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Ultrastructure of ionocytes from osmoregulatory integumental windows of *Tachidius discipes* and *Bryocamptus pygmaeus* (Crustacea, Copepoda, Harpacticoida) with remarks on the homology of nonsensory dorsal organs of crustaceans

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Abstract

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The ultrastructure of the integumental windows of the polyhaline species *Tachidius discipes* (Tachidiidae) and the freshwater inhabitant *Bryocamptus pygmaeus* (Canthocamptidae) is described. The cuticle as well as the underlying cells exhibit fine structural features, which show that these windows are sites of ion exchange. The thick epidermal cells possess apical membrane amplifications and many mitochondria, which in the case of *B. pygmaeus* are ramified in a very unusual style. The osmoregulatory function can also be shown by vital staining with silver nitrate. The integumental windows of both species are not innervated. This is also evidenced by SEM observations. An overview about the distribution of integumental windows within the Harpacticoida is given. The question of the homology of nonsensory embryonic and non-embryonic dorsal organs is discussed.

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Introduction

The so-called integumental windows (Reid 1994) are widespread among the Harpacticoida: Good descriptions of the integumental windows of the taxa Tachidiidae, Canthocamptidae, *Mesochra* and *Moraria* were given by Gurney (1932). Reid (1994) gave an overview for the Parastenocarididae. A SEM micrograph of the cephalothorax window of *Pseudostenhelia wellsi* (Stenheliidae) can be found in Williams-Howze and Fleeger (1987). The integumental windows of *Harpacticella paradoxa* (Harpacticidae) were described by Itô and Kikuchi (1977). Hosfeld and Schminke (1997) provided light micrographs of the windows of *Chappuisius inopinus* (Chappuisiidae). A SEM micrograph of the cephalothorax window of *Heteropsyllus nunni* was given by Coull and Grant (1981). Kikuchi *et al.* (1993) described the windows of *Limnocletodes angustodes*, and Cicchino and Ringuelet (1977) described those of *Antarctobiotus longifurcatus*. Integumental windows have not been mentioned for any other copepod taxon except the Cyclopoida: *Halicyclops caneki* (Fiers 1995). The different known distribution patterns of the integumental windows of Harpacticoida are shown in Fig. 1.

Until now the function of the integumental windows has only been revealed for two harpacticoid species: *Parastenocaris vicesima* (Parastenocarididae) and *Chappuisius inopinus* (Chappuisiidae). The windows of these species are osmoregulatory (Hosfeld and Schminke 1997).

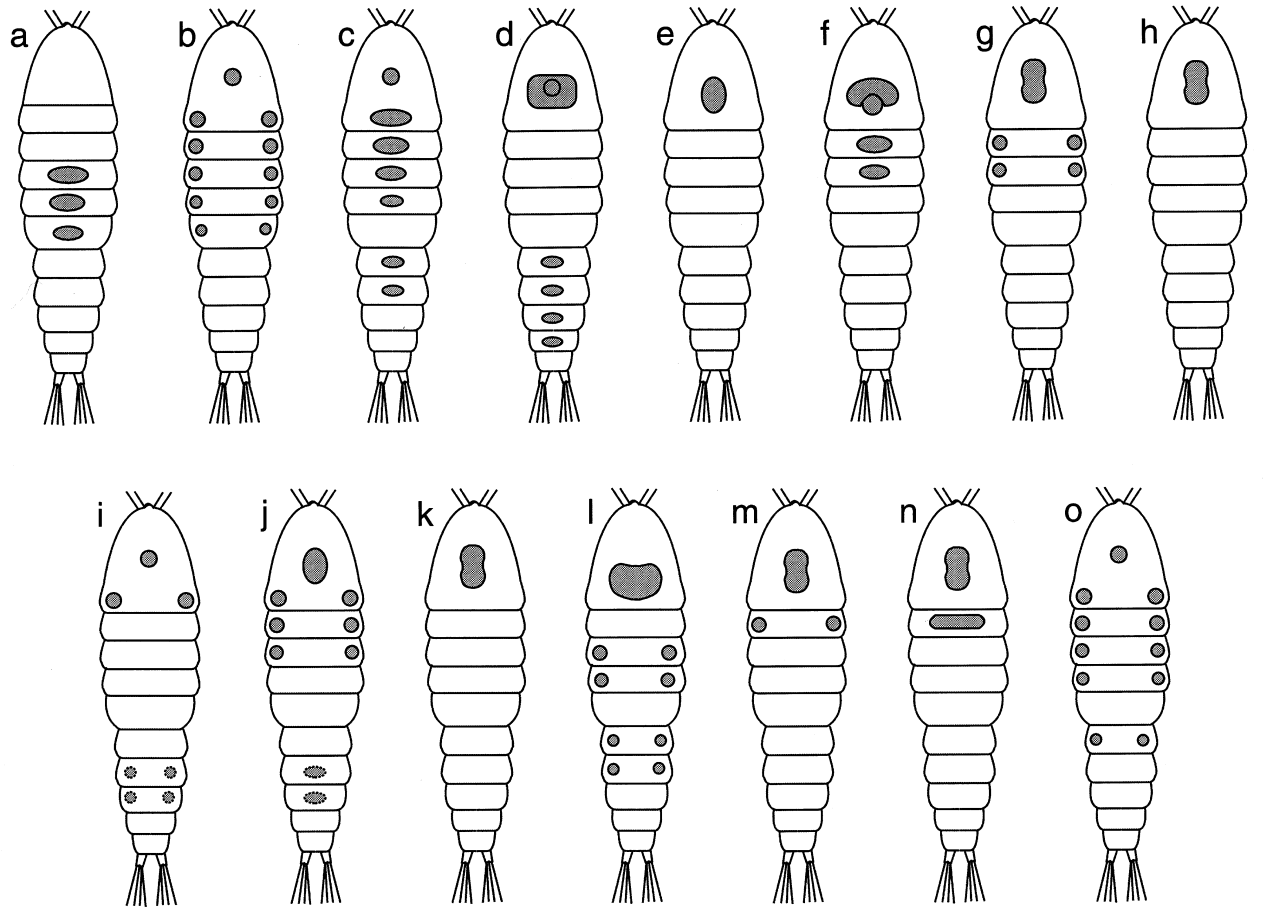


Fig. 1.—**a-o,** Distribution patterns of the integumental windows within males of the Harpacticoida and *Halicyclops caneki*, dorsal view. **a.** *Chappuisius inopinus* (Chappuisiidae). — **b,** Tachidiidae. — **c,** *Harpacticella paradoxa* (Harpacticidae). — **d,** Parastenocarididae. — **e,** *Pseudostenhelia wellsi* (Stenheliidae). — **f,** *Antarctobiotus longifurcatus* (Canthocamptidae). — **g,** *Moraria brevipes* (Canthocamptidae). — h, *Moraria varica* (Canthocamptidae). — **i,** *Mesochra aestuarii*. — **j,** *Mesochra rapiens*. — **k,** *Heteropsyllus nunni*. — **l,** *Limnocletodes angustodes* (Cletodidae). — **m,** *Bryocamptus pygmaeus* (Canthocamptidae). — **n,** *Canthocamptus dentatus* (Canthocamptidae). — **o,** *Halicyclops caneki* (Cyclopoida). Dotted lines (i and j) indicate a ventral position of the windows. For references see text.

Since sensory windows (sensory dorsal organs) also exist within the Crustacea (Laverack *et al.* 1996) and especially the Copepoda (Nishida 1989), it seemed suitable to the author to extend the earlier investigations. Therefore, two species, *Tachidius discipes* (Dana, 1848) and *Bryocamptus pygmaeus* (Sars, 1862) of two different families (Tachidiidae and Canthocamptidae) and two different habitats (a polyhaline and a freshwater species) were examined.

