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# Fine structure of the naso with median eye and trichobothria in the prostigmatid mite *Rhagidia halophila* (Rhagidiidae, Actinotrichida)

**Gerd Alberti[1] and Rainer Ehrnsberger[2]***

[1] Allgemeine und Systematische Zoologie, Zoologisches Institut und Museum, Ernst-Moritz-Arndt-Universität Greifswald, J.-S.-Bach-Str. 11/12, 17487 Greifswald, Germany

[2] Institut für Didaktik der Naturwissenschaften, Universität Vechta, Driverstr. 22, 49377 Vechta, Germany

* Corresponding author, e-mail: alberti@uni-greifswald.de

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## Abstract

*Rhagidia halophila*, as other Rhagidiidae, possesses a distinct frontal idiosomatic protuberance, the naso. It bears an unpaired eye (ocellus) that is directed ventrally and consists of four receptor cells provided with numerous rhabdomeric microvilli. The cuticle overlying the microvilli is thin and smooth in contrast to the dorsal cuticle of the naso that shows a fine, spiny sculpture. Details of the fine structure of the receptor cells of the eye are reported. It seems that there is a high membrane turnover which is indicated by numerous dense stacks of membranes. The peculiarity of the median eye and the naso of actinotrichid mites is highlighted and interpreted as plesiomorphic within Arachnida. On the dorsal side of the naso, a pair of small setae (internal verticals) is located in deep sockets thus representing trichobothria. Each sensillum is innervated by two dendrites which terminate with prominent tubular bodies. The axons of the receptor cells of these trichobothria like those of the median eye leave the naso through a narrow passage bordered by specialized cells.

Keywords Acari | evolution | ocellus | sensilla | tubular bodies | ultrastructure

## 1. Introduction

Rhagidiidae (Prostigmata, Actinotrichida = Acariformes) is a mite family of worldwide distribution comprising about 125 species living mostly in soils as fast running, voracious predators of small arthropods (Ehrnsberger 1973, 1981, Zacharda 1980, Walter et al. 2009). *Rhagidia halophila* (Laboulbène, 1851) is found in the upper eulitoral of rocky or stony coasts of the North Sea and Atlantic hunting for Collembola and small mites (Ehrnsberger 1981). In contrast to the mostly pale-whitish coloured species of other habitats, *Rhagidia halophila* is brightly red at least in the older instars.

Since some years there is growing evidence, that Acari is not a monophyletic taxon comprising two subgroups, i.e. Anactinotrichida (= Parasitiformes s.l.) and Actinotrichida (= Acariformes) as was believed by the majority of authors for many years (see review in Dunlop & Alberti 2008), but that these two groups represent taxa with different relationships. The actinotrichid mites are apparently most closely related to Solifugae, whereas Anactinotrichida are closer to Tetrapulmonata as is evident from morphological (Alberti 1980a-c, 1984, 2000, 2006, Alberti & Peretti 2002, Klann et al. 2009, Dunlop et al. 2012) as well as from molecular data (Dabert et al. 2010, Pepato et al. 2010). Alberti (2006) provided an overview on fundamental morphological differences between the two groups mentioned updating earlier such reports (e.g., Grandjean 1969, Lindquist 1984, Bernini 1986, Hammen 1989, Evans 1992). One very striking character of early derivative actinotrichid mites is a dorsomedian, frontal protuberance of the idiosoma, for which the term ‘naso’ became established Grandjean (1943, 1958, Hammen 1980, 1989, Evans 1992, Alberti & Coons 1999, Krantz 2009). It is found in some so-called Endeostigmata, and early derivative


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Prostigmata and Oribatida and thus certainly represents a fundamental character of Actinotrichida (Grandjean 1932, 1939, 1943, Hammen 1980, 1989, Weigmann 2006, Norton & Behan-Pelletier 2009, Walter 2009, Walter et al. 2009). Hammen (1980, 1989) suggested that the naso may have an acronal or precheliceral origin. From most of these mites the naso is known to bear two (paired) eyes or one (unpaired) eye representing ocelli (see, e.g., Grandjean 1958, Coineau 1970, Wachmann et al. 1974, Hammen 1980, 1989, Witte 1991, 1995, Alberti & Coons 1999, Haupt & Coineau 2002, Alberti & Moreno-Twose 2012, Olomski 2012). In many actinotrichids, the naso may be less distinct or may disappear together with the median eye(s) (many Prostigmata, most Oribatida and Astigmata – Norton & Behan-Pelletier 2009, OConnor 2009, Walter et al. 2009), in others the median eye may persist without showing a naso (e.g. in certain Bdellidae – Alberti 1975, Hydrachnidia – Mischke 1981, Walter et al. 2009, Olomski 2012, few Oribatida – Norton & Behan Pelletier 2009, Alberti & Moreno-Twose 2012). On the other hand, there are reports on mites possessing a naso, but eyes in it were not seen. This applies for example to Rhagidiidae (e.g., Zacharda 1980) and Saxidromidae (Coineau et al. 2006).

On the contray, a pair of lateral eyes is evident in Rhagidiidae (Zacharda 1980, Walter et al. 2009) and two pairs of lateral eyes are known from Saxidromidae (Coineau et al. 2006).

Remarkably, a pronounced naso may be present in prelarvae (the first instar in Actinotrichida) including species which lack a naso in the following instars (e.g., Grandjean 1958, Ehrnsberger 1974, Alberti 1975, Otto 1996).

None of the other arachnid groups possess a protuberance like the naso, but many have a pair of median eyes (Scorpiones, Uropygi, Amblypygi, Araneae, Opiliones, Solifugae) others not (Pseudoscorpiones, Ricinulei, Anactinotrichida). Opiliones have only median eyes, Ricinulei have lateral lucent (eye-?) spots and Palpigradi have no eyes at all (e.g., Moritz 1993, Hammen 1989, Alberti et al. 2008). The organization of median eyes and lateral eyes may differ (e.g., Araneae – Foelix 1996). Since the naso is such a peculiar and an evidently basal character of Actinotrichida on the one hand and the median eyes are such fundamental for Arachnida (or even Arthropoda [Paulus 1979, Weygoldt & Paulus 1979, Gruner 1993, Moritz 1993]; their absence must be regarded as apomorphic), it seemed reasonable to clarify the organization of this structure for a further mite taxon.

The naso in many species bears a pair of small so-called internal vertical (iv, vi) or rostral (ro) setae, which in certain taxa have been described as trichobothria (e.g., Sphaerolichida, Saxidromidae and related taxa – Coineau et al. 2006, Walter et al. 2009), in others they are simple setae (e.g., certain Endeostigmata – Walter 2009; all Oribatida [mostly without naso] – Norton & Behan-Pelletier 2009). With regard to Rhagidiidae the status of these setae is uncertain (Zacharda 1980, Walter et al. 2009).

Thus this study – with respect to *Rhagidia halophila* – presents information to the following:

- the fine structure of the naso clarifying whether there is an unpaired eye or not,
- the fine structure of the putative median eye of the naso,
- the status of the pair of setae located on the naso.

## 2. Materials and methods

*Rhagidia halophila* (Laboulbène, 1851) mites were collected in summer 2012 and 2013 at rocky or stony places covered with algae or organic debris in the upper eulitoral of the North Sea coast close to Weddewarden and Wilhelmshaven (Germany). The mites were transferred into plastic boxes provided with a bottom made of Plaster of Paris that was kept moist. After transport of the mites into the lab, 11 specimens were transferred to 70% ethanol. These specimens were used for scanning electron microscopy (SEM). They were dehydrated through a series of ethanols (60%, 70%, 80%, 90%, 95%, abs.) and critical point dried using liquid $CO_2$ as final medium. The specimens were mounted on Al-stubs, coated with palladium-gold and examined with a Zeiss EVO LS 10. Since these specimens turned out to be quite shrunken, in addition two specimens fixed as for TEM (see below) were studied.

For transmission electron microscopy (TEM), 8 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% $OsO_4$-aqueous solution. After rinsing again, specimens were dehydrated with graded ethanols and embedded in Spurr's medium (Spurr 1969). Sections were done with a Leica UCT using a Diatome diamond knife. The sections (75 nm) were stained with uranylacetate and lead citrate (Reynolds 1963) and studied with a JEOL JEM-1011. For general orientation with a compound light microscope semi-thin sections (400 nm) were stained using Richardson's solution (Richardson et al. 1960). For more technical information see Alberti & Nuzzaci (1996).

## 3. Results

### 3.1. External aspects

The anterior border of the idiosoma projects dorsomedially with a distinct protuberance, the naso (Fig. 1A). The naso is a flattened sphere which is dorsally covered by a spiny cuticle (Fig. 1B, C). These spines are much smaller than those of the more posterior idiosomatic cuticle. Close to the anterior border of the naso, a pair of finely barbed setae, the internal verticals iv, is situated (Fig. 1A, B; designation of setae acc. to Zacharda 1980). The setae are set into deep sockets, bothridia, like the more posteriorly located, prodorsal trichobothria (tr, Fig. 1A, B, D). This insertion differs from other idiosomatic setae, which do not show such deep sockets (Fig. 1D). Thus, the setae of the naso have to be considered as trichobothria. At its anterior and lateral borders, the cuticle of the naso abruptly changes towards the ventral side, where it is smooth. This sharp border is pronounced by a fine furrow (Figs. 1B, C; 3B).

