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Fine Structure of the Rhagidial-Organs of the Prostigmatid mite *Rhagidia halophila* (Laboulbène, 1851) (Actinotrichida, Rhagidiidae)

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Abstract

The rhagidial organs of *Rhagidia halophila* were studied using scanning (SEM) and transmission (TEM) electron microscopy. They are located on the legs I and II and consist of 4 recumbent setae on tarsi I and of 4 setae on tarsi II and 1 on tibiae I. In addition to the 4 anterior setae there is a very small stellate setae at the medial base of the second proximal recumbent seta. All setae are innervated and thus represent sensilla. The recumbent sensillae are innervated by three sensory cells with distal dendrites and a lattice-like cuticular sheath and many wall pores (wp-sensillum, likely olfactory). The innervation of the stellate sensillum could only partly been observed. This sensillum bears a terminal pore (tp-sensillum, likely gustatory) and is provided with an additional internal cuticular layer, likely bearing the birefringence properties of almost all setae of actinotrichid mites, except for the so-called solenidia. We regard the recumbent setae as peculiar solenidia and the stellate setae as reduced so-called eupathidia. Certainly, the rhagidial organs are an important chemical receptor for these mites and deserve further experimental research.

Keywords Rhagidial-organs | Actinotrichida | fine structure

1. Introduction

The rhagidial-organs are enigmatic structures of recumbent dorsal solenidia that occur in a more or less complex form in some of the eupodoid mites (*Rhagidia*, *Linopodes*, *Eupodes*, *Coccorhagidia*). They are mostly positioned in small groups on the tarsi of the legs I and II (in some species also on legs III and other leg segments; (see Zacharda 1980 for further details regarding rhagidiids) and are probably most obvious in the predatory family Rhagidiidae, hence the name (Fig. 1).

Grandjean (1935) defined the setiform-like structures on the body of actinotrichid mites, mainly based on their shapes and the presence of birefringence (containing some or no actinochitin/pilin within their shafts). Until now, the following setiform-like structures are distinguished in actinotrichid mites.

The tactile seta, the trichobothrium, the eupathidum and the famulus, that are all provided more or less with birefringence (see also Krantz 2009, Zacharda 1980). An exception is the solenidion which never shows birefringence (for further details see also: Hammen 1989, Evans 1992, Alberti & Coons 1999, Krantz 2009).

Walter et al. (2009) described the solenidia of eupodoids as being 'usually inserted in individual or communal grooves or troughs and typically modified so that each solenidion takes the form of a pick hammer with the offset stem representing the shortened handle'. This applies perfectly to the rhagidial organs of *Rhagidia halophila* (Fig. 1). We have studied these peculiar organs using scanning and transmission electron microscopy and revealed a very complex fine structure.

2. Material and methods

Rhagidia halophila (Laboulbène, 1851) mites were collected during summer in 2012, 2013, and 2014 at rocky places covered with algae or organic debris in the upper

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eulitoral of the North Sea coast close to Weddewarden and Wilhelmshaven (Germany).

For transmission electron microscopy (TEM), specimens were shortly placed into isopropanol to break the hydrophobic surface of the mites. Then the still living mites were transversely cut into halves and fixed in cold 2.5% glutaraldehyde (pH 7.2, phosphate buffer 0.1M + 1.8% sucrose) for several hours. After rinsing with buffer solution, the tissues were postfixed with 2% OsO4-aqueous solution. Specimens were dehydrated with graded ethanols and embedded in Araldite using propylenoxide as intermedium. Sections were done with a Leica UCT using a Diatome diamond knife. The sections (70 nm) were stained with uranylacetate and lead citrate (Reynolds 1963) and studied with a JEOL JEM-1011. For general orientation semi-thin sections (400 nm) were stained using methyleneblue (Richardson et al. 1960). The same fixation procedure was carried out for scanning electron (SEM) microscopy. Subsequently specimens were dehydrated using graded ethanol, critical point dried using liquid CO2 as final medium. The specimens were mounted on Al-stubs, coated with palladium-gold and examined with a Zeiss EVO LS 10. For macrophotogaphy a SLR camera with a converter 2X, a bellow with a 135 mm lens and a 28 mm lens in retro position was used. For more technical information see Alberti & Nuzzaci (1996).

