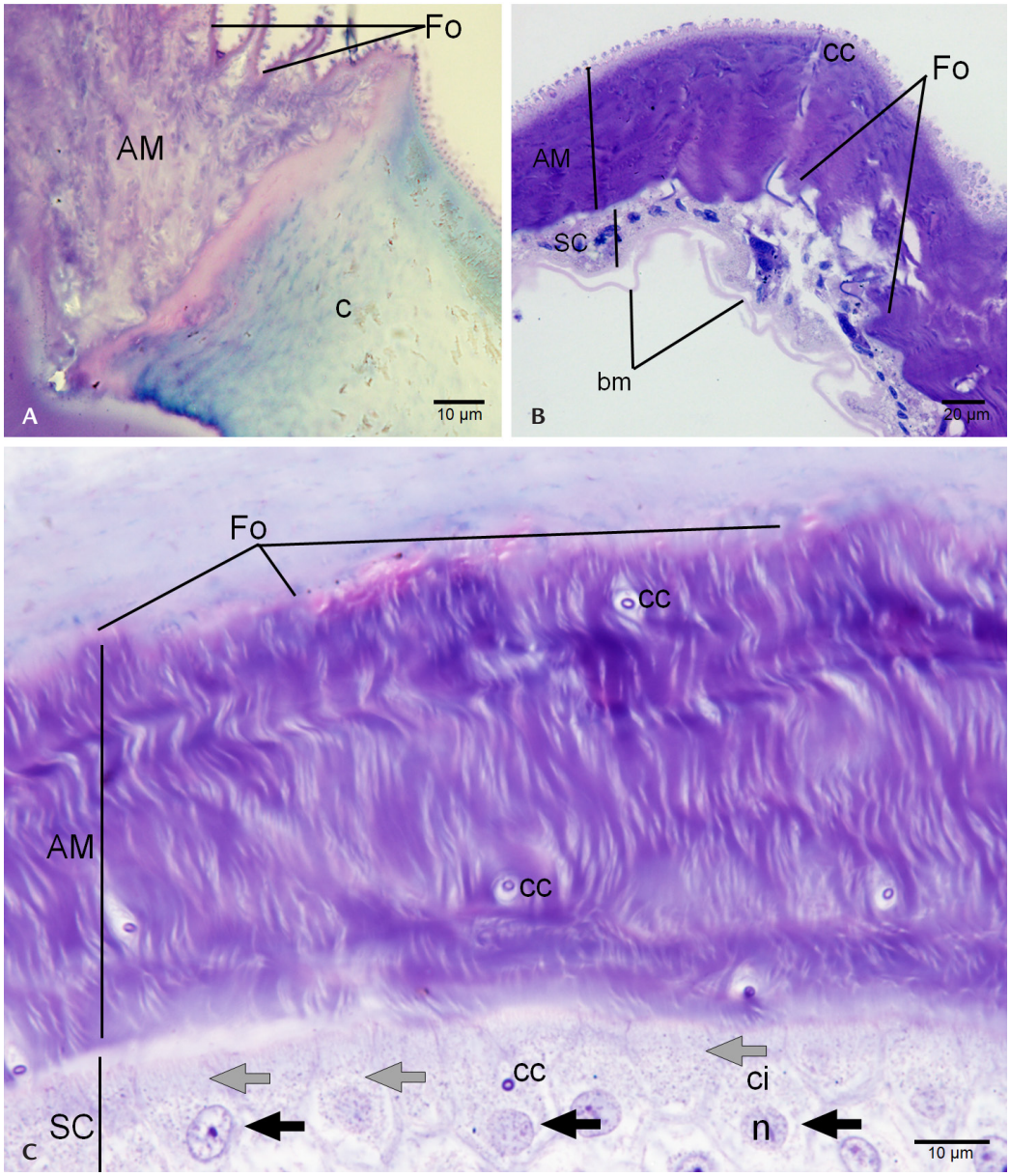

Figure 3. Sagital sections through an arthrodial membrane of the coxa-trochanter articulation of a leg IV in a male harvester *Mischonyx squalidus*: (A) arthrodial membrane cuticle (AM) and cuticle (sclerite cuticle) (c); (B) arthrodial membrane and basal membrane of secretory cells; (C) secretory cells (sc) with glandular prismatic cells (black arrows), granules (gray arrows) and cuticular canals (cc) stained with hematoxylin and eosin. (bm) Basal membrane, (ci) cytoplasm, (Fo) folds, (n) nucleus.

have been reported in arthrodial membranes of insects, but not with such prismatic cells (Grandperrin and Cassier 1983, Hackman and Goldberg 1987).