Materials and Methods

Specimens of the polyhaline species *Tachidius discipes* were collected in the North Sea, on Mellum Island (June 1985). The adult males and females used in this study came from a single-female culture. Specimens of the freshwater inhabitant *Bryocamptus pygmaeus* were collected from a swamp near Oldenburg (Fintlandsmoor), Germany, in June 1994. Voucher specimens are deposited in the authors collection.

Animals were fixed for TEM in a sodium cacodylate buffered (pH 7.4) mixture of glutaraldehyde, formaldehyde, acrolein, dimethyl sulphoxide and sucrose for 16 h at 4 °C (Lake 1973). In the case of the marine specimens this trialdehyde fixative was made up of a mixture of Millipore-filtered seawater and distilled water (2 : 1) according to Wingstrand (1978). After rinsing in cacodylate buffer, specimens were pos-tfixed in 2% OsO_4, then washed in buffer for 45 min, dehydrated and embedded in Epon (Luft 1961). For light microscopy, 1-μm-thick sections were cut with glass knives on a Sorvall MT-5000 ultramicrotome and stained with toluidine or Richardsons blue and covered with cedar oil. Ultrathin

sections were cut with a diamond knife and collected on 200 mesh copper grids coated with pioloform support films. They were stained with uranyl acetate followed by lead citrate and examined with a Zeiss EM 109 electron microscope. Animals to be observed by SEM were fixed in the same way, dehydrated in an ethanol series, and critical-point-dried. They were sputter-coated with gold and observed with a Zeiss DSM 940.

Vital staining with silver nitrate: living specimens were placed in a 0.1–0.2% silver nitrate solution ($AgNO_3$) for 10 min in darkness. Subsequently the specimens were washed and fixed in 70% ethanol and then exposed to bright light for 15 min.

Results

The integumental windows of Tachidius discipes

Tachidiidae in general: Only 13 species of the family Tachidiidae are known worldwide, and they possess a more or less uniform distribution pattern of the integumental windows: 'Cephalothorax with dorsal nuchal organ; paired accessory nuchal organs present on cephalothorax and somites bearing P2 to P4 (sometimes P5), (Huys *et al.* 1996: 220). Members of the genera *Tachidius* and *Geeopsis* possess lateral windows on the P5-bearing somite (Fig. 2), while those of the genus *Microarthridion* do not. Concerning the monotypic genus *Cithadius*, the lateral windows on the P5-bearing somite are also lacking (Bowman 1972). The unpaired median cephalothoracic window is already present in the first nauplius of *Tachidius discipes*. The paired lateral cephalothoracic windows arize later, probably upon the metamorphosis into the first copepodid stage. The paired lateral windows on the P2-P5-bearing somites arize with the corresponding somites in the subsequent copepodid stages.

External morphology (SEM): The cuticle of all windows is smooth and naked and does not differ from the surrounding cuticle (Fig. 3A-D). No pits, pores, spines or sensory structures were observed. The boundary of the windows is always the only visible structure. As seen in Fig. 3D, the anterior part of a lateral window may sometimes not be visible in the SEM, because it may be covered by the frill of the cephalothorax or the preceding somite. Sometimes the windows of the P4 and P5-bearing somite could not be detected at all, because the somites were telescoped too much.

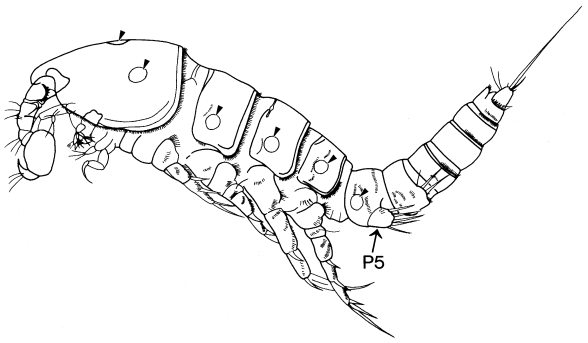


Fig. 2.—Male of *Tachidius discipes*, lateral view. The arrowheads indicate the position of the integumental windows; peraeopod 5 (P5). After Gurney (1932).

Ultrastructure of the unpaired cephalothoracic window: This more or less round window has a dorsomedian position on the cephalothorax and measures about 28 μm in diameter (Fig. 4). It is separated from the surrounding cuticle by a transition zone (tz), which is about 1 μm wide. The cuticle of this transition zone has low electron density and lacks the laminated layers of microfibrils, as well as the p2-level of the procuticle (Fig. 5A). The thickness of the tz-cuticle is reduced to 250–300 nm. The surrounding cuticle measures 1.2 μm in thickness and the window cuticle 1.3 μm. The window cuticle and the surrounding cuticle also differ in the thickness of the p1-and p2-level of the procuticle. The p1-level of the surrounding cuticle measures 470 nm, and the p2-level 620 nm, on average. The p1-level of the window cuticle is thinner (270 nm) and the p2-level is much thicker, measuring 970 nm. Although the epicuticle of the window is thinner (35 nm) than that of the surrounding cuticle (50 nm), it has a higher electron density. The cephalothorax cuticle is smooth, whereas the window cuticle shows many small protuberances and depressions. In some areas the window cuticle is even interspersed with channels, which are present in the p1-as well as in the p2-level of the procuticle (Fig. 5B). The channels are connected to the extracellular space between the cuticle and the underlying epidermal cells. The distance between the cuticle and the underlying cells measures about 40–60 nm.

The epidermis of the window, which is composed of one cell type, has a maximum thickness of about 8.75 μm in the middle of the window. Toward the border the thickness decreases to 5–6 μm (Fig. 4). The cytoplasm in the apical part of the cells is characterized by a pronounced tubuli system. The basal region of the cells contains abundant glycogen granules and many mitochondria (Fig. 5C). The apical tubuli region is up to 2 μm high. The tubuli are arranged irregularly and less than 2 μm long. Most of them measure about 1–1.5 μm in length. The tubuli are connected to the apical plasma membrane only exceptionally. Nevertheless, it is noteworthy that there are many vesicles in the neighbourhood of the apical plasma membrane, which indicate the possibility of endo-or exocytotic processes (Fig. 5D). The vesicles do not belong to the type of coated vesicles. Their content as well as the content of the tubuli consists of electron-dense granules, which measure 60 nm in diameter on average. Sometimes the granules seem to possess an electron-lucent core, in this case having a more or less ringlike appearance. The tubules measure about