3. Results

The rhagidial organs of *Rhagidia halophila* are located dorsally on the two fore legs (Fig. 1, 4). In the adult mite the first two legs bear four setae on the tarsi and one on tibiae I. The setae are directed in parallel with their tips pointing oblique/antiaxially-anteriorly. They are positioned with their stalks in an insertion site, which could allow a slight bending (Figs 1B–D). Each seta is located in an individual small and smooth depression and is surrounded by trichomes which cover the whole body of the mite. The trichomes bordering closely the seta are slightly longer (Fig. 1E).

In addition to the group of four setae on the first leg there is a single, tiny, stellate seta close to the median side of the second proximal recumbent seta (Figs 1B, C). Each recumbent seta is innervated by three short dendrites with typical ciliary regions (Figs 2B–E). The posterior part (behind the stalk) is filled with dense material. The main part (pointing anteriorly) of the seta is densely filled by numerous vesicles (Figs 2B, C). Most striking is the complex cuticle that covers the seta (Figs 2C, F, G). Whereas the offset stem and the proximal cuticle are made of the usual, densely staining cuticle, the more distal part is highly modified. Here the epicuticle is forming separate, almost transparent ribs parallel to the length axis of the sensillum. The ribs are located above the also rib-forming densely staining procuticle which is passed almost transversely, thus forming a lattice-like network. Tiny spaces lie between the crossing ribs. Due to the very dense and irregular cellular equipment in the legs, we were not able to trace the innervation further proximally.

The stellate seta (Figs 3A–C) shows a crown of star-like emergences on a short stem. In the centre it is a bit extended and at its tip a terminal pore is present (Fig. 3B). The stellate seta reveals a third type of more lucent, internal cuticle, which was neither seen in the adjacent surface cuticle of the body nor in the recumbent setae (Figs 3C–D). The stellate seta is also innervated, but we were not able to detect its innervation completely, due to its tiny size (Figs 3C–D).

4. Discussion

Our study shows that the rhagidial organs have a chemoreceptive structure, as already suggested by Zacharda (1980). This conclusion can be drawn by the complex external cuticular cover of each recumbent seta, a solenidion, which bears numerous entrances for the passage of volatile components. Hence we think that these are olfactory organs (as is assumed in general for solenidia now; Evans 1992). It is remarkable, how these entrances are formed. Unlike in most olfactory sensilla of arthropods (e.g., Altner 1977; Steinbrecht 1984) including mites and ticks (e.g., Alberti & Coons 1999, Coons & Alberti 1999) they do not exhibit additional merely numerous pores in the shaft of a seta-like sensillum (so-called wall-pore (wp-) sensilla), but they have additional transversely-crossing ribs of epi-and densely staining procuticle, which leaves tiny passages between the ribs. The transparency of the external ribs is certainly the reason why the rhagidial organs are usually drawn in the light microscope as being transversely-striated due to the internal transversely oriented ribs of densely staining procuticle as is the case for solenidia in general (e.g., Vitzhum 1940/43, Hammen 1989, Alberti & Coons 1999, Krantz 2009). However, the peripheral epicuticular ribs were not known from other solenidia until now. Also a slight movement of a solenidion against its insertion has always been denied (e.g., Krantz 2009). Yet this could be true for the individual solenidia of the rhagidial organ shown here.