### 3.2. Internal aspects

The sections reveal that a median eye is located in the naso (Figs. 2–5A, 6). It consists of four photoreceptor cells which fill most of the volume of the naso. Two of these cells (1, 2) are located posterolaterally. The other

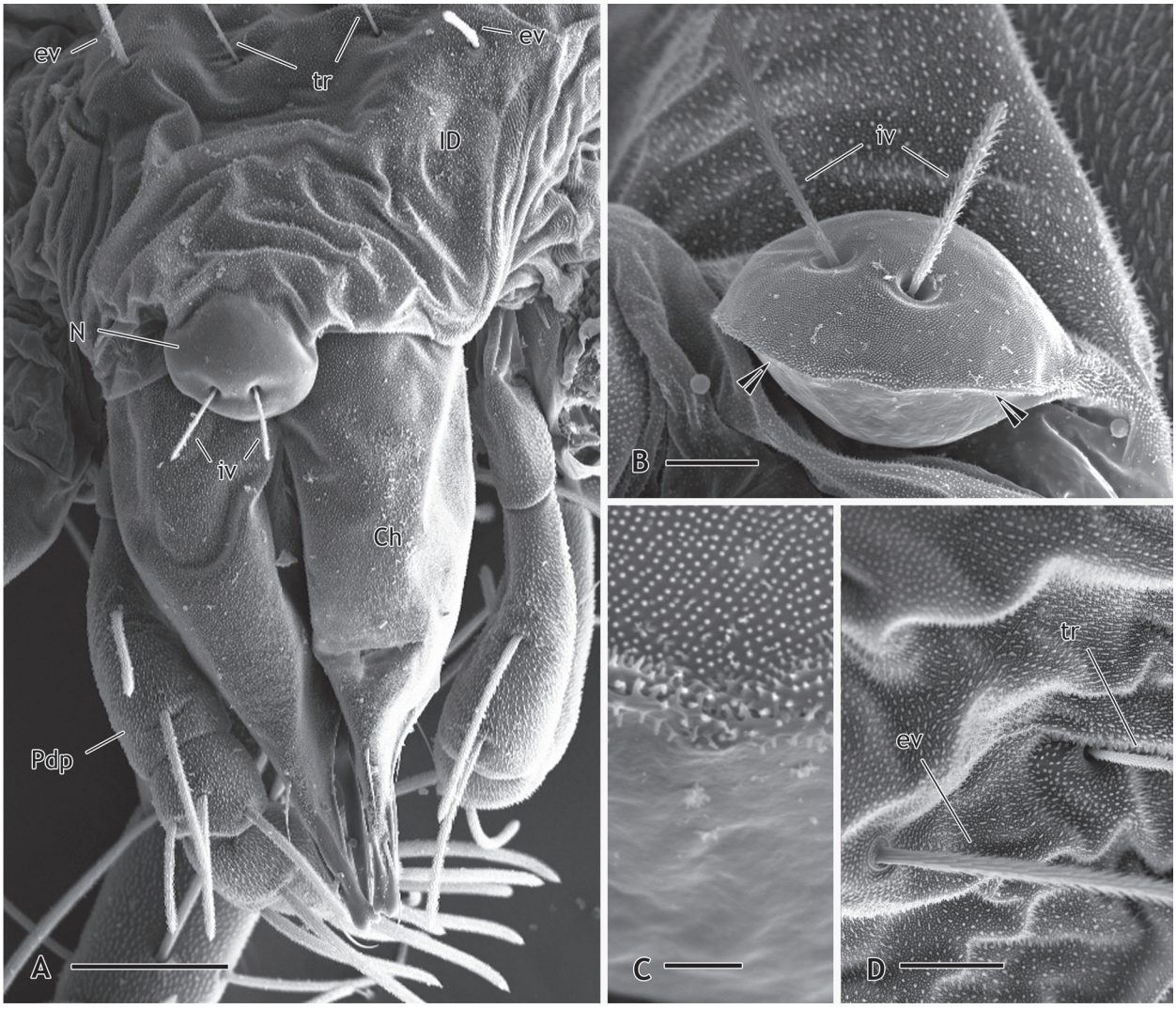
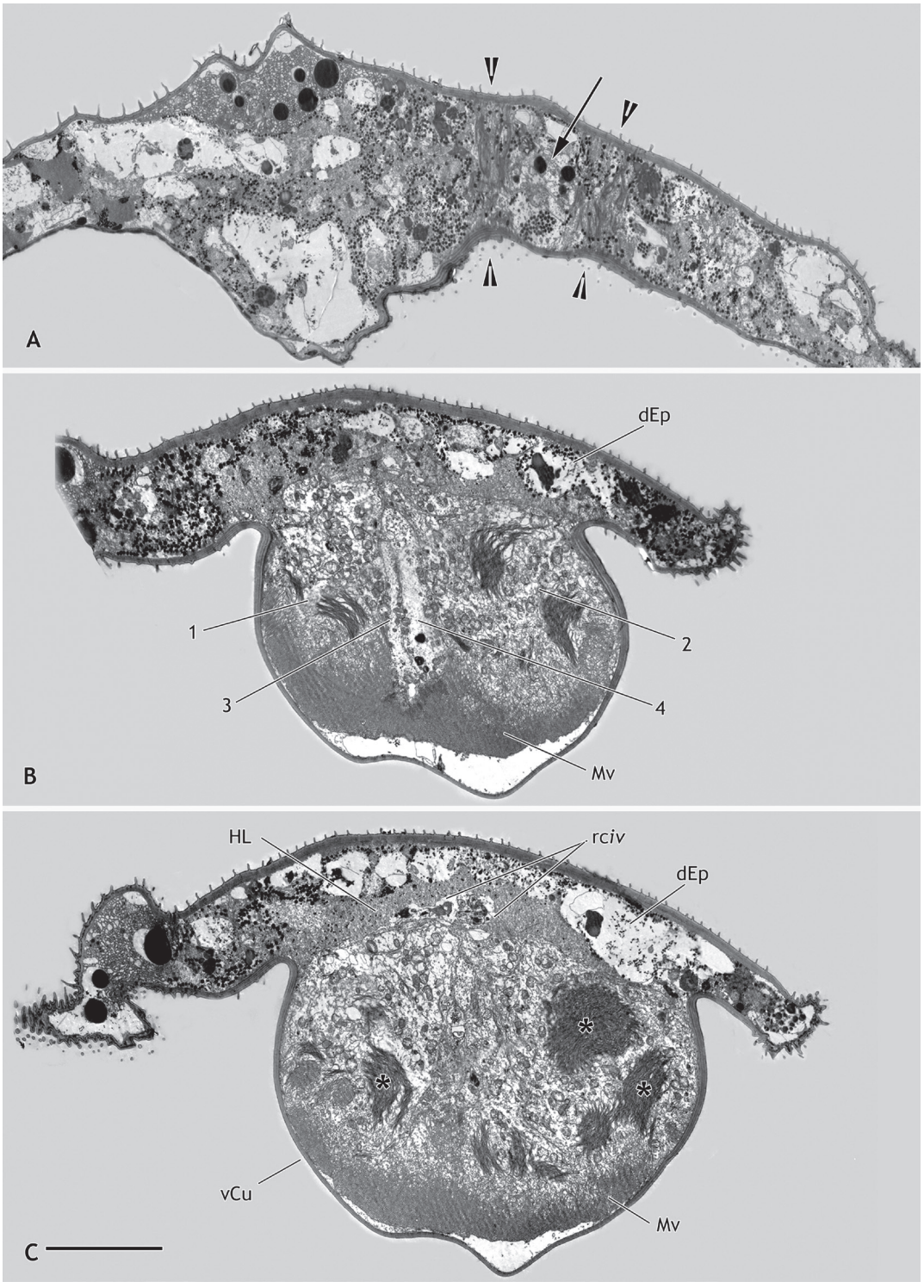



**Figure 1**. SEM-figures of *Rhagidia halophila* (setal designation acc. to Zacharda 1978). (**A**) Dorsal view of gnathosoma and anterior part of idiosoma. Scale bar: 50 µm. (**B**) Naso seen in an anteroventral direction. Note distinct border (arrowheads) between spiny dorsal surface and smooth ventral surface of naso. Setae *iv* are inserted into deep sockets thus representing trichobothria (compare Fig. 1D). Scale bar: 10 µm. (**C**) Detail of Fig. 1B in higher magnification. Scale bar: 2.5 µm. (**D**) Prodorsal setae *tr* and *ev.* Note *tr* being inserted in a deep socket (bothridium) like setae *iv* on naso in contrast to *ev*. Scale bar: 10 µm.