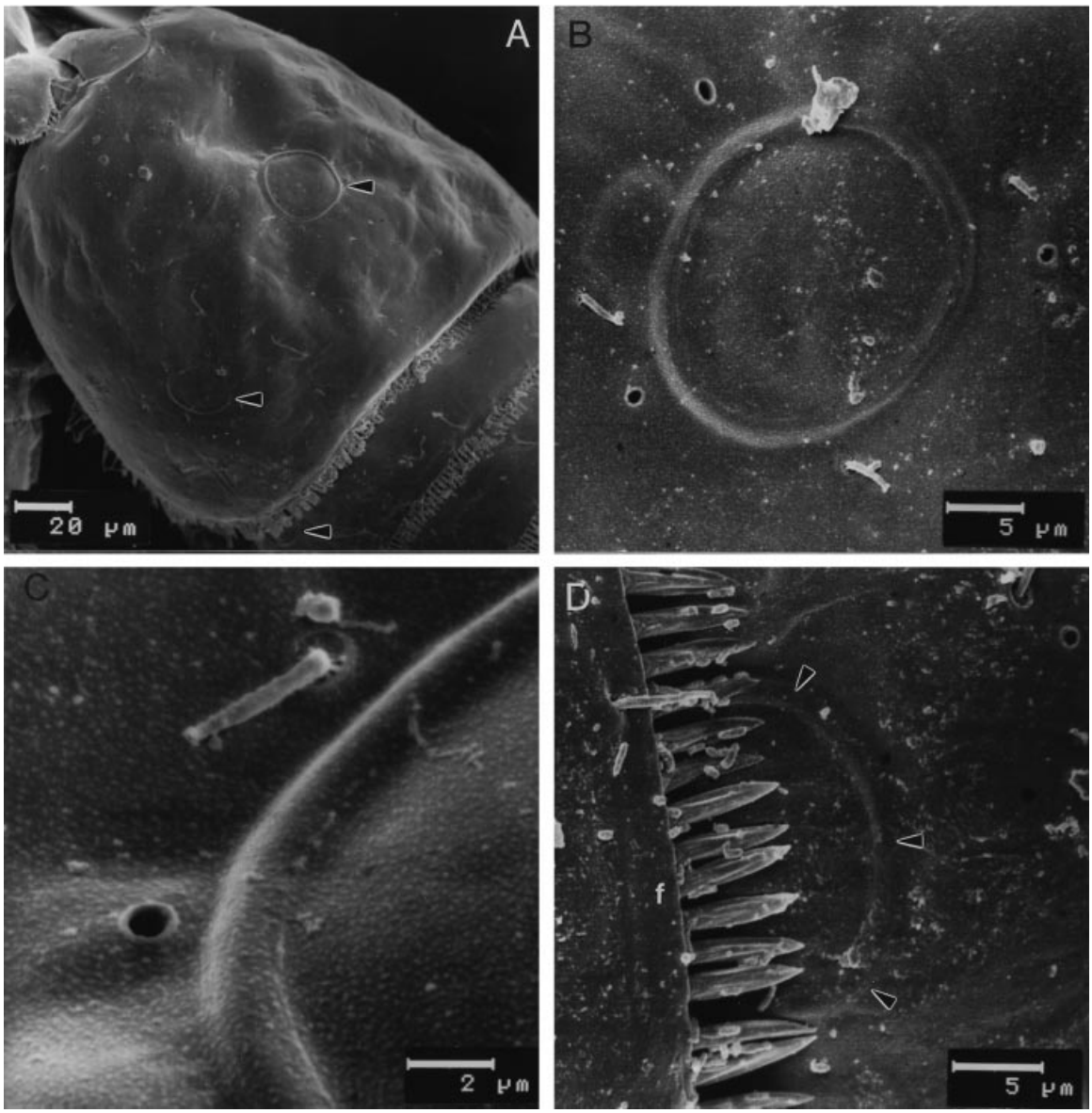


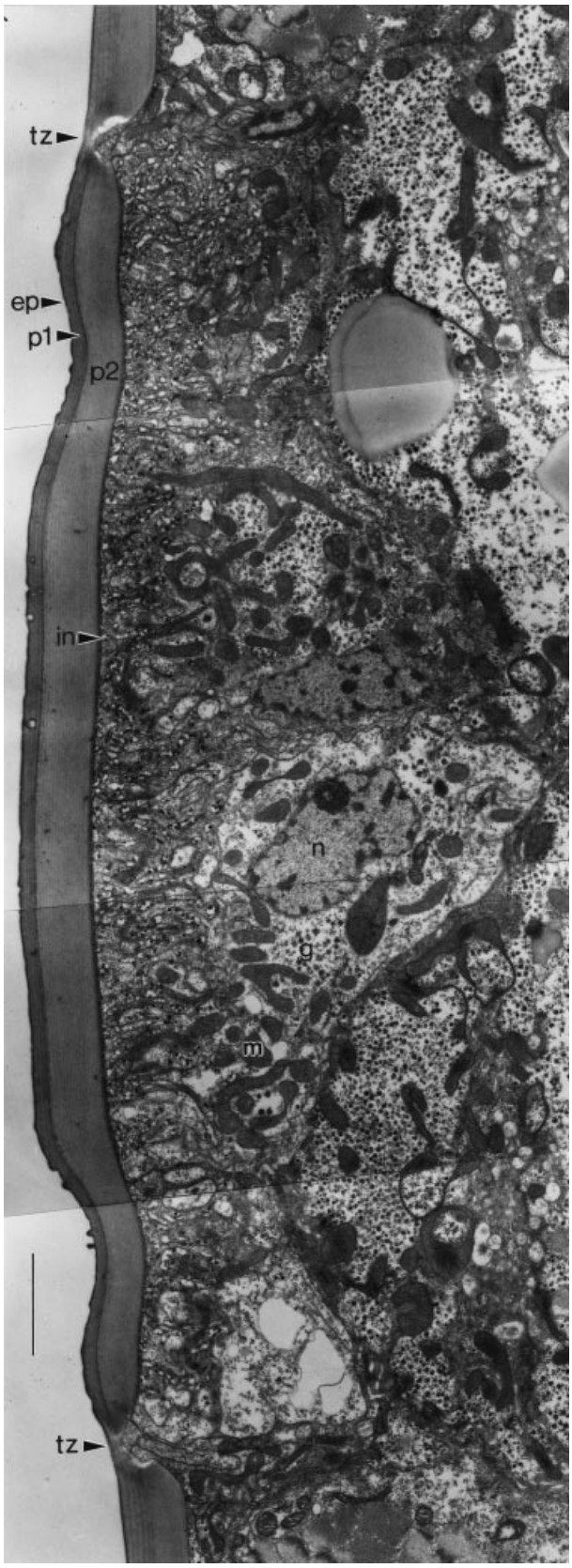
Fig. 3.—*Tachidius discipes*. SEM micrographs of the integumental windows. — **A,** Male, dorsolateral view. Arrowheads point to the median and a lateral cephalothoracic window as well as to the lateral window on the P2-bearing somite. — **B,** Left cephalothoracic window. The cuticle of the window does not differ from the surrounding cuticle. — **C,** Boundary of median cephalothoracic window. — **D,** The lateral window (arrowheads) on the P3-bearing somite is partly covered by the frill (f) of the preceding somite.

115–120 nm in diameter, but there are also collapsed ones with a diameter of only 25 nm. All in all the apical region of the cells gives the impression of being a very dynamic and flexible system with high transport activity, and it is probably a part of the smooth endoplasmic reticulum.

Vital staining with silver nitrate: It was possible to stain all integumental windows of both male and female specimens. However, staining was successful only if the specimens had not been reared in pure seawater. For staining the integumental windows of T. discipes, it was necessary to adapt the specimens to a much lower salinity (about 10%) for several hours. The stain reaction did not show the same contrast as for pure freshwater inhabitants. The whole specimen became amber, while the darkened integumental windows differed only slightly.

The integumental windows of Bryocamptus pygmaeus

Canthocamptidae in general: Apart from more complex distribution patterns (see Introduction), many representa-



tives of the family Canthocamptidae only possess one integumental window situated in the dorsomedian position on the cephalothorax. The window differs much in size, form and ornamentation from species to species (e.g. Hamond 1988). Por and Hadel (1986) showed SEM micrographs of the cephalothoracic windows of *Attheyella jureiae* and *A. vera*. The array of integumental windows in *Gulcamptus huronensis* may be the most elaborate known in the Canthocamptidae (Reid and Ishida 1996).

Besides the cephalothoracic window, some representatives of the Canthocamptidae possess additional windows on the P2-bearing somite. Gurney (1932: 127/128) described the windows of *Bryocamptus pygmaeus* as follows: 'Nuchal organ very large and elongated, slightly constricted in the middle. . . . Somite 2 of thorax (first free somite) often shows a lateral oval area in the cuticle enclosed by a double contour similar to that in some *Mesochra* and *Moraria*. Its significance is unknown, (Fig. 6). In the literature there are no data about the first appearance of the windows in the course of post-embryonic development. Dahms (1987: Fig. 1C) showed a dorsal view of a second nauplius of *Bryocamptus pygmaeus*, but a cephalothoracic window is absent. Subsequent examination of the mounted specimens revealed that all larval stages (nauplii and copepodids) of *Bryocamptus pygmaeus* lack the cephalothoracic window.