Remarkable is the innervation of the solenidia by three dendrites with ciliary regions. This is not observed in mechanoreceptors and trichobothria of mites which

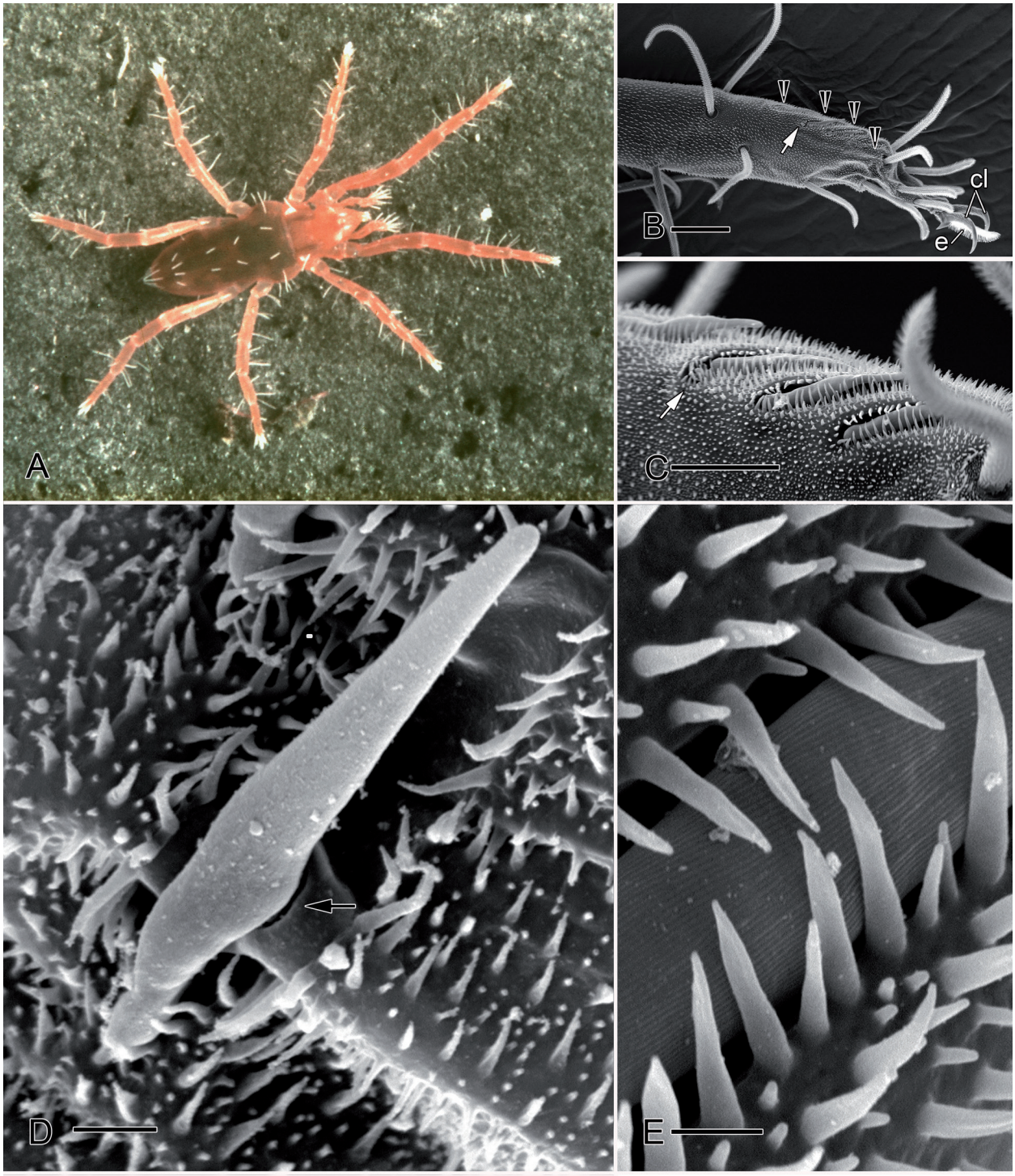
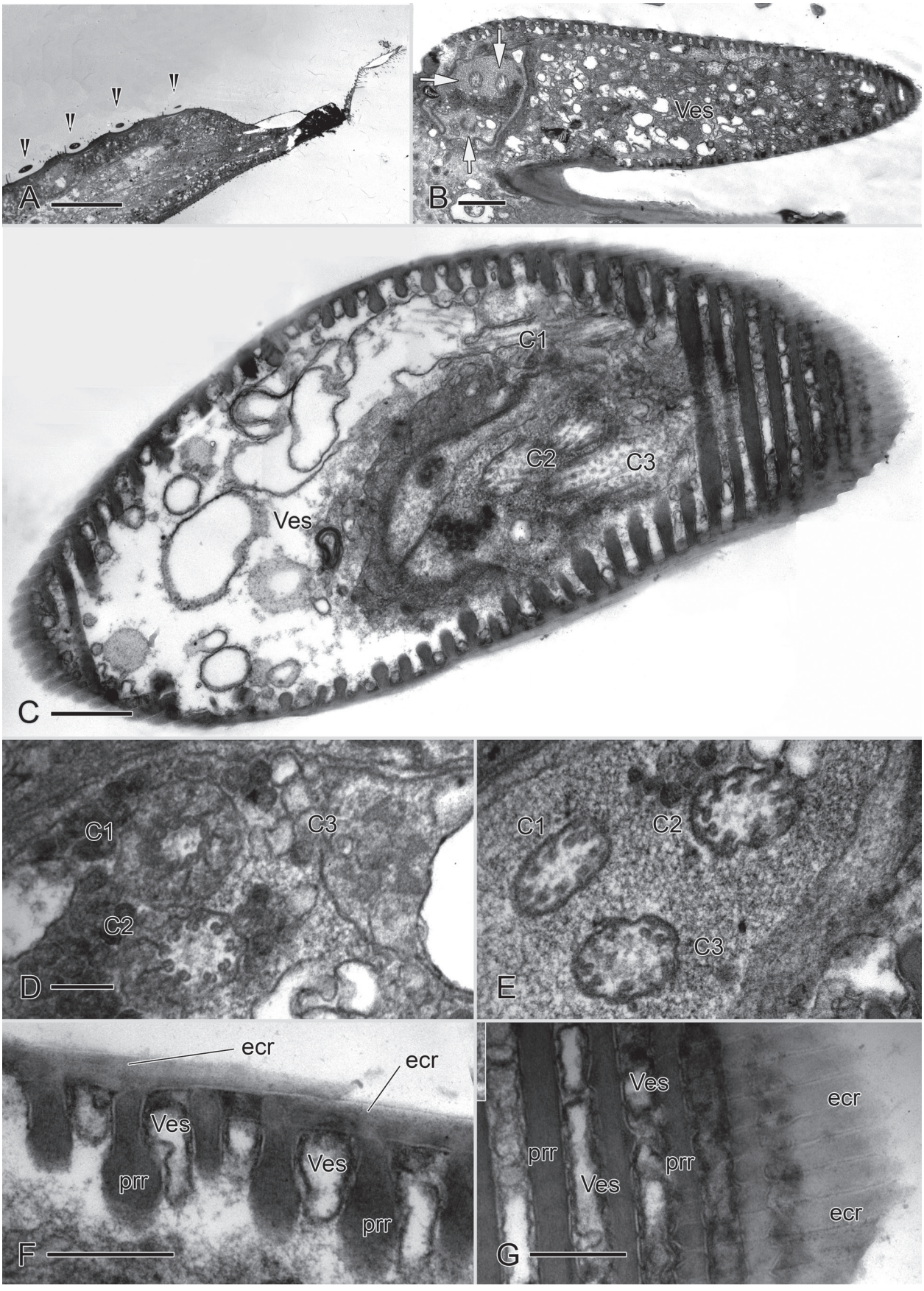