Abbr.: **Ch** – chelicera, ***ev*** – external vertical seta, **ID** – idiosoma, ***iv*** – internal vertical seta (or rostral seta), **N** – naso, **Pdp** – pedipalp, ***tr*** – (prodorsal) trichobothrium.

two (3, 4) pass medially between these cells to reach a more anterior position where they broaden. All four cells bear apically numerous rhabdomeric microvilli which are directed posteroventrally (Figs 2B–D, 3A, D; 4B, 5A). The cuticle of the ventral side of the naso is smooth and thin consisting of four procuticular layers and a thin epicuticle. Pore canals were not observed. A very thin epidermal layer is located underneath the cuticle (Figs 3A, D; 4A, B, D), which may be interrupted in a central area over the rhabdomeric microvilli. The receptor cells contain basally a roundish nucleus with few heterochromatin. The cytoplasm shows numerous mitochondria with few cristae, some rough cisternae of endoplasmic reticulum (ER), many vesicles and, most



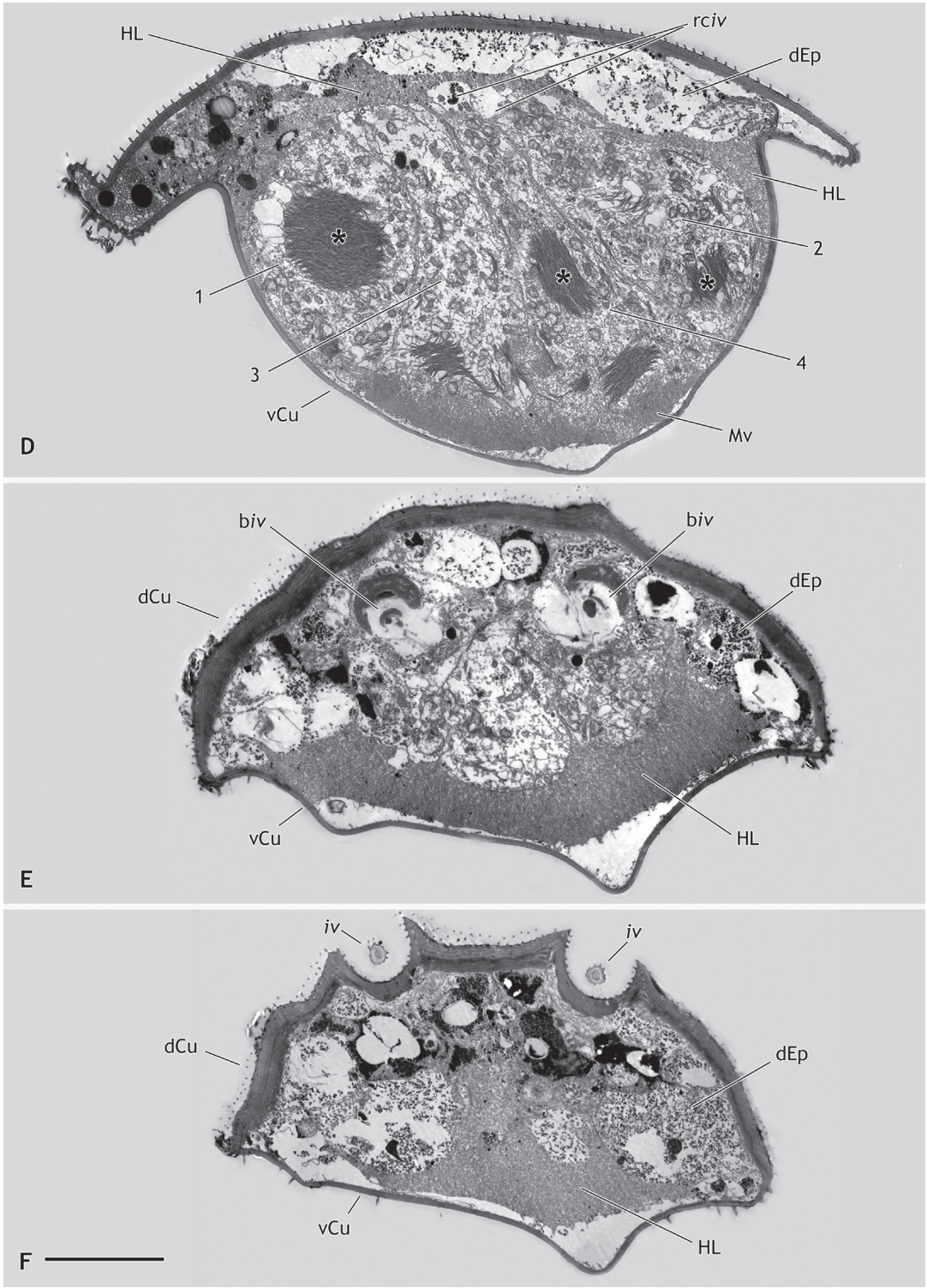



**Figure 2**. TEM-figures of a series of slightly oblique cross-sections through naso of *Rhagidia halophila* in posterior (**A**), anterior (**F**) sequence (all figures in same scale). Note narrow passage (arrow) for axons of nasal trichobothria and photoreceptor cells bordered by specialized epidermal cells (arrowheads; see Fig. 4E, F). Two photoreceptor cells (1, 2) are located posteroventral, the other two (3, 4) extend between them more anteriorly. The rhabdomeric microvilli are only met in the posterior sections because of their posteroventral orientation. In the photoreceptor cells (1–4) conspicuous dense stacks of membranes (asterisks) are present. Note that the dorsal cuticle appears thicker in Figs **2E**, **F** because it is sectioned slightly tangentially due to the anterior curvature of the naso (compare Figs 1B, 3A, 5A). Scale bar (in Figs **2C**, **F**): 10 µm.

**Abbr**.: **1–4** – photoreceptor cells 1-4; **b*iv*** – base of nasal setae *iv,* **dCu** – dorsal cuticle, **dEp** – dorsal epidermis, **HL** – hemolymph, ***iv*** – internal vertical seta, **Mv** – (rhabdomeric) microvilli, **rc*iv*** – receptor cells of setae *iv,* **vCu** – ventral cuticle.

conspicuous, large stacks of electron-densely staining membranes (Figs. 2, 3). These membranes are sometimes found close to bundles of microvilli which reach deeply into the cell (Fig. 3D). Microtubules are also found. Neigbouring receptor cells are connected via desmosome-like cell junctions (Fig. 4B,C). The axons of these cells are hard to detect within the naso, but evidently leave the naso through a narrow passage between specialized cells that are formed by modified epidermal cells. These cells of the dorsal and ventral epidermal layer are closely adjoining at their bases, interdigitated and connected via conspicuous microtubule-associated desmosome-like cell junctions. They are connected to the overlying cuticle via hemidesmosome-like junctions. This passage is also used by the axons of the trichobothria. Thus, eight axons are found in cross-sections through this region (Figs 2A, 4E,F, 5A).

Specialized pigment cells are not involved in the formation of the median eye of *Rhagidia halophila*. But the epidermis of the flat idiosomatic part that connects