The composition of the secretion granules showed differences in electro densities. That could be related to the degree of maturation of these granules (though we cannot discard the possibility that these are granules of different substances), and be evidence of secretory activity in the specimen studied (Sobotnik et al. 2003). Moreover, the shape of these granules suggests their composition include proteins (Sobotnik et al. 2003, Billen 2009). This would be in agreement with a study on beetles, where the semi-solid lubricating substance

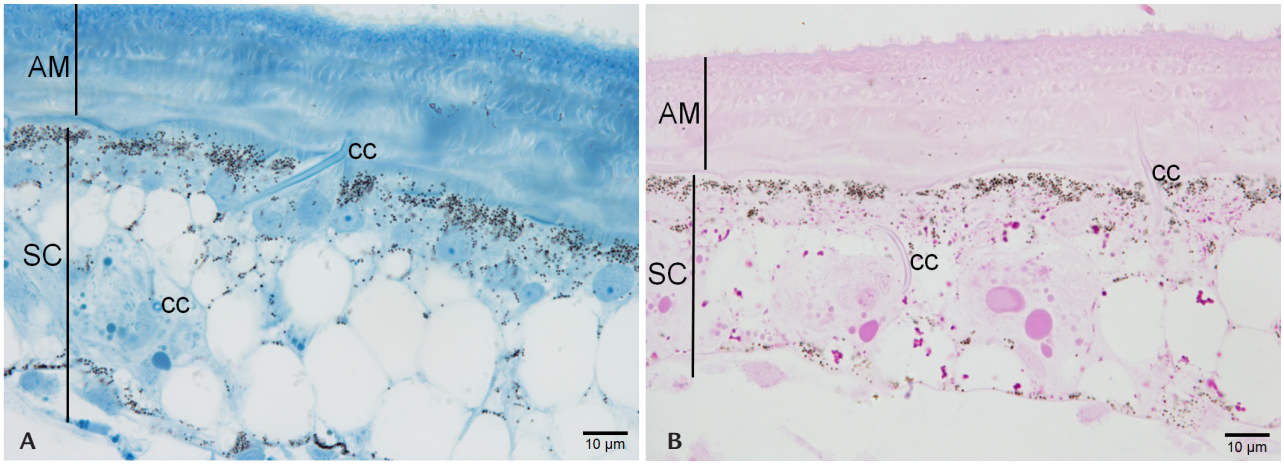

Figure 4. Sagital sections through an arthrodial membrane of the coxa-trochanter articulation of a leg IV in a male harvester *Mischonyx squalidus*: (A) staining with bromophenol blue; (B) staining with PAS. (AM) Arthrodial membrane, (SC) secretory cells, (cc) cuticular canals.