Figure 1. (**A**) *Rhagidia halophila*, adult living mite in dorsal view. Idiosoma length 1050 µm. (**B**) Distal left tarsus I seen from medial. Arrowheads indicate recumbent setae, white arrow points to position of tiny stellate setae. Scale bar: 20 µm. (**C**) Enlarged vision of rhagidiid organ, almost same view (white arrow points to stellate seta). Scale bar: 5 µm. (**D**) An individual recumbent seta. Note its short stalk into which it is inserted with a fold (black arrow). Scale bar: 2 µm. (**E**) Detail of recumbent seta showing longitudinally arranged epicuticular ribs (compare Fig. 2F, G) and accompanying protecting trichoms. Scale bar. 1 µm.

Abbr.: **cl** – claw, **e** – empodium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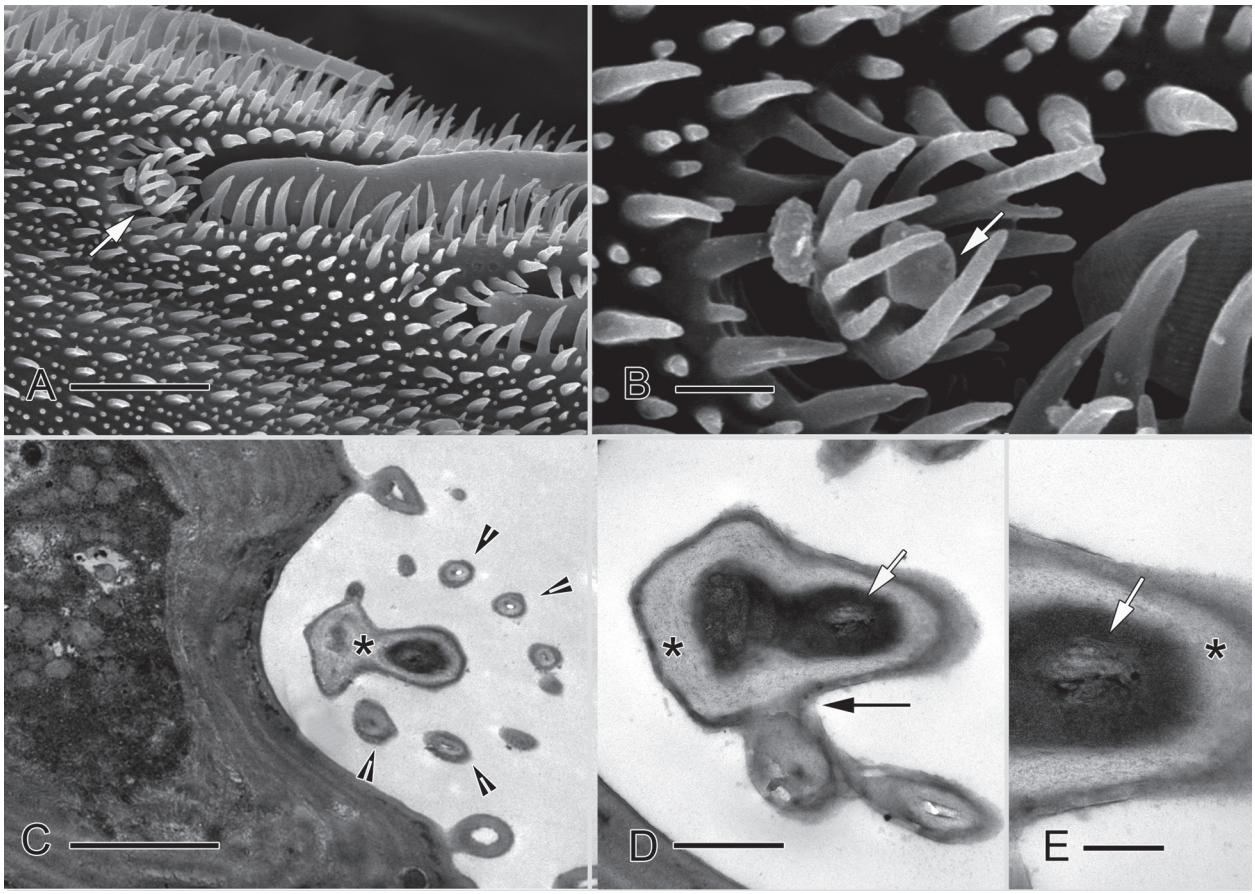

▲ **Figure 3**. (**A**) SEM of stellate seta close to second proximal recumbent sensillum (white arrow). Scale bar: 5 µm. (**B**) Enlarged detail. White arrow points to median process with terminal pore. Scale bar: 1 µm. (**C**) TEM section through stellate seta showing branches of the star-like crown around median process. Asterisk indicates additional cuticular layer. Scale bar: 2 µm. (**D**) Detail of 3C showing branches of stellate seta (black arrow). Asterisk shows additional cuticular layer. White arrow indicates probably innervations (?). Scale bar: 0.5 µm. (**E**) Detail showing innervations (white arrow) and additional cuticular layer (asterisk). Scale bar: 0.2 µm.