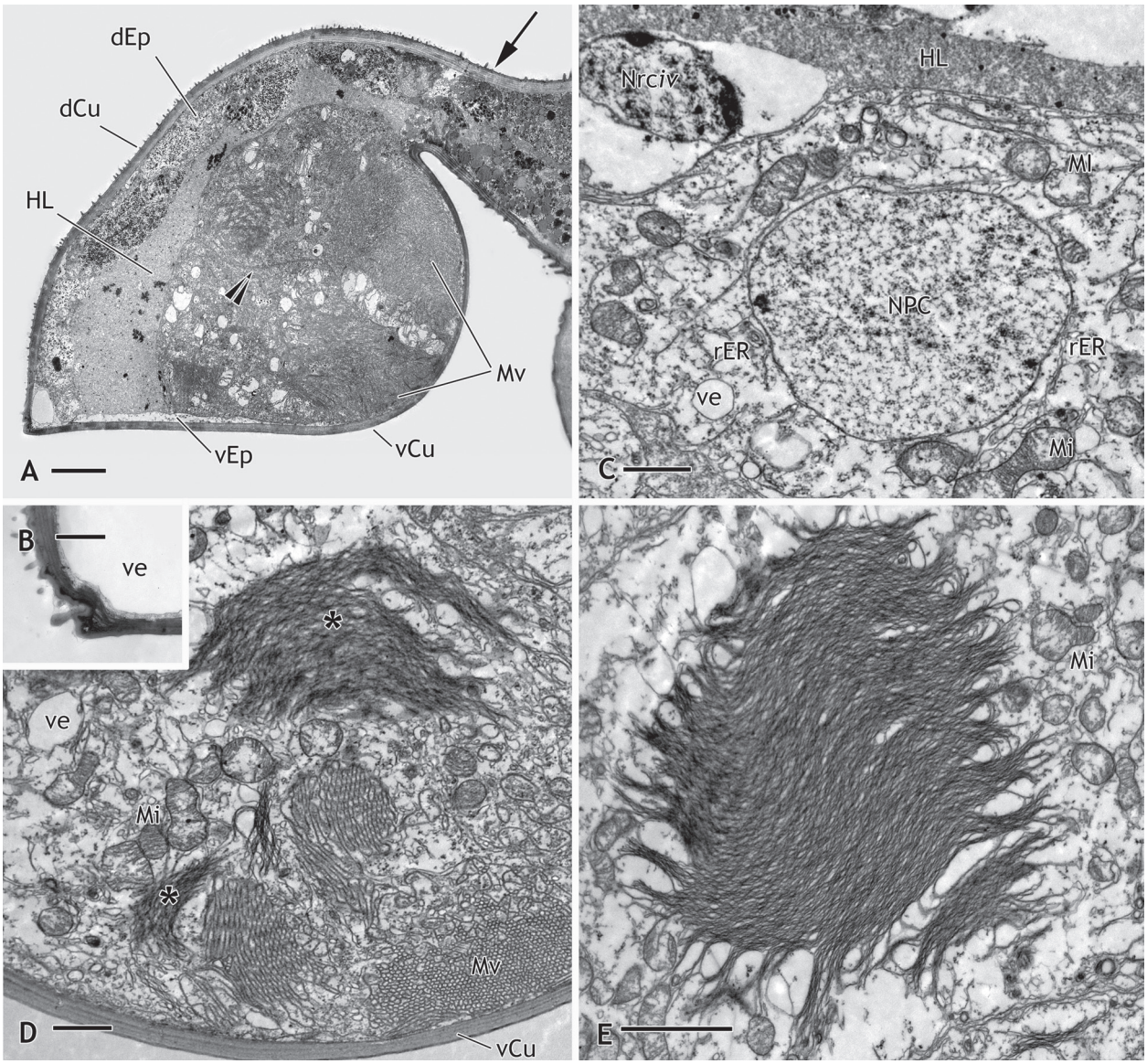



**Figure 3**. TEM-figures of sections through naso of *Rhagidia halophila* (**A**, **B** from sagittal; **C**–**E** from transversal sections). (**A**) Overview of a mediosagittal section showing naso and its flat and narrow connection (arrow) with the main part of the idiosoma. Arrowhead indicates cell border between two photoreceptor cells. Scale bar: 5 µm. (**B**) Detail showing abrupt border between dorsal, spiny and ventral smooth cuticle. Scale bar: 1 µm. (**C**) Nuclear region of photoreceptor cell. The nucleus has a roundish shape with few heterochromatin. Few rough ER, vesicles and cristae mitochondria are also present. In the upper left, a nucleus of a receptor cell of the nasal seta *iv* is visible. Scale bar: 1 µm. (**D**) Part of a photoreceptor cell with rhabdomeric microvilli, some reaching deep into the cell body being close to membrane stacks (asterisk). Scale bar: 1 µm. (**E**) A membrane stack in higher magnification. Scale bar: 2 µm.

**Abbr**.: **Ep** – epidermis, **HL** – hemolymph, **Mi** – mitochondrium, **Mv** – (rhabdomeric) microvilli, **NPC** – nucleus of photoreceptor cell, **Nrc*iv*** – nucleus of receptor cell of seat *iv,* **rER** – rough endoplasmic reticulum, **ve** – vesicle.

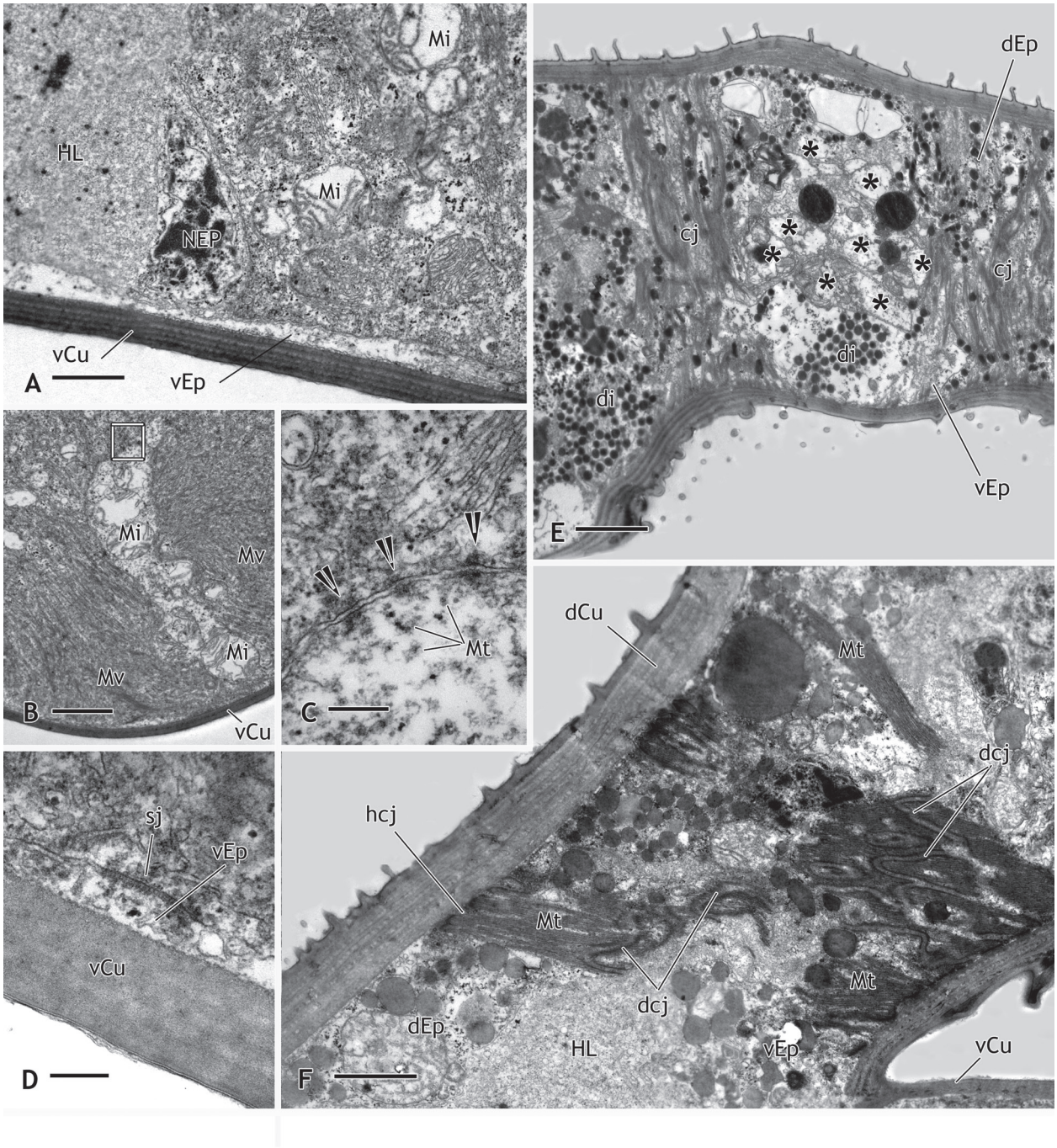



**Figure 4**. TEM-figures of sections through naso of *Rhagidia halophila* (A–D, F from sagittal, E from transversal sections). (**A**) Periphery of a photoreceptor cell bordering the hemolymph space (compare Fig. 3A). Note thin ventral epidermis and smooth ventral cuticle. Scale bar: 1 µm. (**B**) Detail of adjacent photoreceptor cells. Squared area is shown in higher magnification in Fig. 4C. Sale bar: 2 µm. (**C**) Detail of Fig. 4B showing desmosome like cell junctions (arrowheads) between adjacent photoreceptor cells. Scale bar: 0.2 µm. (**D**) Detail of smooth ventral cuticle and epidermal layer with septate cell junction between adjacent cells. Scale bar: 0.2 µm. (**E**) The narrow passage through which the axons (asterisks) of photo- and receptor cells of setae *iv* pass. The epidermal cells bordering this passage interdigitate basally and are connected via desmosome-like cell junctions. They are connected to the dorsal and ventral cuticle via hemidesmosome-like junctions. Note numerous small dense inclusions, likely representing pigment granules. Scale bar: 2 µm. (**F**) Sagittal section through the connected specialized cells bordering the narrow passage. Scale bar: 1 µm.

**Abbr**.: **cj** – cell junctions of narrow passage, **dcj** – desmosome-like cell junction, **dCu** – dorsal cuticle, **dEp** – dorsal epidermis, **di** – dense inclusion, **hcj** – hemidesmosome-like cell junction, **HL** – hemolymph, **Mt** – microtubules, **NEP** – nucleus of ventral epidermis cell, **PC** – photoreceptor cell, **sj** – septate junction, **vCu** – smooth ventral cuticle, **ve** – vesicle, **vEP** – ventral epidermis.