External morphology (SEM): The cuticle of the integumental window, which is positioned centrally on the cephalothorax, is smooth and naked. Pores, sensilla or other structures are completely lacking (Fig. 7A,B). Only the raised boundary of the cephalothoracic window is visible. The window cuticle does not differ from the surrounding cuticle. Even the lateral windows on the P2-bearing somite, which are partly covered by the posterior margin of the cephalothorax, are discernible because of the raised boundary. The cuticle of these lateral windows is as smooth and naked as the cuticle of the cephalothorax window.

Light microscopy: In contrast to the windows of *Tachidius discipes*, the cephalothoracic window of *Bryocamptus pygmaeus* is rather large, and the transition zone as well as the cells underlying the window are visible with the light microscope (Fig. 7C,D). It is striking that the cells underlying the window are tapered toward the ventral side and therefore exhibit a conical shape. The cells are striated perpendicular to the cuticle. Larger 'cell inclusions' are visible adjacent to the cuticle. In front of

Fig. 4.—*Tachidius discipes*. TEM micrograph of the median cephalothoracic window, cross-section. The arrowheads indicate the borders (tz, transition zone) of the window. The ionocytes are characterized by apical infoldings (in), many mitochondria (m) and an abundance of glycogen granules (g). Epicuticle (ep), nucleus (n), p1-and p2-level of the procuticle (p1, p2). Scale bar: 2 μm.

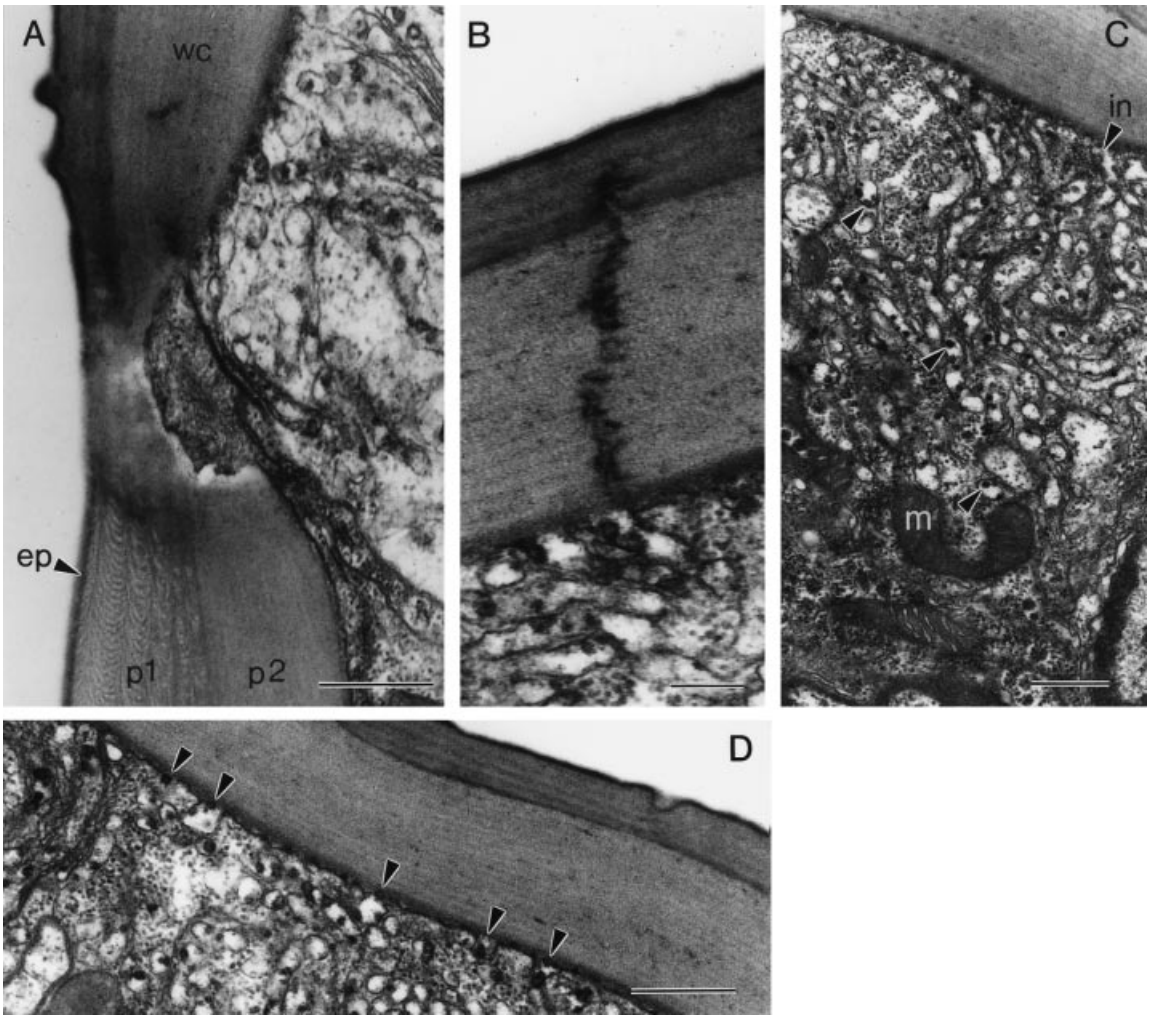


Fig. 5.—*Tachidius discipes*. TEM micrographs of the median cephalothoracic window, cross-sections. — **A,** Transition zone. Epicuticle (ep), p1-and p2-level of procuticle (p1, p2), window cuticle (wc). Scale bar: 0.5 μm. — **B,** Window cuticle interspersed with channel. Scale bar: 0.2 μm. — **C,** Ionocyte. Apical region with many infoldings (in) containing electron-dense granules (arrowheads). Basal region with many mitochondria (m) and glycogen granules. Scale bar: 0.5 μm. — **D,** Apical plasma membrane of ionocyte. The arrowheads point to vesicles. Scale bar: 0.5 μm.

and below the anterior end of the cephalothorax window a large gland is situated, the pore of which lies just anterior to the window. It is not yet clear whether there is a functional relationship between the window and the gland. Below the cells of the cephalothoracic window there is a larger system of lacunae connected to the anterior part of the midgut. This small, short anterior region of the midgut is separated from the following main midgut by a constriction.

Ultrastructure of the cephalothorax window: The cephalothorax window has an elongated form and is laterally depressed in the middle. It is about 65 μm long and 30 μm wide. The transition zone, which separates the window from the surrounding cuticle, is very broad: 3.2 μm (Fig. 8A-C). It has a much lower electron density than the window cuticle, and the microfibrillar structure seems to be mostly absent. The cuticle of the transition zone is very thin (about 0.7–0.8 μm), but both levels of the procuticle (p1 and p2) are present. Compared to the surrounding cuticle (thickness 1.9 μm) the window cuticle is much thicker (about 3.2 μm) because of the thickness of the p2-level of the procuticle: the p2-level of the window cuticle measures 2.7 μm, while the p2-level of the surrounding cuticle measures only 1.3 μm. The p1-level of the window cuticle is 0.5 μm thick. The window cuticle is also striking because of its high electron density (Fig. 8C). The same is valid for the specialized epidermal cells underlying the window cuticle.

The cells below the window taper toward the ventral side. Measuring from this tip, the cells are about 6 μm high. Adjacent to the cuticle the cells are 11–13 μm wide.

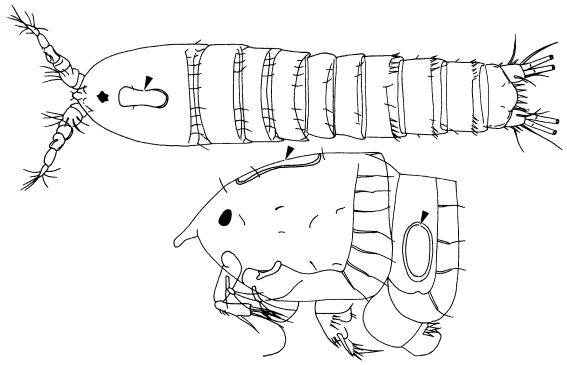


Fig. 6.—Male (dorsal view) and female (lateral view) of *Bryocamptus pygmaeus*. The arrowheads indicate the position of the integumental windows. After Gurney (1932).

