JOURNAL OF CRUSTACEAN BIOLOGY, 21(3): 631–639, 2001

ULTRASTRUCTURAL FEATURES OF THE FUNNEL OF *GAMMARUS OCEANICUS* (AMPHIPODA)

Kevin Halcrow

Biology Department, University of New Brunswick, Saint John, New Brunswick, Canada E2L 4L5
(e-mail: Halcrow@unbsj.ca)

A B S T R A C T

The funnel of *Gammarus oceanicus* extends as a fold of the foregut wall into the midgut lumen. It comprises an anterior segment, to which both foregut epithelium and cuticle contribute, and a posterior segment that lacks the epithelium. The latter segment consists of two layers of foregut cuticle that are in close contact and extend distally beyond the point where the foregut epithelium turns back on itself; contact between the two cuticular layers develops as the epithelium retracts from between them, in postmolt. Light microscopy does not consistently distinguish between the funnel's posterior segment and the peritrophic membrane found within mid- and hindguts; however, their ultrastructural organizations are distinctly different, and an origin of peritrophic membrane from the funnel of some amphipods, as suggested in the literature, is not the case for *Gammarus oceanicus.* Scattered within the posterior segment cuticle are clusters of short, highly curved, electron-lucent rods. Such features have not been described from other crustacean cuticles. However, rather than being novel structures, they are interpreted as artifacts produced by sectioning of the abundant chitin macrofibrils of the cuticle.

Several accounts of the amphipod digestive tract note the presence of folds of the gut wall that project posteriorly from the general vicinity of the foregut/midgut junction into the midgut lumen (Martin, 1964; Kanneworff and Nicolaisen, 1969; Keith, 1974; Sheader and Evans, 1975; Icely and Nott, 1984; Coleman, 1991, 1992). The entire tubelike structure formed by these folds, which are seen in the same gut region of some other peracarid crustaceans (Metillo and Ritz, 1994; de Jong and Casanova, 1997; Kobusch, 1998), is generally called the funnel.

In *Corophium volutator* (Pallas), the funnel wall consists of an epithelium folded back on itself and therefore is double layered; the apical surface of the epithelium is covered by cuticle so that inner and outer surfaces of the funnel are cuticle lined (Icely and Nott, 1984). A similar arrangement is present in the funnel of *Parathemisto gaudichaudi* (Guerin) (see Sheader and Evans, 1975).

In *Caprella equilibra* Say and *Cyamus boopis* Lütken, epithelial folds similar to those seen in *C. volutator* and *P. gaudichaudi* appear to continue into the midgut lumen as peritrophic membrane (PM; Keith, 1974); a delicate acellular PM surrounds the mid- and hindgut contents of many arthropods (Spence, 1991). However, in neither of the latter two species does the funnel extend posteriorly as PM.

The ultrastructural study reported here, which gives details of the funnel's constitution and development in *Gammarus oceanicus* Segerstråle, was prompted by some preliminary light microscope observations that suggested that this funnel is composed, at least in part, of acellular material continuous with the PM.

MATERIALS AND METHODS

Adult and juvenile *Gammarus oceanicus* were collected and maintained as described elsewhere (Halcrow, 1996). Before being dissected, animals were immobilized in 0.1% tricaine methane sulphonate in cold sea water. For light microscopy (LM), the body cavity of each adult was opened anteriorly by excising the head just posterior to the eyes; pereiopods and all pleonal segments were also removed. Juveniles were bisected at the pereion/pleon junction. For transmission electron microscopy (TEM) of adults, much of the digestive tract's length was exposed by two curving lateral incisions through the dorsal body surface; the region containing the junction between fore- and midgut was excised and dropped into fixative.

Tissue was fixed for LM in aqueous Bouin and embedded in Paraplast; sections were cut at 6–8 μm and stained according to Cason's procedure (Kiernan, 1992). Tissue was processed and sectioned for TEM as described elsewhere (Halcrow and Powell, 1992). Serial thin sections were mounted on slot grids according to Bozzola and Russell (1992). Three-dimensional reconstructions from serial sections were produced by the method of Pignot-Paintrand and Bressac (1992).

