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Fine structure of the urnulae of *Balaustium* mites (Actinotrichida: Erythraeidae) representing peculiar defense organs

Gerd Alberti [a,*], Joanna Mąkol [b]

[a] *Allgemeine und Systematische Zoologie, Zoologisches Institut und Museum, Ernst-Moritz-Arndt-Universität, J.-S.-Bach-Str. 11/12, 17489 Greifswald, Germany*

[b] *Department of Invertebrate Systematics and Ecology, Institute of Biology, Wrocław University of Environmental and Life Sciences, Kożuchowska 5b, 51-631 Wrocław, Poland*

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ABSTRACT

The urnulae, until now the enigmatic paired dorsal protrusions on idiosoma dorsum in active postlarval forms of *Balaustium* mites, were studied using electron microscopy. They consist of walls made of unmodified integument, which form a cylinder covered by a roof of thin cuticle. At the posterior border of the urnula, the roof has a crescent slit. On its inner surface, a rather large muscle inserts with several tendons. The roof forms a flap under which the modified columnar epidermal cells containing numerous lipid inclusions are located. These lipids are probably secreted through pore canals of the overlying cuticle. Materials mainly originating from an extensive vesicular tissue situated underneath the columnar cells of the urnula and under the adjacent unmodified epidermis are extruded through the mentioned slit. Our results support previous studies that have suggested a function of the urnulae as defensive organs. Our study further suggests that the agent that provides the repellent effect comes mainly from the vesicular tissue, whereas the columnar cells with their lipid secretions are likely to restore the external secretion layer of the epicuticle after its destruction during the repellent release. Further structural and functional details are discussed and compared with other putative defensive secretory organs.

1. Introduction

Balaustiinae is a subfamily of Erythraeidae, a quite large family of world-wide distribution. It is represented by moderate to rather large sized mites (*circa* 1000–2500 μm long as adults), usually bright red in color, sometimes with white shagreening on the idiosoma dorsum. They live in a broad range of habitats, including xeric sandy or rocky substrata and are known as pollen-feeders and predators of small arthropods and their eggs. As members of the Parasitengona, these mites have only three active instars: larva, deutonymph and adult. Other instars (prelarva, protonymph, tritonymph) are quiescent. However, in contrast to larvae of other parasitengone mites, including other Erythraeidae, the larvae of Balaustiinae are not parasitic (Grandjean, 1957; Walter et al., 2009; Mąkol et al., 2012; Yoder et al., 2012). One of the most characteristic features of postlarval Balaustiinae is the presence of paired protrusions on the idiosoma dorsum, that have got a variety of names: "Rückenstigmen" (Oudemans, 1916; Schweizer, 1951; Willmann, 1951), "eye-like structures" (Hirst, 1926), "sensory pits" (Womersley, 1934), "verrues dorsales" (Grandjean, 1947, 1959), "glandular openings" (Newell, 1963), and "urnulae" (Southcott, 1961). The latter term suggested by Southcott (1961) became well established and is commonly used in the recent literature (e.g., Kethley, 1990; Gabryś, 2000; Halliday, 2001; Lindquist, 2001; Yoder et al., 2006a; Walter et al., 2009; Mąkol, 2010). There may be one (genera *Balaustium*, *Microsmarialla*, *Neobalaustium*) or two (genera *Microsmaris*, *Wartookia*) pairs of urnulae (Southcott, 1961; Khot, 1963). Until recently, the function of these peculiar structures – not present in other actinotrichid mites – was unknown or speculative as the aforementioned terms indicate. In his milestone-talk presented at the opening session of the 10th International Congress of Acarology, Lindquist (2001) referred to these enigmatic structures as one of the "Future enquiries for Acarology". In the meantime, several studies, mainly of Yoder and co-authors (Yoder et al., 2006a, b, 2007, 2008; 2010; Yoder and Heydinger, 2011), have focused on the urnulae and suggested two main functions: (1) providing a defensive secretion and (2) providing a secretion

* Corresponding author. Tel.: +49 3834 86 4250.
E-mail addresses: alberti@uni-greifswald.de (G. Alberti), joanna.makol@up.wroc.pl (J. Mąkol).

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enhancing resistance against water loss. Both functions were supported by experiments. By exposing postlarval mites to potential predators, e.g. ants, Yoder et al. (2006a, 2008) could observe secretions being extruded from the urnulae that had a repellent effect on ants and other arthropods. Thus, this capability protected the mites from being eaten or injured through trampling by larger arthropods. Also, they could show that conspecifics dispersed when stimulated by such secretions, which include neryl formate (Yoder et al., 2007), a component also found in repellent secretions of other arthropods including mites (Raspotnig, 2010). Mąkol et al. (2012) observed the secretion of urnulae-derived fluid, when handling the specimens from laboratory culture. Yoder et al. (2006a) compared the function of the urnulae with reflex bleeding of certain beetles. On the other hand, the studies of Yoder et al. (2006b) and Yoder and Heydinger (2011) showed that larvae, devoid of the urnulae, represented the instar most sensitive to water loss. Since larvae of, e.g., *Balaustium murorum* (Hermann, 1804) avoid exposure to bright sun in contrast to postlarval instars, the latter function seems to be in accord with observations in the natural habitat (Mąkol pers. observations). Until now, the structure of the urnulae has only been studied using light microscopy and due to limitations of this technique, the structural composition of the urnulae is only incompletely known. For example, from the available data it is not clear whether or not the urnulae have a permanent opening and how the putative glandular tissue is organized. Since the urnulae are evidently quite exceptional within mites, here we present results of our studies on adults and deutonymphs of *Balaustium hernandezi* Mąkol et al., 2012 and *B. murorum* using scanning and transmission electron microscopy, hoping that these will help to clarify the functional morphology of these hitherto enigmatic organs.