The outstanding features of these cells are their mitochondria and the electron-dense invaginations of their apical plasma membrane (Fig. 8C; 9 A, B). It is impossible to differentiate between an apical region with invaginations and a basal region with mitochondria, because both elements are tightly interconnected. The stretched and ramified mitochondria exhibit large numbers of densely packed lamellar cristae, arranged very regularly. The basis of the ramifying mitochondria is situated in the tip of the cell. As the diameter increases toward the dorsal side, more and more new branches of the mitochondria go off. Next to the cuticle the branches of the mitochondria have a diameter of 370 nm on average. It is remarkable that the mitochondrial branches do not touch each other, because the apical invaginations of the plasma membrane serve to separate them (Fig. 9A). The invaginations are arranged in such an orderly array that the distance between the mitochondrial branches always measures exactly 100 nm. In cross section the invaginations are circular and have a diameter of 65 nm. They are filled with a homogenous electron-dense substance. Therefore it is difficult to detect the inner layer of the limiting membrane (Fig. 9B). There is also a distance of 35–40 nm between the invaginations themselves. The invaginations are connected with the mitochondria by a matrix resting on their outside. Longitudinal sections through the invaginations and the associated mitochondria reveal striations with an 18–20-nm periodicity (Fig. 10E).

The distance between the window cuticle and the underlying cells measures less than 100 nm for the most part in those areas, where no electron-dense substance has accumulated below the cuticle (Fig. 9C). This homogenous and amorphous material, nearly hemispherical in shape, exhibits the same electron density as the substance within the invaginations (Fig. 9D). However, the invaginations appear to have a direct contact with this extracellular substance only rarely. Between the window cuticle and their underlying cells there is a second extracellular matrix, which exhibits a lower electron density and fills the extracellular space in general (Fig. 9C). The hemispheric and darker material measures up to 3.8 μm in width and is 1.4 μm high.

The underlying cells of the window cuticle possess delicate offshoots, which (inspected in cross sections) form a lumen below the cell. Sometimes a third, median lumen is also present (Fig. 8A). Another possibility is the formation of only one lumen below the window. There is one more cell type involved with the formation of this lacunae system, and this cell type is connected with the midgut (Fig. 10A) but not with the oesophagus (Fig. 10B). The offshoots also have flattened nuclei and very rarely regions of rER.

Ultrastructure of the lateral windows: The lateral windows of the P2-bearing somite are oval and about 30 m high in the dorsoventral direction. The cells underlying the windows are consistent with the cells of the cephalothorax window. However, both those cells and the cuticle of the lateral windows show special features.

The transition zone (3.5–4 μm wide) is not as well delimited as the transition zone of the cephalothorax window. The tz-cuticle has a higher electron density than the surrounding cuticle, but is not as electron-dense as the window cuticle (Fig. 10C). The p1-and the p2-level of the procuticle are present. Contrary to the transition zone of the cephalothorax window, there is no difference between the thickness of the transition zone cuticle, the window cuticle, and the surrounding cuticle. They all measure 0.8 μm. It is noteworthy that the thickness of the epicuticle increases conspicuously in the region of the window (Fig. 10C). Within the window the epicuticle is 190 nm thick, whereas the epicuticle of the normal cuticle is only 25 nm thick (measured in the dorsomedian region of the P2-bearing somite). The p1-and the p2-level (about 150 and 500 nm thick) of the window procuticle are clearly differentiated.

The cells underlying the window are consistently flat and only 1.2 μm high. There are two per window in cross section. The mitochondria lack the conical shape, but nevertheless are of the ramified type (Fig. 10D). The invaginations of the apical plasma membrane are interspersed between the mitochondrial branches. The invaginations, which have a diameter of 65 nm, are filled with a homogenous and electron-dense substance. They are arranged in such an orderly array that the distance between the mitochondrial branches measures always 100 nm exactly. The mitochondria, which bear many tightly packed cristae, are 1 m high and their unramified basis measures 1.3 μm in width. The mitochondria are connected with the invaginations as tightly as in the cells of the cephalothorax window. The striation exhibits the same periodicity. Because of the better fixation of these more flattened cells, it is much easier to identify the invaginations as such (Fig. 10E). The distance between the apical plasma membrane and the adjacent cuticle measures