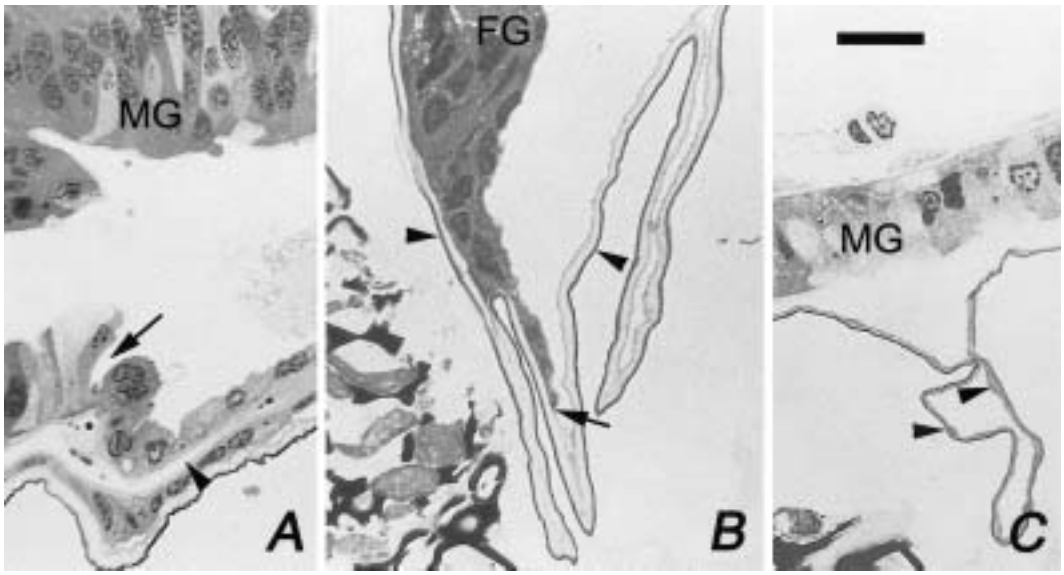

Fig. 1. Light micrographs of anterior and posterior segments of the funnel. The animal's anterior is to the left in A and C, and to the top in B. A, Part of anterior funnel segment near junction of foregut and midgut epithelia; arrow indicates "cleft" at junction of midgut (to left of arrow) and outer foregut layer of the funnel (to right). A narrow haemocoelomic space (arrowhead) separates outer and inner integumental layers of the ASF; the inner layer's epicuticle is distinctly stained. B, Junction between anterior and posterior funnel segments. Arrowheads identify epicuticle of inner cuticle layer. Epicuticle at left is part of funnel inner integument; epicuticle at right is part of posterior (acellular) funnel segment. Funnel inner epithelial layer becomes outer funnel layer where their respective cuticles merge (arrowhead), forming the funnel's posterior segment. The outer cuticle may be seen indistinctly at the surface of the funnel outer epithelium, near arrowhead. C, Part of funnel's posterior segment. Folding shown here is common near the origin of the posterior segment and much less common more distal to this. Patches of stained material are present within the funnel cuticle (arrowheads). FG, foregut epithelia (two layers); MG, midgut epithelium. Scale bar represents 25 μm (A) and 20 μm (B, C).

RESULTS

In *Gammarus oceanicus*, the posterior region of the foregut (FG) epithelium folds back on itself before joining to the midgut (MG) epithelium. The inner and outer layers of this epithelial fold, facing the FG lumen and MG surface respectively, are separated by a narrow haemocoelic space (Fig. 1). Each layer carries to its outside a layer of cuticle. Although thicknesses of inner and outer epithelia are similar, the layer of cuticle overlying the inner FG epithelium is substantially thicker than that associated with the outer layer.

The entire double integumental structure comprises that part of the funnel described here as the anterior segment of the funnel (ASF). The posterior segment (PSF) is acellular and develops as a posterior extension of each layer of cuticle beyond the point where the epithelium folds back on itself. At this point, inner and outer cuticular layers previously separated by the epithelia and haemocoel become associated with each other and project into the MG lumen as a single layer about 2 μm thick.

In light micrographs (Fig. 1), a densely stained layer is visible at the surface of the funnel's inner layer. Other, less densely stained material occurs as discrete patches within the inner layer; these patches, often associated with localized cuticular thickenings, are restricted to the inner layer where this is part of the PSF.