2. Material and methods

The specimens of *B. hernandezi* Mąkol et al., 2012 were obtained from the laboratory culture at Biobest, Belgium (for details see Mąkol et al., 2012), whereas those of *B. murorum* (Hermann, 1804) were collected on a brick wall of a balcony, Wrocław, Poland. *B. murorum* was used for SEM examination only, whereas the main study was done on the much larger *B. hernandezi*. The specimens were processed as follows:

2.1. Transmission electron microscopy (TEM)

Adult living mites were placed in a drop of cold fixative (2.5% glutaraldehyde in phosphate buffer, pH 7.4; 0.1 M) and cut into halves with a razor blade. The mites were then transferred to small vials and kept in the refrigerator for about 2 h. The specimens were then mailed to Greifswald and transferred to buffer solution for about 2 h. Subsequently postfixation with buffered 2% OsO_4-solution was carried out for about 2 h. Afterward, the mites were rinsed for 10min in buffer solution and dehydrated in graded ethanol series (60%, 70%, 80%, 90%, 96%, abs. ethanol). Embedding occurred in Spurr's resin (Spurr, 1969). Ultrasections were cut with a Leica Ultracut microtome using a Diatome diamond knife. The sections (70 nm) were transferred to copper grids (100 mesh), stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a JEOL JEM-1011 transmission electron microscope. Semithin sections (400 nm) were used for general orientation and were stained according to Richardson et al. (1960). They were examined under a compound light microscope.

2.2. Scanning electron microscopy (SEM)

For SEM we used fresh specimens preserved in 70% ethanol, dehydrated in graded ethanol series and critical point-dried using liquid CO_2 as final medium. Part of the mites was previously treated with an ultrasonic cleaner, whereas another part was macerated with lactic acid and cut open to study the interior of the urnula. The specimens were transferred to Al-stubs with double-stick carbon tape, sputtered with palladium-gold and studied with Zeiss EVO LS10 and LS15 scanning electron microscopes (for more technical details see Alberti and Nuzzaci, 1996).

3. Results

SEM: Each urnula presents a cylindrical elevation on the prodorsum, located postero-medially to the eyes (Fig. 1A and D). The wall of the cylinder is structured like the surrounding integument bearing small parallel cuticular ridges and also some barbed setae (Fig. 1B, C, E and G). Each ridge consists of small, more or less elongated platelets. In contrast, the roof of the elevation is devoid of ridges and contains small, low bulges (Fig. 1E–G). At high magnification, tiny pores are detectable (Fig. 1F). These fine structures (platelets and pores) are best seen in SEM preparations that have been cleaned with an ultrasonic cleaner. In non-ultrasound-treated specimens the integument, including the roof of urnula, is covered by a thin layer of secretion (secretion layer = cerotegument in the acarological literature) (Fig. 1B). The roof of the urnula slopes gently towards the posterior rim of the cylindrical wall, where a sickle-shaped slit is visible (Fig. 1B, C, E and G). In one specimen, this slit was incomplete as the flap was not separated from the rim *circa* in the mid point of its boundary (Fig. 1G). The posterior border of the cylindrical wall of the urnula is sharp-edged contrary to the anterior border where a smooth fold continues into the roof.

In specimens treated with lactic acid, the internal aspect of cuticular structures could be examined (Fig. 1H–K). The wall of the urnula contains numerous small pores, which lead into pore canals traversing the procuticle (see below) and some sockets of the setae. The roof of the urnula when seen from underneath is also provided with numerous, however slightly larger, pores. A bundle of tiny tendons (cuticular strands) is connected to the middle part of the posterior edge of the roof bordering the aforementioned slit. The cuticular sheet adjoining the opposite border of the slit is directed inward, distinctly folded and provided with numerous fine teeth.

TEM: The wall of the urnula is structured as the surrounding integument (Figs. 2A, 3 and 5A, 6A–C, 7A and 8). A thin epidermis is overlain by a cuticle consisting of a procuticle, which is traversed by pore canals that terminate under small indentations of the inner epicuticle (Fig. 6A–C). Thus, a thin layer of the inner epicuticle remains enclosed by a slight enlargement of the terminal portion of each pore canal. Small elevations are present in the center of these indentations (Fig. 6B). A thin secretion layer is usually present on the urnula wall, but may be also disrupted (Fig. 6A and C). The epidermal cells contain small lightly staining granules, probably being lipids (Figs. 5 and 6A and B). The wall bears barbed setae, inserted in sockets forming movable bases (Figs. 2A and 5A). The setae are innervated by two dendrites with typical tubular bodies at their distal ends, reaching a receptor lymph cavity bordered by semicircles, an arrangement indicating their mechanosensitivity (Fig. 5B; Thurm, 1984; Alberti and Coons, 1999). The small nerves innervating the setae are found underneath the unmodified epidermis.

The cuticle of the anterior wall of the urnula becomes thinner towards its distal rim (Figs. 2A, 3A and 5A, 7A and 8). It bends here forming the anterior part of the roof. Towards its posterior part extending to the slit, it becomes thicker again at *circa* mid part of the roof. Here, the cuticle forms many small processes extending into a long tendon cell (Fig. 3A–C). These processes soon join with stronger tendons, which run obliquely and in anterior direction through the tissue which fills the urnula (Figs. 2B and 3A, D and 5A).