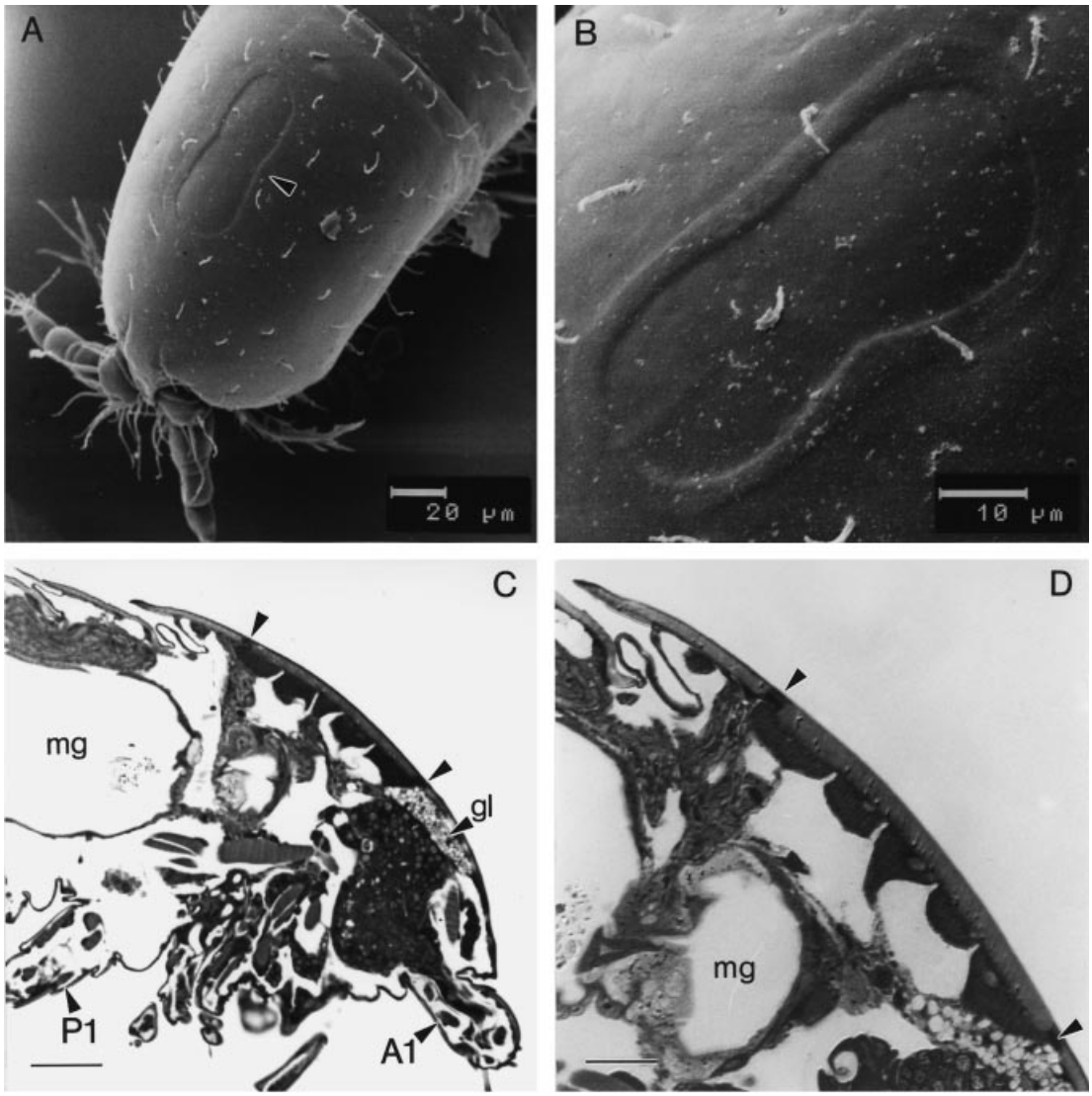
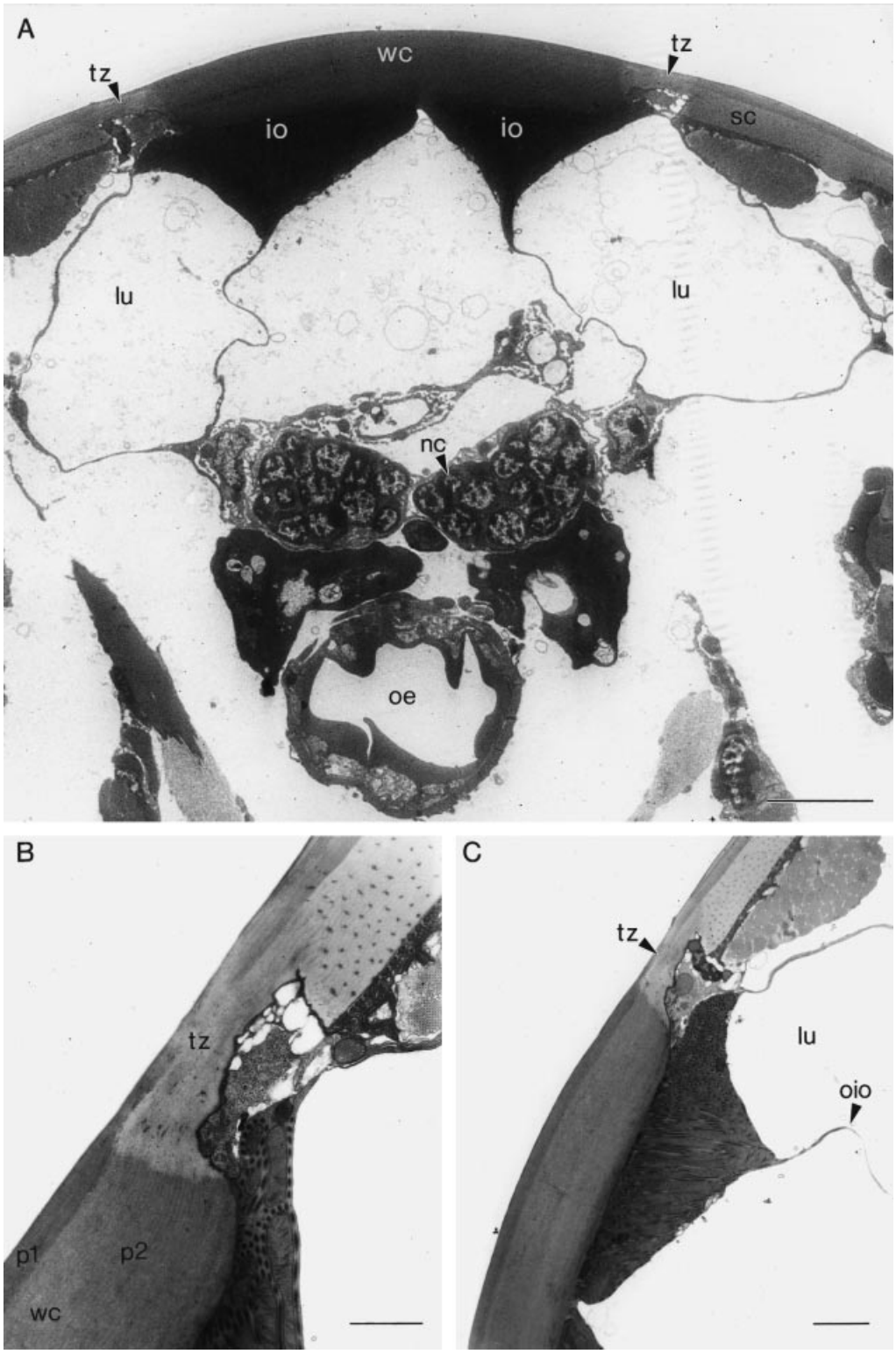


Fig. 7.—*Bryocamptus pygmaeus*. SEM **(A** and **B)** and light micrographs **C, D**; longitudinal sections of the cephalothoracic window. — **A,** The laterally depressed window (arrowhead) is situated in the dorsomedian position on the cephalothorax. — **B,** The cuticle of the window is smooth and naked. — **C,** The arrowheads indicate the anterior and posterior end of the cephalothoracic window. Antennule (A1), gland (gl), midgut (mg), peraeopod 1 (P1). Scale bar: 20 μm. — **D,** The ionocytes, which exhibit a conical shape in cross-sections, are sectioned peripheral. Scale bar: 10 μm.

80 nm on average. This extracellular space is filled with a homogenous substance, which possesses a lower electron density than the material within the invaginations (Fig. 10D,E). The large and hemispheric 'inclusions' of the extracellular space of the cephalothorax window are totally absent. The cells also lack the delicate offshoots. Nevertheless, the nuclei are situated 'outside' the proper cell. Below the cells a basal lamina is present.

Vital staining with silver nitrate: It was possible to stain the cephalothorax window as well as the lateral windows

Fig. 8.—*Bryocamptus pygmaeus*. TEM micrographs of the median cephalothoracic window, cross-sections. — **A,** Overview. The thick and electron dense window cuticle (wc) is separated from the surrounding cuticle (sc) by the transition zone (tz). The ionocytes (io) are of conical shape and also of high electron density. Lumen (lu), oesophagus (oe), nerve cells (nc). Scale bar: 5 μm. — **B,** Detail of Fig. A. Transition zone (tz). p1-and p2-level of procuticle (p1, p2), window cuticle (wc). Scale bar: 1 μm. — **C,** Detail of Fig. A. Ionocyte. The electron dense cytoplasm is characterized by the mitochondria and the invaginations. Lumen (lu), offshoots of ionocyte (oio); transition zone (tz). Scale bar: 2 μm.



