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FINE STRUCTURE OF THE HUMERAL ORGAN OF JUVENILE EDWARDZETES EDWARDSII (CERATOZETIDAE, ORIBATIDA) COMPARED WITH POROSE AREAS OF THE ADULTS

by Gerd ALBERTI¹, Andrej KLIMEK² and Stanislaw SENICZAK²

SUMMARY: The oribatid integument is well known for a number of porose organs. The distribution of these organs has taxonomic importance, especially in Brachypylina. Until recently, the function of these organs was conflictingly interpreted. It is now known that porose organs may represent either respiratory or secretory porose organs. According to NORTON et al. (1997), in adult Poronota the porose organs may be grouped into several series. In addition to the octotaxic system of the notogaster, a marginoventral series of the ventral plate, an apodemato-acetabular system of tracheae, and a humerosejugal series of the sejugal and lateral podosomal region can be recognized. In juveniles the lateral parts of the bodies are usually not provided with porosities. However, in some taxa, predominately belonging to Ceratozetoidea, the juveniles possess a rather conspicuous humeral organ (oh). We have studied this organ for the first time with TEM and SEM in Edwardzetes edwardsii (Ceratozetidae). It evidently represents a secretory porose organ, probably homologous to the humeral porose organ Ah of the adults, which may be modified into a conspicuous vesicle in Ceratozetoidea (and some other taxa).


1. Zoological Institute and Museum, University of Greifswald, Bachstr. 11/12, D-17489 Greifswald, Germany.

INTRODUCTION

The integument of Oribatida bears a number of peculiar porose organs, which have received much interest due to their taxonomic importance (see, e.g., Willmann, 1931; Grandjean, 1954; Balogh and Balogh, 1992). They were studied most intensively by Grandjean (e.g., 1934, 1959), who developed the idea that all these organs have a respiratory function (see also Wallwork, 1969; Woolley, 1988). Other investigators considered these structures to be at least in part involved in secretory processes (e.g., Oudemans, 1913; Vitzthum, 1940-43; Jones, 1954; Woodring and Cook, 1962; van der Hamm, 1980; Alberti et al., 1981). In a recent series of studies, on the distribution of these organs in Oribatida, the fine structure of a number of different types, and the ecological and evolutionary implications of these organs have been investigated and discussed (Alberti et al., 1997a, b; Norton & Alberti, 1997; Norton et al., 1997). It was confirmed that these structures comprise two different types of organs: secretory porose organs and respiratory porose organs. Based on cuticular characteristics alone these two types cannot be distinguished with certainty. It is necessary to investigate the cellular components located under the modified porose cuticle (or, more precisely, procuticle). Most prominent are the porose organs in Brachypylina, a sub-taxon of which, Poecinota, is in part characterized by four pairs of secretory notogastral porose organs (octotaxic system). A further peculiarity of most brachypyline oribatid mites is a series of porose organs in the sejugal and lateral region of the podosoma (humerosejugal series of secretory porose organs). In some poronotic oribatids, one of the humeral porose organs (organ Ah) may show a morphology different from other porose organs, i.e., its cuticular region may be invaginated (funnel-like) or evaginated (mushroom-like) (Grandjean, 1929; Norton et al., 1997). Such modified porose organs, termed also humeral vesicles, are known from, e.g., many Ceratozetoida and Achipteriidae (Grandjean, 1929; Norton et al., 1997). Alberti et al. (1997a, b) have shown that this organ also differs with respect to fine structural details from other porose organs but is evidently a secretory structure.

The function of the secretory organs is still a matter of speculation. Alberti et al. (1997a, b) and Norton & Alberti (1997) suggest that it may originally be related to the maintenance of cuticular functions but may have at least in some taxa received additional importance, perhaps in providing a hydrophobic component in the region of the stigmata or in secreting pheromones or repellents.