In transmission electron micrographs, each cuticular layer is seen to be subdivided into an electron-dense outer layer overlying a thicker, electron-lucent inner layer (Fig. 2). At higher magnification, the dense layer at the surface of the inner cuticular layer is resolved as an outer multilaminar region, about 30 nm thick, and an inner amorphous layer, about 250 nm thick (Fig. 3). In location, appearance, and relative thicknesses, these components are comparable to, respectively, outer

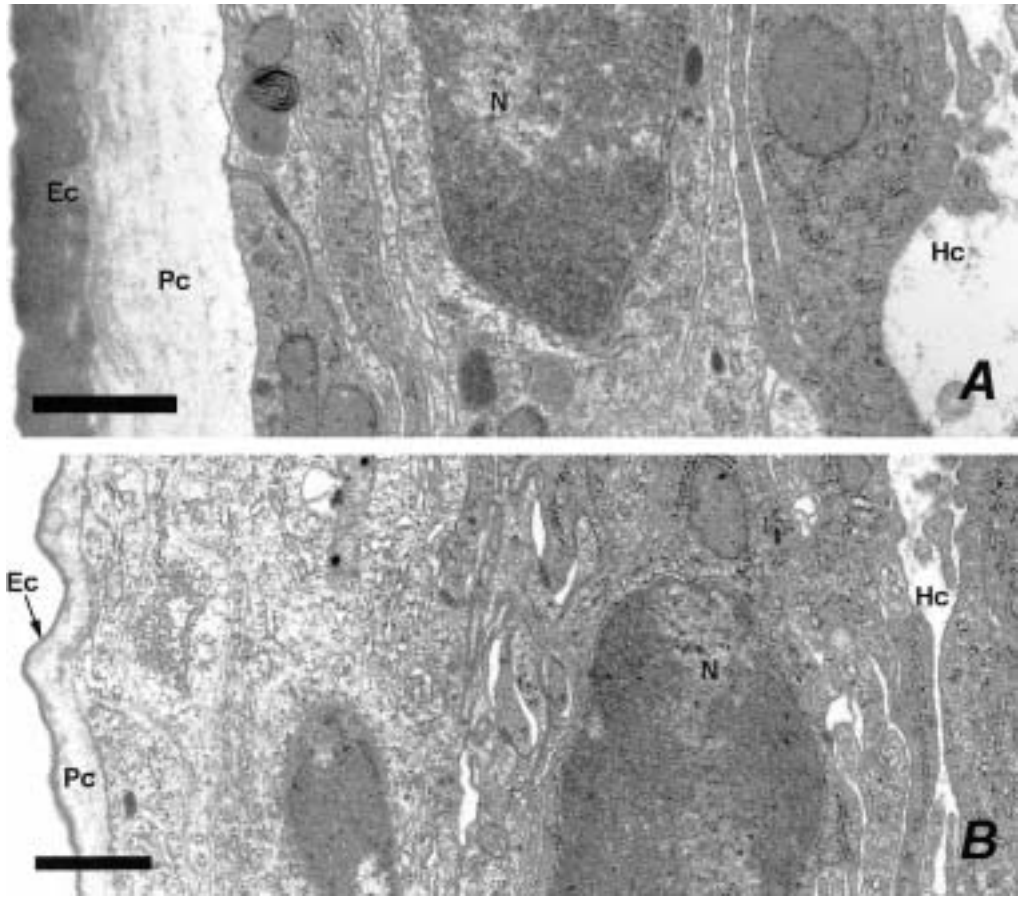

Fig. 2. Transmission electron micrographs of the inner (A) and outer (B) integumental layers of the funnel anterior segment. Ec, epicuticle; Pc, procuticle; N, nucleus; Hc, haemocoel. Scale bar in each micrograph represents 1 μm.

and inner epicuticles of the general body cuticle. Only an outer epicuticle is present in the outer layer of the funnel cuticle.

The multilaminar character of the outer epicuticle is clearly seen in perpendicular sections of PSF cuticle (Fig. 3A). However, such sections do not reveal as obviously the lamellations within the material that separates the two epicuticles of the PSF. Tangential sections (Fig. 3B) of this material show distinctly its multilamellate nature; at least three lamellae may be distinguished. Sections taken at the edge of the epithelial fold show that these distinct lamellae are derived from the inner layer's contribution to the PSF (Fig. 4). The order seen indistinctly in the outer cuticle layer's contribution (Fig. 3B) suggests that the arcuate-pattern orientation here is different from that of the inner layer. This difference in orientation arises as a direct consequence of the fact that both layers of cuticle originate from the same epithelial layer, folded back on itself.