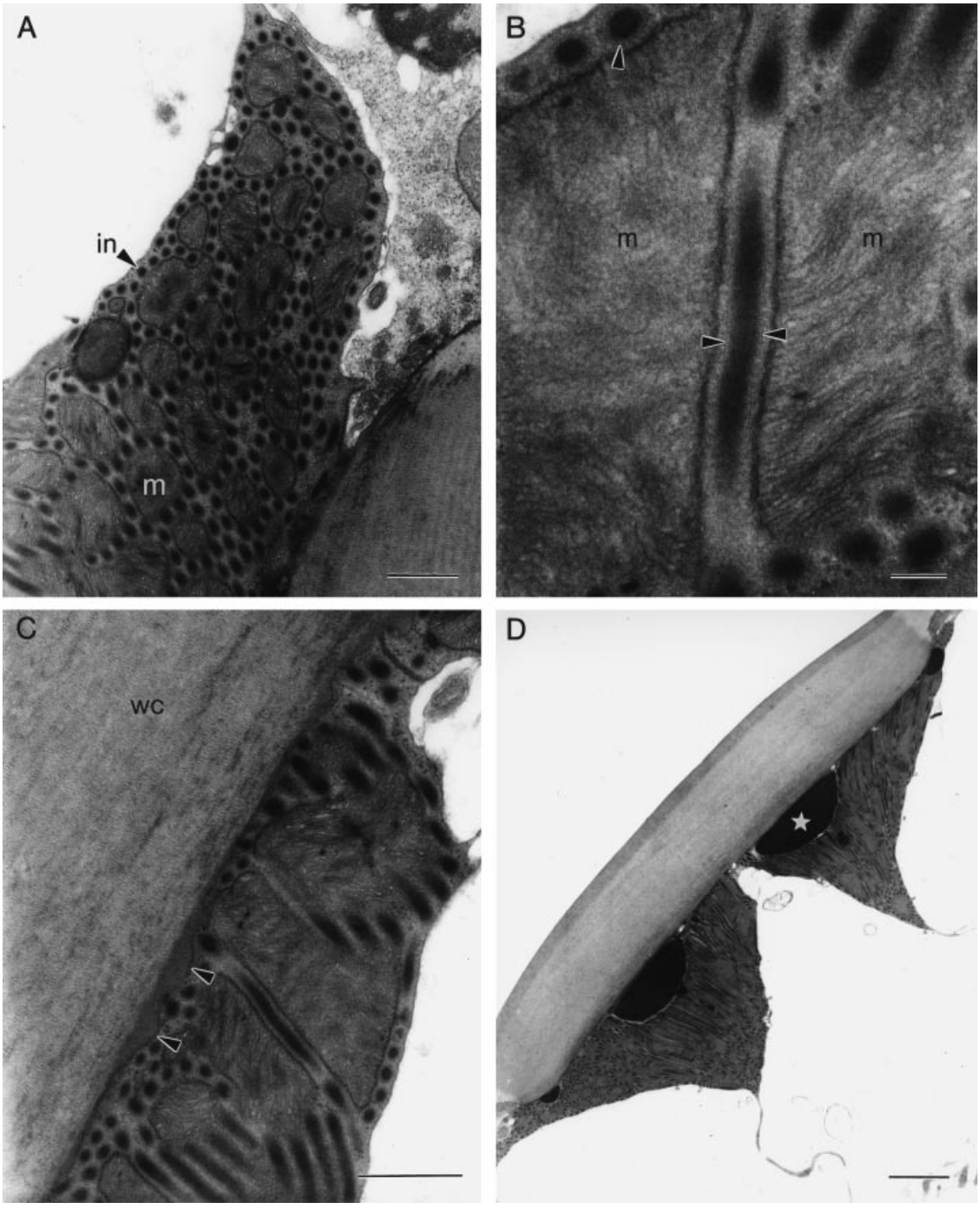


Fig. 9.—*Bryocamptus pygmaeus*. TEM micrographs of the ionocytes, cross-sections. — **A,** Mitochondrial branches (m) and the invaginations (in) of the apical plasma membrane are cross-sectioned. Scale bar: 0.5 μm. — **B,** The distance between the mitochondrial branches (m) always measures exactly 100 nm. The arrowheads point to the limiting membrane of the invaginations. Scale bar: 0.1 μm. — **C,** The arrowheads point to the apical plasma membrane. The extracellular space beneath the cuticle is filled with a homogenous matrix. Window cuticle (wc). Scale bar: 0.5 μm. — **D,** The extracellular space also often bears a second component (star), which exhibits a hemispheric shape. Scale bar: 2 μm.

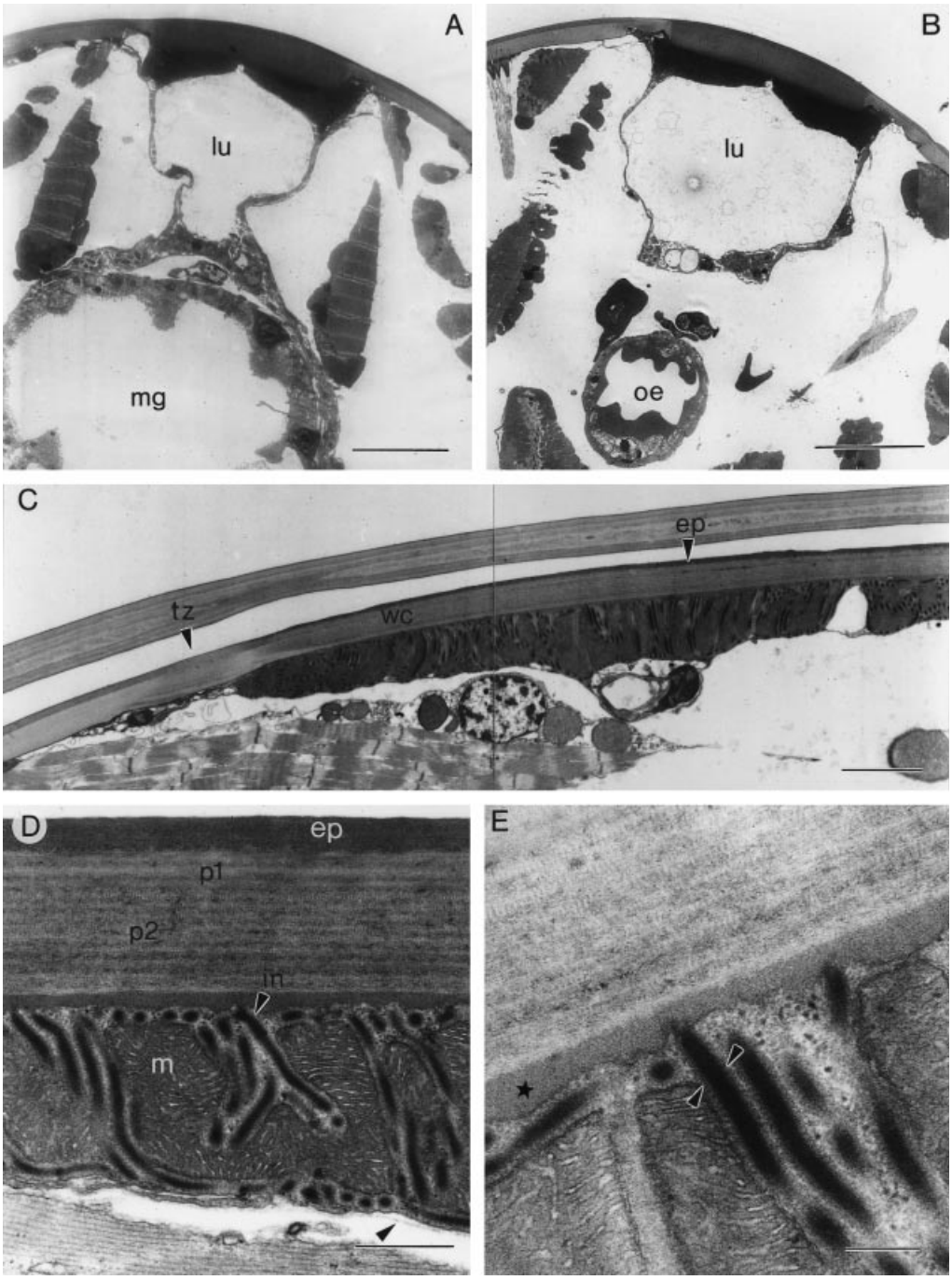


Fig. 10.—*Bryocamptus pygmaeus*. TEM micrographs of the cephalothoracic window (A, B; cross-sections) and the lateral window on the P2-bearing somite (C-E; cross-sections). — **A,** Overview. The lumen (lu) below the ionocytes is connected with the midgut (mg). Scale bar: 10 μm. — **B,** Overview. The lumen below the ionocytes is not connected with the oesophagus (oe). Scale bar: 10 μm. — **C,** Overview, lateral window on P2-bearing somite. The transition zone (tz) is not as delimited as the tz of the cephalothorax window. The ionocytes are rather flat and the epicuticle (ep) of the window cuticle (wc) is very thick. Scale bar: 2 μm — **D,** Ionocyte. The cytoplasm is characterized by the branched mitochondria (m) and the invaginations (in). The arrowhead points to the basal membrane. Epicuticle (ep), p1-and p2-level of the procuticle (p1, p2). Scale bar: 0.5 μm. — **E,** Ionocyte, apical part. The limiting membrane (arrowheads) of the invaginations is clearly visible. The extracellular space is filled with a homogenous matrix (star). Scale bar: 0.2 μm.