Porose organs of juvenile oribatid mites have not been studied in such detail, and in particular no transmission electron microscopical observations have been made until now. In contrast to adult brachypyline Oribatida, the juveniles generally lack porose organs in the humeral region. There are only a few exceptions, found mainly in the Ceratozetoida, which are very distinct and have been termed humeral organs (oh). The adults of such species always have a humeral porose organ (Ah), which may be modified, suggesting that both structures are homologous (Norton & Alberti, 1997). But since these organs are not always correlated, i.e. oh may be lacking in juveniles of adults with Ah, the interpretation of these organs is difficult. Although histological investigations by Jones (1954) and Woodring & Cook (1962) in species of Scheloribates and Ceratozetes suggest that the humeral organs represent a secretory organ, ultrastructural information could prove this and would provide further details.

We have studied the humeral organ in juveniles of Edwardzetes edwardsii (Nicolet) using light microscopy (LM), scanning (SEM) and transmission electron microscopy (TEM), and compared it with porose organs of adults of the same species from the octotaxic system and humerosejugal series to resolve some of these questions.

MATERIAL AND METHODS

Specimens of Edwardzetes edwardsii were collected from the litter horizon of spruce (Picea) forests in Karkonosze National Park, 1100m a.s.l. (Poland) and near Heidelberg (Königsstuhl, 400 m a.s.l.; Germany). They were prepared for study as follows:

LM: Juvenile as well as adult specimens were macerated with lactic acid and studied with an Olympus compound microscope (bright field).
FIG. 1: Drawing showing humeral organ (arrow) of a tritonymph of *Edwardzetes edwardsii*. Note lens-shape of organ and small tubercles behind the organ (from Seniczak et al., 1990).

SEM: Ethanol preserved specimens were carefully washed by sucking them several times into a Pasteur pipette, then dehydrated in a graded ethanol series. Adult specimens were then air-dried, in contrast to juveniles which were dehydrated with the critical point method (see below). To allow observation of interior cuticular structures, some specimens were macerated with lactic acid, subsequently cut into pieces, and dehydrated with graded ethanol solutions. They were then transferred to formaldehyde dimethyl acetal and subsequently to liquid CO$_2$. The specimens were finally dried in a Balzer critical point apparatus. After mounting on Al-stubs with double sided sticky tape, specimens were coated with gold in a Hummer sputter apparatus. SEM: Philips SEM 505.

TEM: Living specimens were transferred to cold (4°C) fixative (3.5% or 2.5% buffered glutaraldehyde; Sörensen phosphate buffer: pH 7.4; 0.1M) and cut into pieces with a razor blade. Specimens were transferred for 2 hr into small vials containing the fixative, then rinsed in buffer solution for 2 hr. After postfixation in buffered 2% OsO$_4$ solution for 2 hr and short rinsing with the buffer solution, specimens were dehydrated with graded ethanol solutions. The material was then embedded in Araldite using propylenoxide as an intermedium. Polymerization occurred at 60°C. Ultrathin sections were made with a Reichert OM U2 ultramicrotome using a diamond knife. The sections were stained with uranyl acetate and lead citrate. TEM: Zeiss EM 9 S2 and Zeiss EM 10CR.

RESULTS

Humeral organ (oh) of juveniles

The humeral organ is located in the lateral (humeral) region of the podosoma approximately above the basis of leg III. In light microscopic investigations it looks like a small, lenticular vesicle. Posterior to it there is a number of small tubercles (Fig. 1; Seniczak et al., 1990). In SEM preparations the organ is not easily recognized since it is hidden in a cuticular depression (Figs. 2, 5a). This peculiarity is not recognizable in the light microscope because of the very thin cuticular layer covering the porose region. The small tubercles behind the organ are very distinctive.
FIG. 2: Humeral organ. a. — SEM of humeral organ of a nymph. The small tubercles behind the organ are visible in this external view. Note secretory material coming from the pore (arrowheads), under which the organ is hidden. × 1930. b. — TEM of humeral organs. Overview of humeral organ of a larva. Note thick epithelium underlying the cuticular part of the organ, which is dome-shaped. The organ is included in a small pocket, the lumen of which contains some secretory products and contacts the exterior by a pore. × 4800. Inset: humeral organ of a nymph. Note that organ is not located as deeply as in the larva but seems to be slightly everted. × 3600.