◄ **Figure 2**. (**A**): Distal tarsus I in a sagittal section, black arrowheads indicate recumbent setae. Scale bar: 10 µm. (**B**) Longitudinal section through one recumbent seta revealing its innervations by three ciliary regions (white arrowheads). Note numerous distal vesicles within the sensillum. Scale bar: 1 µm. (**C**) Enlarged, almost tangentially sectioned sensillum showing the three ciliary regions and the cuticular sheath forming the peculiar wall of the sensillum. Scale bar: 0.5 µm. (**D**) The three ciliary regions close to their basis. Scale bar: 0.2 µm. (**E**) The three ciliary regions more distally. Scale bar: 0.2 µm. (**F**) Longitudinal section through cuticular wall of recumbent sensillum. Scale bar: 0.2 µm. 2G: Tangential section through cuticular wall of recumbent sensillum. Scale bar: 0.2 µm.

Abbr.: **C1-C3** – sensory cilia, **ecr** – epicuticle rib, **Ves** – vesicle, **prr** – procuticle rib

always have two receptor cells with dendrites ending in typical tubular bodies (as no-pore (np-) sensilla; e.g., Alberti & Coons 1999, Alberti et al. 2011, Alberti & Dabert 2012, Alberti & Kitajima 2014).

Zacharda (1980) collected numerous details on rhagidiids living habits and found that all are depending on humid conditions, which certainly applies also to the species investigated here. It was also suggested that cave-dwelling rhagidiids which may bear more solenidia than others (e.g., species of *Troglocheles*) may be better adapted to finding their prey in 'challenging habitats' (Walter et al. 2009). However, since the organs (though not studied in detail) occur also on mites which are considered to be purely phytophagus (e.g., *Penthaleus* species; Walter et al. 2009) we think of a broader function, e.g., highly evolved humidity receptors. Of course, we cannot conclude any definite function of these organs and the way of transduction of the volatile stimuli based on our morphological studies. However, the numerous vesicles

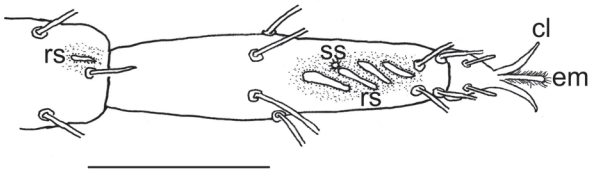

Figure 4. *Rhagidia halophila*, diagram of the rhagidial sensory organ with four recumbent setae on tarsus and one tiny recumbent seta on tibia of the left leg I, dorsal view. Scale bar: 100 µm.

Abbr.: **cl** – claw, **e** – empodium, **rs** – recumbent sensilla, **ss** – stellate seta

that are included in each solenidon seem to be remarkable and may play a role in the transduction process (e.g., by different swelling degrees during various humidity conditions). This evidently is at the moment speculative and needs an experimental approach. Perhaps those vesicles could be artefacts.

An even more curious structure is the tiny stellate seta occurring close to the rhagidial organ on legs I. Evans (1992) stated that famuli are 'aporous' and the function of them remains mysterious. This contrasts with Krantz (2009), who stated that the famulus has a terminal pore and thus is similar to an eupathidium. In our study, we could show, that the stellate seta is probably an innervated sensillum and is indeed provided with a terminal pore and an additional layer of lucent cuticle. The latter may bear the birefringence (compare Alberti et al. 2011). Thus, this is a sensillum, which is indeed closely related to a reduced, but peculiar, eupathidum as e.g. (Krantz 2009) already suggested.

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