Where the PSF cuticle folds back on itself (at the distal end of the funnel), the inner layer is thicker here than anywhere else in the funnel. As result of this, and of the separation of the two layers often seen here, the end of the PSF has an irregularly inflated appearance in section (not illustrated). Two other features of the thickened inner layer are noteworthy. Pore canals are clearly visible within the lamellae (Fig. 5) but are absent from any other part of the funnel. In some regions within the uppermost lamella, small clusters of short, curved, rodlike objects with electron-dense surfaces and electron-lucent interiors are visible. These objects are distributed irregularly along the length of the PSF, in which they are clearly visible (Fig. 6); they

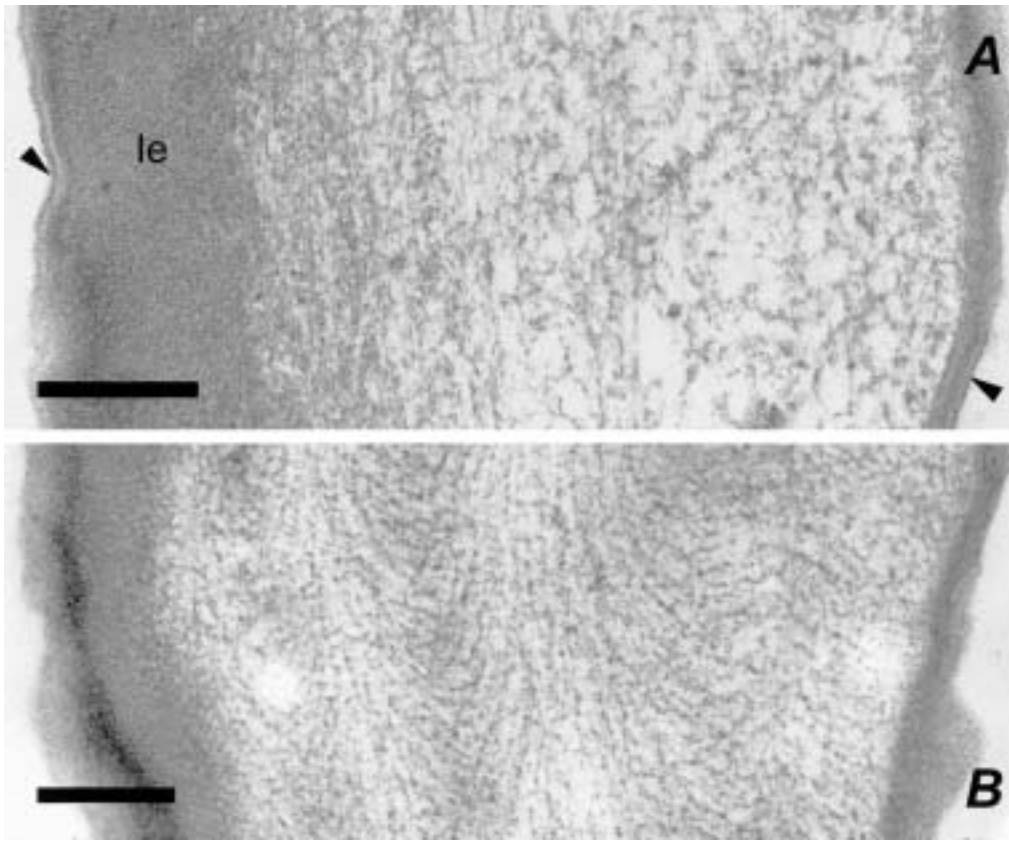

Fig. 3. The two cuticular layers of the funnel posterior segment; inner and outer layers merge imperceptibly. A, Near-perpendicular section showing the multilaminate outer epicuticle of both layers (arrowheads). An inner epicuticle (Ie) is distinct in the inner layer, but absent from the outer layer. B, Tangential section showing arcuate patterns within the procuticle of the inner layer. Scale bar in each micrograph represents 2 μm.