Abbr.: C, cuticle; Ep, unmodified epidermis; M, mitochondria; Mv, microvilli; N, nucleus of cell of humeral organ; S, cutegument-like secretion; Scs, subcuticular extracellular space.
in SEM, however. In our preparation, some material comes from the small pore connecting the porose organ with the surface of the body.

From TEM it is evident that the humeral organs represent secretory porose organs (Figs. 2-4). The cuticle is thin and its procuticle is penetrated by numerous broad pore canals. The peripheral terminations are covered by the epicuticle. There is a quite extensive subcuticular extracellular space filled with some fine-granular material, which is also seen in the pore canals. The underlying cells are much larger than adjacent epidermal cells. They converge towards the cuticular, porose plate. Apically, the cells are provided with long microvilli which form bundles and originate from deep crypts in the cell apex. The cytoplasm of the cells looks quite homogeneous. There is an extensive smooth endoplasmic reticulum (ER). Many long mitochondria and some dense lysosomes are present. The nuclei are ovoid and contain large nucleoli. There is only a thin basal lamina. Externally from the cuticle, a distinct material composed of several layers of electron-lucent material and a thick layer of dense secretion which is composed of subunits are present. Both components extend through the small pore onto the surface of the animal. This material corresponds to the material seen in SEM (see above).
The humeral organs of larvae and nymphs are rather similar. However, the specimens differed with respect to the width of the small pore: the opening of the nymphs seemed to be wider (cf. Fig. 2b). It needs further investigation to decide whether this difference truly depends on the instar, represents an artefact, or if the width of the opening may be actively altered by the individual.

**Porose organs of adults**

Porose organs of the octotaxic system are all of a similar shape. They are distinct, small, round fields on the notogaster (cf. SENICZAK et al., 1990). In SEM they are detectable externally due to a concentration of small tubercles within the tuberculated surface of the animal (Fig. 5b). There are many small and shallow pits in this region, also on the tubercles. When viewed from the interior of a macerated and dissected animal, the porose areas are distinct depressions in the cuticle (procuticle), which show numerous small pores (pore canals). In TEM these organs represent the typical aspect of secretory porose organs, i.e., they are underlain by large cells provided with long microvilli and containing numerous lipid inclusions (Fig. 6). The microvilli are at least in some areas very

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**Fig. 4**: Nuclear region of cells of the humeral organ of a tritonymph (same specimen as in Fig. 3a). Note abundant smooth ER and sections of small bundles of microvilli which originate from deep crypts in the apical plasmalemma and are hence seen level with the nuclei. × 14,250.
Abbr.: M, mitochondrion; Mv, microvilli; N, nucleus
long and arranged in bundles. Long mitochondria and smooth ER are further conspicuous elements in these cells.

There are two humeral porose organs, which are located under the pteromorphs, in the investigated species: an anterior ‘normal’ porose area (Am) and a posterior modified area (Ah).

In SEM both areas are covered externally by cero­
 tegument (Figs. 7, 9). When viewed from the interior of a dissected and macerated specimen, the porose organs are very conspicuous because of the concentra-
tions of pores (pore canals) located in a distinct region of thinner procuticle. The porose region of Am is flat, whereas that of Ah protrudes slightly into the interior of the animal. In a closer view it is also apparent that the porose procuticle is different in both areas. In Am the pores are slightly more irregularly shaped than in Ah.

In TEM the porose area Am is rather similar to the porose area of the octotaxic system described above. Most conspicuous are the bundles of very long microvilli originating from deep apical crypts (Fig. 8). The cells contain many lipid droplets. Both charac-
ters are not as conspicuous in the porose organ Ah (Figs. 9, 10). Microvilli are shorter and instead of lipid droplets, dense lysosomes are more prominent. The shape or position of Ah slightly differed in our specimens. Sometimes the cuticular, porose portion was freely exposed at the surface and showed a distinct convexity (Fig. 9), sometimes it was partly included in a slit-like depression. Again we cannot decide whether this is a result of an artefact or is a consequence of an active movement prior to fixa-
tion.
DISCUSSION