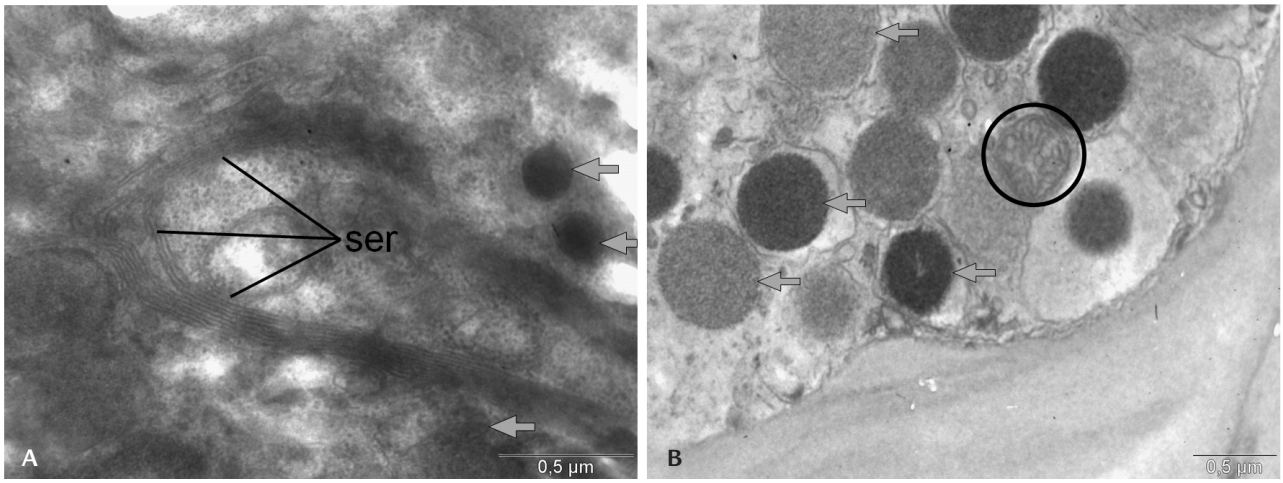

Figure 5. Interior of a prismatic cell in the arthrodial membrane of the coxa – trochanter articulation of a leg IV in a male harvester *Mischonyx squalidus*: (A) smooth endoplasmic reticulum (ser); (B) mitochondrion (circle) and granules (gray arrows).

is mostly insoluble protein (Naiden et al. 2021). Considering that harvesters joints are exposed to the environment, insoluble proteins allow that they will not be lost in contact with humidity, which is an important feature for harvester living in humid regions (see Curtis and Machado 2007). It should be mentioned that Silva et al. (2021) have reported proteins related to transport, lysis, storage of lipids and with possible antimicrobial function in this exact gland this harvester species. Such proteins may also be related to the vesicles described herein. Finally, the presence of mitochondria suggests some secretory activity in these cells.

The works that described exocrine glands (histological) in intersegmental membrane regions are almost exclusively carried out in insects, mainly ants and wasps (Sobotnik et al. 2003, Billen 2009, Nijs and Bilen 2015). The coxal-gland was described in the same region of the arthrodial membrane between the coxa-trochanter of harvesters (Willemart et al. 2007, Billen and Plancken 2014, Nijs and Billen 2015). This gland differs from ours in being a bicellular gland (class-3, see Noirot and Quennedey 1991) while that of *M. squalidus* has only one cell unit (probably class-1 – Noirot and Quennedey 1991). The other works described several other exocrine

glands, mostly class-3 secretory cells, belonging to other regions of the intersegmental membrane. Almost unanimously, the authors suggest that these glands have a lubricating function. The main reasons for this conclusion, which also apply to the species we have studied, are the presence of the gland close to articulation regions, as well as canals that open close or properly in intersegmental articulation membranes such as trochanter-femur, distal femur, proximal and distal junction of tarsomeres, femur-tibia, proximal and distal tibia, coxa-thorax (Schoeters and Billen 1993, Billen 2009, Billen and Plancken 2014, Nijs and Bilen 2015). Other possible reasons that likely also apply to harvesters (though we have not tested) are the presence of these glands in all legs, and in in both sexes, the repetition of these glands in segments (such as the tarsomeres) and, finally, the presence of organelles such as a smooth endoplasmatic reticulum (Schoeters and Billen 1993, Billen 2009, Billen and Plancken 2014, Nijs and Billen 2015). It has been shown that lubricants prevent physical contact between surfaces, absorbs energy and minimizes friction and wear (Naiden et al. 2021).

Because other joints of harvesters also have similar morphologies including pore openings, they possibly also have intersegmental glands (NFS Silva, personal observation). Based on the several suggestions found in the literature, such as gland location, pore opening site, proteins substances, presence of organelle with possible indication of production of oily substances (Attygalle et al. 1996, Billen and Ito 2006, Billen 2009) and exclusivity of the prismatic cells in the arthrodial membrane, we also suggest that the cells of the epidermal region of the arthrodial membrane of *M. squalidus* may be a gland with a lubricating function. Based on the similarities in the morphology of other intersegmental regions and joints to the one studied, such function would also be present in other body regions in harvesters.

We provided a first structural characterization and described general patterns of the chemicals present in an arthrodial membrane in Opiliones. Evidences from both areas suggest a secretory function, with the function to be determined.

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Competing Interests

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