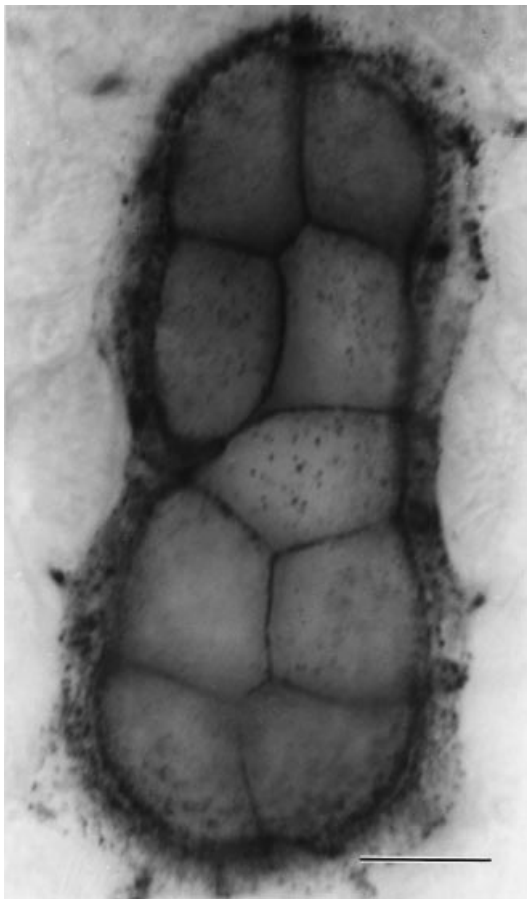


Fig. 11.—Cephalothoracic window of *Bryocamptus pygmaeus* stained with silver nitrate. It is possible to detect nine single cells. Light microscopy, dorsal view. Scale bar: 10 μm.

on the P2-bearing somite of adult male and female specimens. In addition, the cephalothorax window exhibited a better staining reaction than the lateral ones. In contrast to the lateral windows, it was possible to detect the single cells within the cephalothorax window (Fig. 11). There are always nine cells, the middle ones always being of irregular shape. It was impossible to identify the single cells of the lateral windows. Copepodid stages showed no staining reaction at all.

Discussion

The epidermal cells underlying the cuticle of the integumental windows of *Tachidius discipes* and *Bryocamptus pygmaeus* are specialized for ion transport. This is evidenced by ultrastructural features as well as by the staining reaction of these cells (ionocytes) with $AgNO_3$. The integumental windows of both species are not innervated and therefore are not sensory.

A striking feature of the epidermal cells underlying the median cephalothoracic window of *T. discipes* is the apical tubuli system. The maximum diameter of the tubuli (115–120 nm) is much larger than the corresponding invaginations of *B. pygmaeus* (65 nm). The tubuli of *T. discipes* contain electron dense granules, while the invaginations of *B. pygmaeus* are filled with a homogeneous substance of high electron density. Moreover, the presence of vesicles at the apical plasma membrane and the collapsed tubuli give the impression of a transport activity in the ionocytes of *T. discipes*, completely different from that in the ionocytes of *B. pygmaeus*. Because of the lack of physiological studies, e.g. enzyme proof or element analysis, it is impossible to make a concrete statement about the polarity and quality of the ion transport in either species examined here. Because the specimens of *T. discipes* were reared in pure seawater at normal salinity, it is not surprising that there were differences from the ionocytes of *B. pygmaeus*.

Lang (1948) described the cytoplasm of the cells underlying the cephalothoracic window of *Canthocamptus staphylinus* as clearly striped. Such a distinct striation is also visible with the light microscope in the ionocytes of *Bryocamptus pygmaeus*. The ultrastructural study reveals that the striation is linked with a special ramified type of mitochondrion and the regularly arranged invaginations between the mitochondrial branches. These ionocytes are very characteristic. It seems to be important to find out if this cell type is widely distributed, or possibly limited to a particular subtaxon of the Canthocamptidae.

More than 50 specimens of *B. pygmaeus* were stained with $AgNO_3$. All individuals showed the constant number of nine cells in the cephalothoracic window, the cells always arranged identically. Hamond (1988: 1092) also described nine cells in the cephalothoracic window of *Fibulacamptus victorianus*: '...the divisions inside the nuchal organ represent imperfectly preserved soft parts', which are arranged in the same manner as in *B. pygmaeus*. This is another indication that the organization of the cephalothoracic window within a particular subtaxon of the Canthocamptidae may be more or less uniform.

According to presently available information, the large hemispheric accumulations of electron-dense material below the cuticle of the cephalothoracic window of *B. pygmaeus* are only comparable with the so-called 'Konus' in the extracellular cavity of the embryonic dorsal organ of the amphipod species *Orchestia cavimana* and *Niphargus puteanus*. Meschenmoser (1996) showed that the 'Konus' does not contain polysaccharides.

In general the cuticle above ionocytes shows a different ultrastructure and is remarkably thinner than the 'normal' cuticle (Taylor and Taylor 1992). The cuticle of the cephalothoracic window of *B. pygmaeus* is

remarkably thicker than the surrounding cuticle, and the function of the very thick epicuticle of the lateral discs on the P2-bearing somite also remains a matter for further investigation.

The question of the homology of embryonic and non-embryonic dorsal organs

Martin and Laverack (1992) as well as Meschenmoser (1996) stated that the dorsal organs of larval and adult Crustacea are not homologous with the embryonic dorsal organs. However, certain arguments narrow the gap between the non-sensory dorsal organs of embryonic, larval, and adult Crustacea. Moreover, those coincidences concern not only the placement but also the function and the presence in time or developmental stages, respectively: Meschenmoser (1996) stated that the embryonic dorsal organ of different amphipod and isopod species is osmoregulatory in function, and that this osmoregulatory function is an adaptation for living in fresh- or brackish water. According to Meschenmoser, none of the dorsal organs from marine species that never enter brackish or freshwater habitats show ultrastructural features indicating an osmoregulatory function. Interestingly, Meschenmoser also speculated that the embryonic dorsal organ is generally involved in the embryonic moult: this moult is linked with an uptake of water and a regulation of the inner milieu. This physiological situation in this phase of development is different from the remaining development for marine as well as for freshwater species: 'Vor diesem Hintergrund läßt sich vermuten, daβ das Dorsalorgan eine besondere Rolle bei der Volumenvergrößerung und damit der embryonalen Häutung spielt' (Meschenmoser 1996: 254). Pyatakov (1926: 168, 170) described the identical mechanism for the larval dorsal organ of *Argulus foliaceus* (Branchiura): 'Imbibing water and swelling up the second cuticle pushes the halves of the egg-shell more and more asunder. The embryo straightens itself and at last bursts the second cuticle, escaping as a free-swimming larva.'

Coincidences in time: The osmoregulatory cephalothoracic dorsal organ of the harpacticoid copepods is present from the first nauplius onward (the only known exception is *Bryocamptus pygmaeus*). This dorsal organ of harpacticoid copepods is identical in time or developmental stage with the 'embryonic' dorsal organ of the Peracarida. Because Meschenmoser (1996) never used the terms nauplius or metanauplius while describing the dorsal organ of the different stages, it has given the wrong impression that non-embryonic and embryonic dorsal organs are completely different objects. In fact, all larval stages of the Peracarida are passed through inside the egg. The Peracarida have direct development with no free larval stages (Gruner 1993). Meschenmoser designated the whole development inside the egg until hatching as 'embryonic stages'. Fioroni (1980) stated that the 'embryonic' dorsal organs of the Malacostraca (Phyllocarida, Syncarida, Decapoda, Pancarida and Peracarida) are structures that also occur in the nauplius and metanauplius.

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