Three secretory porose organs have been studied in the adults of *Edwardzetes edwardsii*. The porose areas of the octotaxic system and the humeral area Am are quite similar and similar areas have been observed in a number of poronotic species by Alberti et al. (1997a, b). Ah is somewhat different in having shorter microvilli and, apparently, fewer lipid inclusions. Instead there are more lysosomes. It is difficult to decide whether this quantitative difference has any relevance. However, microvilli shorter than those in other porose organs have also been observed in the Ah of *Achipteria coleoptrata* (Alberti et al., 1997b). The Ah of both species are always covered by thick layers of cerotegumental material.

The humeral organ of the juveniles are evidently secretory porose organs. The position of the porose region within a cuticular invagination is reminiscent of the modifications seen in Ah of some adults (Norton et al., 1997). In all our specimens there was a distinct amount of secretion above the porose region.
Fig. 7. a. — Overview of an adult specimen of *Edwardzetes edwardsii*. × 110. b. — Humeral region showing cerotegument under pteromorphs. × 625. c. — Porose areas Am (right) and Ah (left) from humeral region (view from the interior after maceration). × 965. Abbr.: Pt, pteromorph.
This secretion was similar to the material forming the cerotegument of adult oribatid mites. The cellular components appeared to be intermediate between those of 'typical' porose areas (i.e., Am; octotaxic system) and the modified humeral area Ah.

In Achipteria coleoptrata, the cuticle of the organ Ah forms a deep invagination, whereas in Achipteria nitens the organ seems to be evaginated (Grandjean, 1929). Our investigations have provided some evidence that the cuticle of this organ may at least allow some movement of the organ, resulting in a different exposure of the modified, porose region. Further studies are needed to confirm whether this putative capability plays a functional role. Is the animal able to regulate the amount of extrusion of the secretion? Such a mechanism could help to understand the differentiation of humeral vesicles. Their products could perhaps be added only occasionally to the secretion of the 'normal' areas of the humeral series.

What is the function of the humeral organ in these juveniles? Why do some species possess humeral organs and others do not need them? Since juveniles of most oribatids do not have a respiratory system, it is unlikely that the humeral organs are functionally related to a respiratory system as may be the case with the humeral areas in the adults which likely help
FIG. 9. Overview of humeral porose area Ah (animal transversely sectioned). Note that the surface of the area is convex and exposed to the exterior. It is covered by cerotegument (cf. Fig. 6d). × 3550. Abbr.: C, cuticle; Ce, cerotegument; Mv, region of microvilli; N, nucleus of area cell; Pt, basis of pteromorph; Tr, trachea
FIG. 10. Subcuticular region of same area. Microvilli are less regularly arranged as in area Am and subcuticular space is less extensive than in the humeral organs of the juveniles. Note small vesicles (arrowheads) containing material similar to that found in the extracellular space above the cell apex and within the pore canals. \( \times 28,500 \). Abbr.: C, cuticle; Mv, microvilli

provide a hydrophobic lateral body region (see Alberti et al., 1981; Pugh et al., 1987; Messner et al., 1992; Alberti et al., 1997a, b; Norton & Alberti, 1997).

It seems most likely to us that the humeral organ of the juveniles is related to the humeral organ Ah of the adults. It is unlikely that the humeral organ is a plesiomorphic structure since it occurs in only a few taxa of rather derived poronotic Oribatida. It seems more plausible to assume that it is an expression of accelerated development, as suggested by Norton et al. (1997). Perhaps Ah, a differentiation of the areas of the humerosejugal series, adds a component to the 'normal' secretions of the areas of this series that has a positive selective value when also already appearing in the juveniles. After the eventual appearance of the organ in early ontogeny by accelerated development, this putative value may have helped to fix this character. Both organs, Ah and oh, may have evolved rather independently in the various taxa. Such a scenario could help to explain the restricted occurrence of the humeral organ in poronotic Oribatida and the lack of strict correlation with the occurrence of Ah in the form of a vesicle. The exact function of the organ still needs to be elucidated.

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