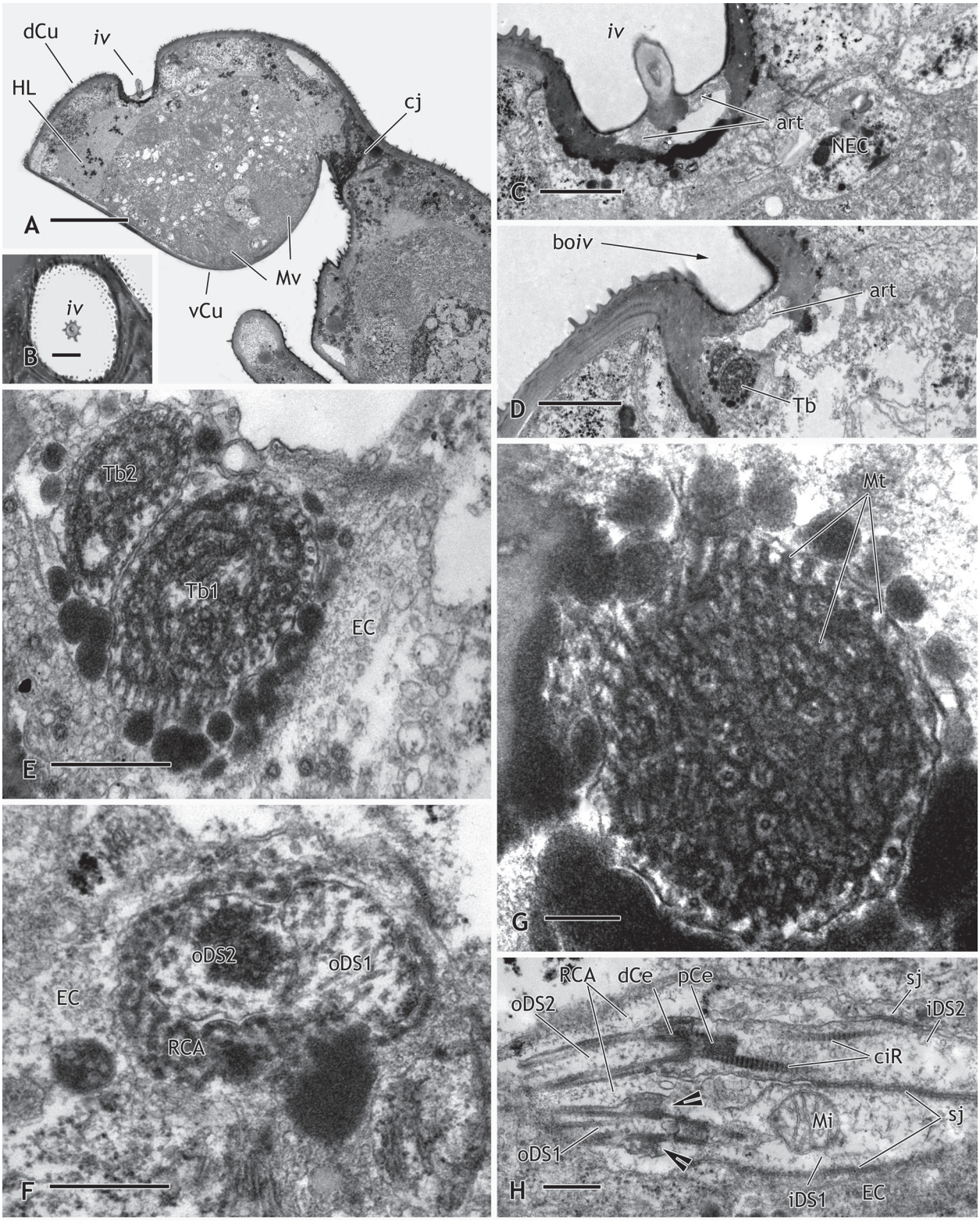



**Figure 5**. TEM-figures of sections through naso of *Rhagidia halophila* (all except of B are from sagittal sections). (**A**) Overview with bothridium and base of a nasal seta *iv* located in a deep socket (bothridium). Scale bar: 10 µm. (**B**) Cross section through seta *iv* close to its base within the bothridium. Scale bar: 2 µm. (**C**) Base of seta *iv* in higher magnification (compare Fig. 5A). Though the section does not meet exactly the center of the setal insertion, the modified articulating membrane with suspension fibers is visible. Scale bar: 2 µm. (**D**) The anterior cuticular wall of the bothridium projects internally. The terminations of the two dendrites of the receptor cells are placed here contacting the articulating membrane with their tubular bodies. Scale bar: 2 µm. (**E**) Detail from Fig. 5D at higher magnification showing the two tubular bodies. Dense globular secretions fill the receptor lymph cavity. Scale bar: 0.5 µm. (**F**) More

the naso with the main part of the idiosoma contains numerous dense droplets, probably lipids, that are likely responsible for the red colour of the mite (Fig. 4E).

The epidermal layer except the region underneath the ventral, smooth cuticle is thicker and contains numerous vesicles of different size and density (Figs 2, 3A, 5A). Aggregates of glycogen are also frequent. The nuclei are small and inconspicuous (Fig. 4A). Neighbouring cells are connected via septate desmosomes (Fig. 4D). The dorsal cuticle is only slightly thicker than the ventral cuticle, but is provided with small spines, which are made of the epicuticle (Fig. 4F, 5C). A rather voluminous space is left between the receptor cells of the eye and the dorsal epidermal layer. It is filled with hemolymph, which appears granular in the sections. Some very densely staining aggregates of granules are present.

Epidermis and photoreceptor cells are underlain by a very thin, almost inconspicuous basal lamina.

The nasal setae *iv* are inserted into deep sockets (bothridia) as was already evident from the SEMs (Figs 1A, B; 2E, F; 5A–D, 6). The setal base is connected to the densely staining lateral walls of the bothridium by an articulating membrane consisting of a thin epicuticle and an underlying electron lucent procuticle provided with a number of suspension fibers. A dense procuticular process extends form the anterior wall of the bothridium slightly deeper and here the dendrites of two receptor cells terminate touching the base of the trichobothrium with prominent tubular bodies (Fig. 5D). The tubular bodies are swollen ends of the outer dendritic (ciliary) segments which contain numerous microtubules embedded in a dense material (Fig. 5D, E). The arrangement of this dense material appears quite regular close to the extreme distal end of the dendrite (Fig. 5G). Peripheral microtubules seem to be in contact with the cell membrane of the tubular body. The enveloping cells surrounding the dendrites produce a dense secretion which forms globular structures and fills the narrow intercellular space (receptor lymph cavity). More proximally (deeper in the naso), the dense material of the tubular bodies and the secretion in the intercellular space disappear (Fig. 5F). The dendrites bend medially and posteriorly and continue into the inner dendritic segment (Fig. 5H). The centriolar region of each dendrite consisting of two coaxially arranged centrioles (basal bodies) is slightly widened and the distal centriole is connected with the cell membrane by fine filaments. From the centriolar region, cross-striated ciliary rootlets reach deeper into the inner dendritic segment, which again is slightly wider in diameter. From the ciliary region on, the enveloping cells are closely connected with the dendrites by septate junctions. We observed few small cell processes containing small nuclei close to the base of the bothridium. These likely belong to the enveloping cells (Fig. 5C). More posteriorly, the small perikarya of the receptor cells are located close to the photoreceptor cells (Figs 2C, D; 3C). The axons of the trichobothria leave the naso through the narrow passage described above (Fig. 4E) as can be judged from the number of axons met in this area.

## 4. Discussion

Our study has revealed the presence of an unpaired eye located on the ventral side of the naso. Since it is not provided with pigment cells and the dorsal side of the naso does not show a modified cuticle, e.g., a lens, it is hard to recognize with a light microscope. Though four receptor cells participate in the formation of the median eye, which thus shows a bilateral organization, traces of a putative fusion from a pair of eyes are not evident. Similar to the median eyes of other actinotrichid mites, whether paired or unpaired, the median eye of *Rhagidia halophila* is directed ventrally and slightly posteriorly (Grandjean 1958, Travé 1968, Coineau 1970, Wachmann et al. 1974, Witte 1991, 1995, Alberti & Coons 1999, Haupt & Coineau 2002, Alberti & Moreno-Twose 2012). In contrast to the median eyes of *Microcaeculus steineri delamarei* Coineau, 1971 (Caeculidae, Prostigmata – Wachmann et al. 1974), *Penthalodes ovalis* (Dugès, 1874) (Penthalodidae, Prostigmata – Haupt & Coineau 2002) and *Heterochthonius gibbus* (Berlese, 1910) (Heterochthoniidae, Oribatida – Alberti & Moreno-Twose 2012), which have evolved (secondarily) a cuticular lens on the dorsal side of the naso, such a lens is lacking in

(**figure 5 continued**) proximally, the dendrites achieve a smaller diameter and the dense material of the tubular bodies disappears. Also, the material filling the receptor lymph cavity is less conspicuous. Scale bar: 0.5 µm. (**G**) A tubular body sectioned very close to its distal termination reveals a regular arrangement of microtubules and dense material surrounding them. Peripheral microtubules apparently are in contact with the cell membrane of the dendrite. Note dense globular material in the receptor lymph cavity. Scale bar: 0.2 µm. (**H**) The connection between outer dendritic segments (ciliary region) and inner dendritic segments of the two receptor cells is shown here containing two coaxially arranged centrioles each. Note the distal centriole connected to the cell membrane via fine filaments (arrowheads) and the ciliary rootlets. Scale bar: 0.5 µm.

**Abbr**.: **art** – articulating membrane, **bo*iv*** – bothridium of seta *iv,* **cj** – cell junctions of narrow passage (compare Fig. 4E,F), **dCe** – distal centriole, **dCu** – dorsal cuticle, **EC** – enveloping cell, **HL** – hemolymph: iDS1, iDS2, inner dendritic segments 1 and 2, ***iv*** – seta *iv,* **Mi** – mitochondrium, **Mv** – (rhabdomeric) microvilli, **NEC** – nucleus of enveloping cell, **oDS1**, **oDS2** – outer dendritic segments 1 and 2; **pCe** – proximal centriole, **RCA** – receptor lymph cavity, **sj** – septate junction, **Tb** – tubular body, **Tb1**, **Tb2** – tubular bodies 1 and 2, **vCu** – ventral cuticle.

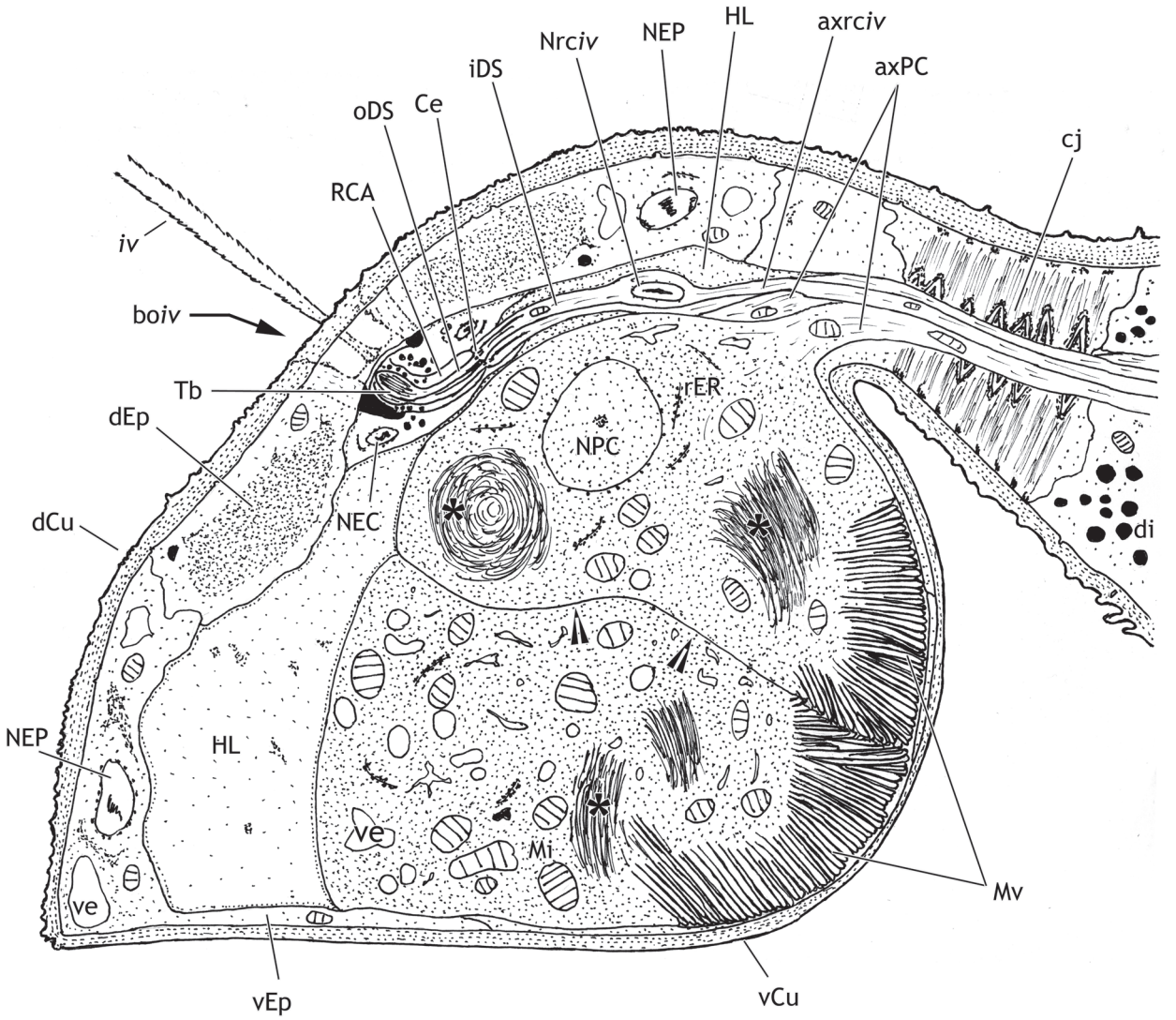



**Figure 6**. Drawing of a mediosagittal section through the naso of *Rhagidia halophila* (slightly simplified). Asterisks indicate stacks of dense membranes. Arrowheads point to cell border between two photoreceptor cells.

**Abbr**.: **axPC** – axons of photoreceptor cells, **axrc*iv*** – axon of receptor cell of seta *iv,* **bo*iv*** – bothridium of seta *iv,* **Ce** – centrioles, **cj** – cell junctions of narrow passage, **dCu** – dorsal cuticle, **dEP** – dorsal epidermis, **di** – dense inclusion, **HL** – hemolymph, **iDS** – inner dendritic segment, ***iv*** – inner vertical seta *iv,* **Mv** – (rhabdomeric) microvilli, **NEC** – nucleus of enveloping cell, **NEP** – nucleus of epidermis, **Nrc*iv*** – nucleus of receptor cell of seta *iv,* **oDS** – outer dendritic segment, **RCA** – receptor lymph cavity, **rER** – rough endoplasmic reticulum, **Tb** – tubular body, **ve** – vesicle.

*Rhagidia halophila*. Pigment cells may be present (e.g., *Penthalodes ovalis*, *Heterochthonius gibbus*) or not (*Microcaeculus steineri*, *Rhagidia halophila*) in median eyes located in a naso. Almost the same was found in median eyes that are not placed in a naso. In the freshwater mite *Hydryphantes ruber* (de Geer, 1778) (Hydryphantidae, Prostigmata), pigment granules are scarce in the eyes, but adjacent epidermal cells send extensions containing pigment granules beween the receptor cells leaving only a central region free (Mischke 1981, see also Olomski 2012 with regard to other freshwater mites). In contrast, the median eye of *Cyta latirostris* (Hermann, 1804) (Bdellidae, Prostigmata) is surrounded by a thin but distinct layer of pigment cells (Alberti et al. 1991, Alberti & Coons 1999). These latter median eyes have receptor (retinula) cells with rhabdomeric microvilli that are close to the dorsal overlying idiosomal cuticle, which forms a distinct lens (everted retina). *Cyta latirostris* is hitherto the only mite known in which the receptor cells of the median eye contain crystalline-like structures.

The cuticle covering the median eye ventrally is thin and smooth in all the naso-eyes and may be called ventral cornea. It is probably transparent but likely has no optical, i.e. refractile function. On the contrary, the cuticle on the opposite, dorsal, side and likely also transparent, may be modified as a (secondary) dorsal cornea or lens

(see above). The epidermal layer underneath corneae of arthropods has been variously called, e.g., corneagen layer (e.g., Gruner 1993, Alberti & Moreno-Twose 2012). The receptor (retinula) cells in all ultrastructurally investigated median (naso-) eyes point away from this dorsal cuticle or secondary lens. These retinae are thus inverted with respect to this dorsal cuticle or secondary lens (Wachmann et al. 1974, Haupt & Coineau 2002, Alberti & Moreno-Twose 2012). On the contrary, the retinae of median eyes, which are not located in a naso but exposed on the prosomal surface, are everted (Mischke 1981, Alberti et al. 1991, Alberti & Coons 1999). These eyes apparently always have lenses (Norton & Behan-Pelletier 2009, Walter et al. 2009), probably homologous to the secondary lens of the naso-eye. Finally, Olomski (2012) reported on certain Hydrachnidia in which the median eyes are sunken under the surface and do not exhibit a lens-like structure.

A hemolymph space of considerable volume in the naso was described by Wachmann et al. (1974) from the median eye of *Microcaeculus steineri*. Such a space was also found in *Rhagidia halophila* but not in *Penthalodes ovalis* (Haupt & Coineau 2002) and *Heterochthonius gibbus* (Alberti & Moreno-Twose 2012). There may be further differences in the morphology of these eyes. For example, Wachmann et al. (1974) described enveloping cells in *Microcaeculus steineri* surrounding and invading the retinae. Such cells were not seen in the other species investigated. Furthermore, a continuous epidermal (corneagen) layer between the ventral cuticle and the photoreceptive (retinula) cells was shown by these authors. In *Penthalodes ovalis*, this layer is at least interrupted above the rhabdomeres, as in *Rhagidia halophila* (Haupt & Coineau 2002, present study). In the oribatid mite *Heterochthonius gibbus,* the corresponding layer is continuous and contains pigment granules.

It is evident that the median eyes, besides a fundamentally similar organization, at least those located in a naso may considerably differ in the details.

We interpret the peculiar organization and orientation of the median naso-eye(s) as a plesiomorphic condition that is retained within Arachnida only in early derivative actinotrichid mites, but may be recapitulated during ontogeny in other Arachnida (e.g., Uropygi – Schimkewitsch [1906], Dawydoff [1949], Opiliones – Moritz [1957], Muñoz-Cuevas [1981], Araneae –Homann [1971], Amblypygi – Weygoldt [1975]), in which the anlage of the median eyes is formed in a deep invagination. This invagination later disappears and an eye with everted retina located under a vitreous body and a cuticular lens – in our interpretation homologs of the corneagen layer and secondary lens of the naso-eye - is finally established (see also Weygoldt & Paulus 1979). These eyes are thus located at the prodorsal part of the prosoma. Only in actinotrichid mites, species are found that have median (naso-) eyes and others without naso that have prodorsal median eyes (see also Coineau 1970, Alberti & Moreno-Twose 2012).

Our study suggests that the median eye in *Rhagidia halophila* has a highly dynamic retina with a rapid membrane turnover (e.g., Waterman 1982, Eguchi 1999). This conclusion is supported by the large stacks or whorls of membranes and numerous vesicles. In some sections, the stacks of membranes are close to bundles of rhabdomeric microvilli and it appears as if there are intermediates between these two structures.

The function of the median eyes of mites is not clear. They may be able to recognize light intensities and perhaps the direction of the incidence of light.

Our study shows that the setae on the naso without doubt represent trichobothria. They are set deeply into sockets (bothridia) like the more posterior located prodorsal trichobothria. As other mechanoreceptors of mites, the naso-trichobothria are innervated by two receptor cells ending with conspicuous tubular bodies. The setal bases are connected to the walls of the bothridium by a thin articulating membrane that allows flexion of the seta which will stimulate the dendrites via the tubular bodies (Thurm 1984). The fine structure of trichobothria in actinotrichid mites has only been rarely studied, i.e. in the prostigmatid mite *Microcaeculus steineri* (Haupt & Coineau 1975) and in the oribatid mite *Acrogalumna longipluma* (Alberti et al. 1994). They are only very exceptionally present in anactinotrichid mites; Alberti 2006). In contrast to these detailed studies on the more posteriorly located prodorsal trichobothira, we could only show some aspects here studying the naso-trichobothria for the first time. The type of innervation is similar in *Rhagidia halophila* as was mentioned already. However, we were not able to observe the basal shape of the seta, which may indicate a directionality (preferred sensitivity) of the sensillum as was shown for the mentioned mites. In any case, the base of the seta and the bothridium is more like that of the caeculid than that of the oribatid mite, which is highly apomorphic in this respect (also when compared to more early derivative oribatid mites; see Alberti et al. 1994, Weigmann 2006, Norton & Behan-Pelletier 2009). Trichobothria are generally considered to perceive substrate or airborne vibrations (Pauly 1956, Reißland & Görner 1985, Alberti & Coons 1999). Ciliary rootlets were not yet reported from trichobothria of mites. The perikarya are rather inconspicuous and, unfortunately, poorly preserved in our specimens. They continue into axons that leave, together with those of the retinula cells, the naso through a very peculiar narrow passage bordered by specialized epidermal cells connected via conspicuous

cell junctions. These junctions are frequently seen at muscle attachment sites between the muscle cell and the epidermal or tendon cell that contacts the overlying cuticle. In the cells of the mentioned passage no muscles are involved and the junctions are made directly between the bases of the dorsal and the ventral epidermal cells. Such epithel-epithel connections are rarely observed, but are also present in the pharyngeal region of actinotrichid mites between the ventral wall of the pharynx and that of the infracapitulum. Interestingly such a site was also observed in the rostral tectum of an oribatid mite (*Archegozetes longisetosus*; c.f. Alberti & Coons 1999, Alberti et al. 2011). These conspicuous attachment sites certainly have a stabilizing effect.

In conclusion, we could show the presence of a median eye in the naso of *Rhagidia halophila* supporting the interpretation of this sturcture as being generally and fundamentally provided with an eye. The presence of an eye-bearing naso seems to be a very ancient character what is obvious from a comparison with other arachnids possessing median eyes and their peculiar development. The presence of median eyes as a fundamental character in actinotrichid mites and its absence in anactinotrichids reflects the phylogenetic distance between these groups as has become more and more evident (e.g., Alberti 2006, Dunlop & Alberti 2007, Dabert et al. 2010, Pepato et al. 2010). The setae on the naso of *Rhagidia halophila* are evidently trichobothria. Considering the setation on the prosoma including the naso it is evident that setae of different positions or homology may be transformed into trichobothria or into unspecialized setae (see also Norton & Behan-Pelletier 2009, Walter 2009, Walter et al. 2009). It seems remarkable that this evolutionary flexibility is not found in anactinotrichid mites, in which trichobothria have evolved only exceptionally (Alberti & Coons 1999, Alberti 2006, Krantz 2009).

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## 6. References

Alberti, G. (1975): Prälarven und Phylogenie der Schnabelmilben (Bdellidae, Trombidiformes). – Zeitschrift für zoologische Systematik und Evolutions-Forschung **13**: 44–62.

Alberti, G. (1980a): Zur Feinstruktur des Hodenepithels und der Spermien von *Eusimonia mirabilis* Roewer, 1934 (Solifugae, Arachnida). – Zoologischer Anzeiger **204**: 345–352.

Alberti, G. (1980b): Zur Feinstruktur der Spermien und Spermiocytogenese der Milben (Acari): I. Anactinotrichida. – Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere **104**: 77–138.

Alberti, G. (1980c): Zur Feinstruktur der Spermien und Spermiocytogenese der Milben (Acari). II. Actinotrichida. – Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere **104**: 144–203.

Alberti, G. (1984): The contribution of comparative spermatology to problems of acarine systematics. – In: Griffiths, D. A. & C. E. Bowman (eds): Acarology VI, I. VIth International Congress of Acarology, Edinburgh. – Chichester: Ellis Horwood lim. Press: 479–490.

Alberti, G. (2000): Chelicerata. – In: Jamieson, B. G. M. (vol. ed.): Progress in Male Gamete Ultrastructure and Phylogeny. – In: Adiyodi, K. G. & R. G. Adiyodi (eds.): Reproductive Biology of the Invertebrates. – Vol. 9, p.B. New Delhi, Oxford & IBH Publishing/Wiley, N.Y.: 311–388.

Alberti, G. (2006): On some fundamental characteristics in acarine morphology. – Atti della Accademia Nazionale Italiana di Entomologia. R. A. LIII - 2005: 315–360.

Alberti, G. & L. B. Coons (1999): Acari - Mites. – In: Harrison, F. W. (ed.): Microscopic Anatomy of Invertebrates. – Vol. 8c. Wiley-Liss, New York: 515–1265.

Alberti, G. & A. I. Moreno-Twose (2012): Fine structure of the primary eyes in *Heterochthonius gibbus* (Oribatida, Heterochthoniidae) with some general remarks on photosensitive structures in oribatid and other actinotrichid mites. – Soil Organisms **84**: 391–408.

Alberti, G. & G. Nuzzaci (1996): SEM and TEM Techniques. – In: Lindquist, E. E., J. Bruin & M. W. Sabelis (eds): Eriophyoid Mites. Their Biology, natural Enemies and Control. – World Crop Pests. Vol. 6. Amsterdam, Elsevier Science Publishing: 399–410.

Alberti, G. & A. Peretti (2002): Fine structure of male genital system and sperm in Solifugae does not support a sister-group relationship with Pseudoscorpiones (Arachnida). – Journal of Arachnology **30**: 268–274.

Alberti, G., Th. Kaiser & N. A. Fernandez (1991): Fine structure of photoreceptor organs in Acari. – In: Dusbábek, F. & V. Bukva (eds): Modern Acarology. Vol. 2. Proceedings of the VIII International Congress of Acarology. – Ceské Budejovice 1990, Academia, Prague and SPB Publishing, The Hague: 343–348, pl. 39 u. 40.

Alberti, G.; A. I. Moreno & M. Kratzmann (1994): The fine structure of trichobothria in moss mites with special emphasis on *Acrogalumna longipluma* (Berlese, 1904) (Oribatida, Acari, Arachnida). – Acta Zoologica, Stockholm **75**: 57–74.

Alberti, G., E. Lipke & G. Giribet (2008): On the ultrastructure and identity of the eyes of Cyphophthalmi based on a study of *Stylocellus* sp. (Opiliones, Stylocellidae). – Journal of Arachnology **36**: 379–387.

Alberti, G., M. Heethoff, R. A. Norton, S. Schmelzle, A. Seniczak & St. Seniczak (2011): Fine structure of the gnathosoma of Archegozetes longisetosus Aoki (Acari: Oribatida, Trhypochthoniidae). – Journal of Morphology **272**: 1025–1079.

Coineau, Y. (1970): A propos de l'oeil antérieur du naso de Caeculidae. – Acarologia **12**: 109–118.

Coineau, Y., P. D. Theron & N. Fernandez (2006): Parades et dimorphismes sexuels compares chez deux noveaux genres de Saxidromidae (Acari, Alycina) d'Afrique du Sud. – Acarologia **46**: 65–87.

Bernini, F. (1986) Current ideas on the phylogeny and the adaptive radiations of Acarida. – Bolletino di Zoologia **53**: 279–313.

Dabert, M., W. Witalinski, A. Kazmierski, Z. Olszanowski & J. Dabert (2010): Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts. – Molecular Phylogenetics and Evolution **56**: 222–241.

Dunlop, J. A. & G. Alberti (2008): The affinities of mites and ticks: a review. – Journal of Zoological Systematics and Evolutionary Research **46**: 1–18.

Dunlop, J. A., J. Krüger & G. Alberti (2012): The sejugal furrow in camel spiders and acariform mites. – Arachnologische Mitteilungen **43**: 8–15.

Dawydoff, C. I. (1949): Développemant embryonnaire des Arachnides. – In: Grassé, P.-P. (ed.): Masson et Cie, Paris, Traité de Zoologie **6**: 320–385.

Eguchi, E. (1999): Membrane turnover of rhadom. – In: Eguchi, E. & Y. Tominaga (eds): Atlas of arthropod receptors. Dynamic morphology in relation to function. – Springer-Verlag, Tokyo: 87–96.

Ehrnsberger, R. (1973): Morphologie, Ökologie und Ethologie bodenbewohnender Milben (Fam. Rhagidiidae). – Doctoral thesis, University of Kiel: 142pp.

Ehrnsberger, R. (1974): Prälarval- und Larvalentwicklung bei Rhagidien (Acari: Prostigmata). – Osnabrücker Naturwissenschaftliche Mitteilungen **3**: 85–117.

Ehrnsberger, R. (1981): Ernährungsbiologie der bodenbewohnenden Milbe *Rhagidia* (Trombidiformes). – Osnabrücker Naturwissenschaftliche Mitteilungen **8**: 127–132.

Evans, G. O. (1992): Principles of Acarology. – C.A.B International, Wallingford: 563pp.

Foelix, R. F. (1996): Biology of spiders. – N.Y., Oxford; Oxford University Press, Georg Thieme Verlag: 330pp.

Grandjean, F. (1932): Au sujet des Palaeacariformes Trägårdh. – Bulletin du Muséum National d'Histoire Naturelle, Paris sér. **2**, 4: 411–426.

Grandjean, F. (1939): Quelques genres d'Acariens appartenant au groupe des Endeostigmata. – Annales des Sciences Naturelles, Zoologie sér. **11** (2): 1–122.

Grandjean, F. (1943): Quelques genres d'Acariens appartenant au groupe des Endeostigmata (2e sér.). Deuxième partie. – Annales des Sciences Naturelles, Zoologie sér. **11** (4): 137–195.

Grandjean, F. (1958): Au sujet du naso et de son oeil infère chez les oribates et les Endeostigmata (Acariens). – Bulletin du Muséum, 2e sér. **30**: 427–435.

Grandjean, F. (1969): Stases. Actinopiline. Rappel de ma classification des Acariens en 3 groupes majeurs. Terminologie ens soma. – Acarologia **11**: 796-827.

Gruner, H.-E. (1993): 19. Stamm Arthropoda. In: Gruner, H.-E. (ed.): Wirbellose Tiere. 4. Teil: Arthropoda (ohne Insecta) (bearbeitet von H.-E. Gruner, M. Moritz und W. Dunger). – 4. Auflage, G. Fischer Verlag, Jena: 11–63.

Hammen, L. van der (1980): Glossary of Acarological Terminology. Vol. I. General Terminology. – Dr. W. Junk Publ., The Hague: 244pp.

Hammen, L. van der (1989): An Introduction to Comparative Arachnology. – SPB Publishing bv, The Hague: 576pp.

Haupt, J. & Coineau, Y. (1975): Trichobothrien und Tastborsten der Milbe *Microcaeculus* (Acari, Prostigmata, Caeculidae). - Zeitschrift für Morphologie der Tiere **81**: 305-322.

Haupt, J. & Y. Coineau (2002): Morphologie comparée du naso des Penthalodidae (Acari, Actinedida, Penthalodidae). – Acarologia **42**: 247-256.

Homann, H. (1971): Die Augen der Araneae. Anatomie, Ontogenie und Bedeutung für die Systematik (Chelicerata, Arachnida). – Zeitshrift für Morphologie der Tiere **69**: 201-272.

Klann, A. E., T. Bird, A. V. Peretti, A. V. Gromov & G. Alberti (2009): Ultrastructure of spermatozoa of solifuges (Arachnida, Solifugae): Possible characters for their phylogeny? – Tissue and Cell **41**: 91–103.

Krantz, G. W. (2009): Form and function. – In: Krantz, G. W. & D. E. Walter (eds): A Manual of Acarology. 3rd ed. Lubbock: Texas Tech University Press: 5–53.

Lindquist, E. E. (1984): Current theories on the evolution of major groups of Acari and on their relationships with other groups of Arachnida, with consequent implications for their classification. – In: Griffiths, D. A. & C. E. Bowman (eds): Acarology VI. Vol. 1. – Chichester: Ellis Horwood: 28–62.

Mischke, U. (1981): Die Ultrastruktur der Lateralaugen und des Medianauges der Süßwassermilbe *Hydryphantes ruber* (Acarina: Parasitengona). – Entomologica Generalis **7**: 141–156.

Muñoz-Cuevas, A. (1981): Développement, rudimentation et regression de l'oeil chez les opilions (Arachnida) - recherché morphologiques, physiologiques et expérimentales. – Mémoires du Muséum Nationale d'Histoire Naturelle. Nouvelle série, série A, Zoologie **120**: 1–117 + 10pl.

Moritz, M. (1957): Zur Embryonalentwicklung der Phalangiiden (Opiliones, Palpatores) unter besonderer Berücksichtigung der äußeren Morphologie, der Bildung

des Mitteldarmes und der Genitalanlage. – Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere **76**: 331–370.

Moritz, M. (1993): Unterstamm Arachnata. – In: Gruner, H.-E. (ed.): Lehrbuch der Speziellen Zoologie (begr. von A. Kaestner). – 4.ed. Bd. 1: Wirbellose Tiere. 4. Teil: Arthropoda. – G. Fischer Verlag, Jena: 64–442.

Norton, R. A. & V. Behan-Pelletier (2009): Suborder Oribatida. – In: Krantz, G. W. & D. E. Walter (eds): A Manual of Acarology. 3rd ed. – Lubbock: Texas Tech University Press: 430–564.

OConnor, B. A. (2009): Cohort Astigmatina. – In: Krantz, G. W. & D. E. Walter (eds): A Manual of Acarology. 3rd ed. – Lubbock: Texas Tech University Press: 565–657.

Olomski, R. (2012): The median eye of freshwater mites (Acari: Parasitengonae, Hydrachnidia) and its fate in the stem lineage of the Euhydrachnidia, Witte & Olomski 1991. – Soil Organisms **84**: 445–458.

Otto, J. C. (1996): Observations on prelarvae in Anystidae and Teneriffiidae. – In: Mitchell, R., D. J. Horn, G. R. Needham & W. C. Welbourn (eds): Acarology IX - Proceedings. – Ohio Biological Survey, Columbus, Ohio: 343–354.

Paulus, H. F. (1979): Eye structure and the monophyly of the Arthropoda. – In: Gupta, A.P. (ed.): Arthropod Phylogeny. – Van Nostrand Reinhold Company, New York: 299–383.

Pauly, F. (1956): Zur Biologie einiger Belbiden (Oribatei, Moosmilben) und zur Funktion ihrer pseudostigmatischen Organe. – Zoologische Jahrbücher. Abteilung für Systematik, Ökologie und Geographie der Tiere **84**: 275–328.

Pepato, A. R., C. E. F. da Rocha & J. A. Dunlop (2010): Phylogenetic position of the acariform mites: sensitivity to homology assessment under total evidence. – BMC Evolutionary Biology **10**: 235.

Reißland, A. & P. Görner (1985): Trichobothria. – In: Barth, F. G. (ed.): Neurobiology of Arachnids. – New York, Springer-Verlag: 138–161.

Reynolds, E. S. (1963): The use of lead citrate at high pH as an electron–opaque stain in electron microscopy. – Journal of Cell Biology **17**: 208–212.

Richardson, K. C., L. J. Jarrett & E. H. Finke (1960): Embedding in epoxy resin for ultrathin sectioning in electron microscopy. – Stain Technology **35**: 313–323.

Schimkewitsch, W. (1906): Die Entwicklung von *Thelyphonus caudatus* (L.) verglichen mit derjenigen einiger anderer Arachniden. – Zeitschrift für wissenschaftliche Zoologie **81**: 1–95, pl. I-VIII.

Spurr, A. R. (1969): A low-viscosity epoxy resin embedding medium for electron microscopy. – Journal of Ultrastructure Research **26**: 31–43.

Thurm, U. (1984): Beiträge der Ultrastrukturforschung zur Aufklärung sensorischer Mechanismen. – Verhandlungen der Deutschen Zoologischen Gesellschaft **77**: 89–103.

Travé, J. (1968): Sur 1'existence d'yeux lateraux depigmentes chez *Eobrachychthonius* Jacot (Oribatei). – Acarologia **10**: 151–158.

Walter, A. D. (2009): Suborder Endeostigmata. – In: Krantz, G. W. & D. E. Walter (eds): A Manual of Acarology. – 3rd ed. Lubbock: Texas Tech University Press: 421–429.

Walter, D. E., E. E. Lindquist, I. M. Smith, D. R. Cook & G. W. Krantz (2009): Order Trombidiformes. – In: Krantz, G. W. & D. E. Walter (eds): A Manual of Acarology. 3rd ed. Lubbock: Texas Tech University Press: 233–420.

Wachmann, E., J. Haupt, S. Richter & Y. Coineau (1974): Die Medianaugen von *Microcaeculus* (Acari, Prostigmata, Caeculidae). – Zeitschrift für Morphologie der Tiere **79**: 199–213.

Weigmann, G. (2006): Hornmilben (Oribatida). – Die Tierwelt Deutschlands. Begr. 1925 von Fr. Dahl. 76. Teil. – Goecke & Evers, Keltern: 520p.

Weygoldt, P. (1975): Untersuchungen zur Embryologie und Morphologie der Geißelspinne *Tarantula marginemaculata* (Amblypygi). – Zoomorphologie **82**: 137–199.

Waterman, T. H. (1982): Fine structure and turnover of photoreceptor membranes. – In: Westfall, J. A. (ed.): Visual Cells in Evolution. – Raven Press, New York: 23–41.

Weygoldt, P. & H. F. Paulus (1979): Untersuchungen zur Morphologie, Taxonomie und Phylogenie der Chelicerata. I: Morphologische Untersuchungen. – Zeitschrift für zoologische Systematik und Evolutionsforschung **17**: 85–116.

Witte, H. (1991): The phylogenetic relationships within the Parasitengonae. – In: Dusbábek, F. & V. Bukva, V. (eds): Modern Acarology. Vol. 2. – Academia, Prague and SPB Academic Publishing bv, The Hague: 163–170.

Witte, H. (1995): Evolution and phylogenetic system of the Erythraeoidea (Prostigmata, Parasitengonae). – In: Kropcsynska, D., J. Boczek & A. Tomczyk (eds): The Acari. – Warszawa. Oficyna DABOR: 117–148.

Zacharda, M. (1980): Soil mites of the family Rhagidiidae (Actinedida: Eupodoidea). Morphology, systematik, ecology. – Acta Universitatis Carolinae, Biologica 1980 (1978): 489–785.