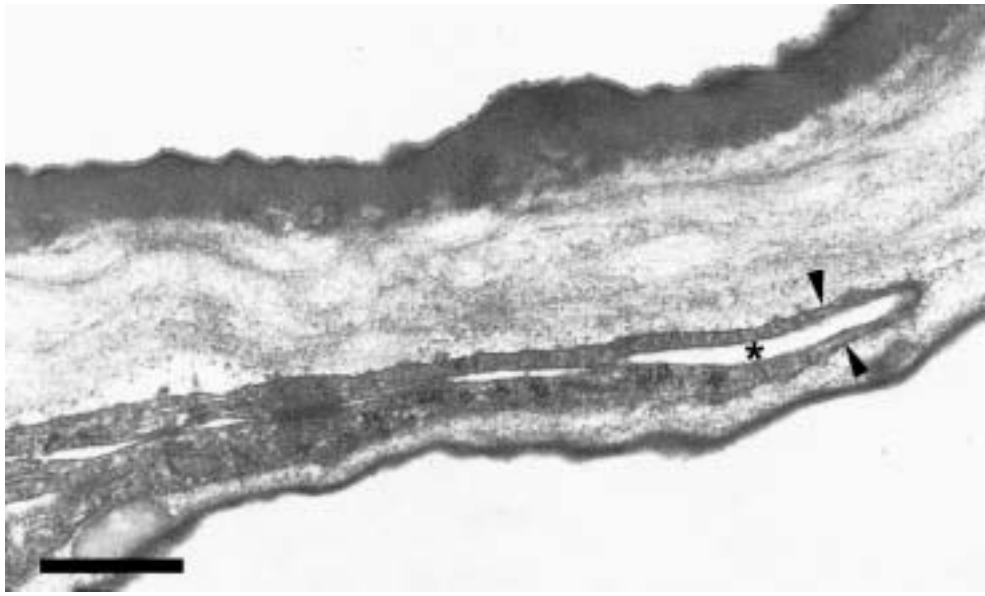

Fig. 4. Termination of funnel anterior segment, where foregut epithelium folds back on itself and the two cuticular layers merge, forming the posterior segment. Arrowheads indicate epithelial cytoplasm folded back and enclosing a haemocoelomic space (asterisk). Scale bar represents 0.5 μm.

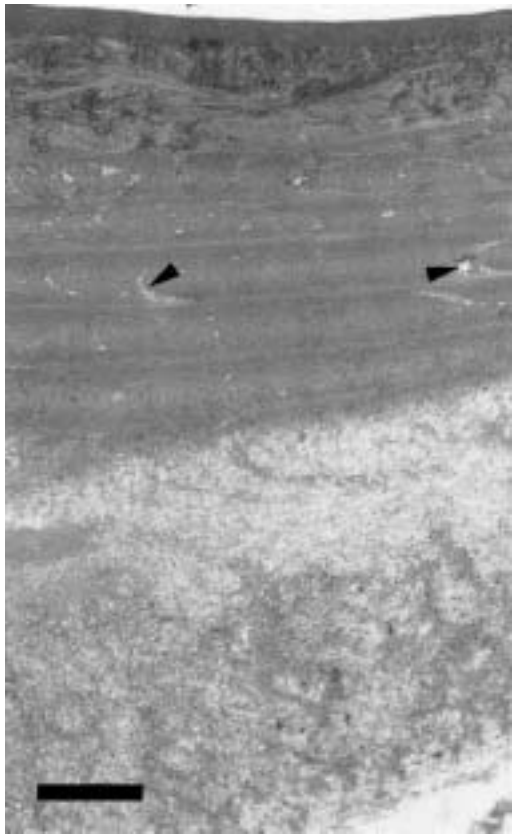

Fig. 5. Section through distal termination of funnel posterior segment. The procuticle of the inner layer is distinctly lamellate and contains pore canals (arrowheads); a few small clusters of curved chitin rods are just visible within the procuticle near the inner epicuticle. Scale bar represents 1 μm.

are seen through the light microscope as the denser patches noted above. They have not been seen in the ASF. Although it might be supposed that the clusters are part of some three-dimensional conformation within the cuticle, analysis of nine consecutive thin sections taken through one cluster did not reveal any structural continuity within the series.

The widths of the rods fall in the range 17–47 nm; chitin macrofibrils indistinctly seen in cross section adjacent to rods have similar widths (Fig. 6A). A reasonable assumption is that the rods are short lengths of macrofibrils, produced then dislodged by sectioning.

Some rods appear to be branched. However, in several sections to which goniometric tilting was applied, the "branching" was seen to arise from overlapping of separate rods (Fig. 6B).

In premolt individuals, funnel cuticle forms at the surface of highly folded apical plasma membranes of foregut epithelia (Fig. 7). Although not conspicuous, microvilli are visible on these membranes; some microvilli contain the electron-dense plaques characteristically seen at the tips of such microvilli on arthropod epithelial cells actively depositing new cuticle.

Through the light microscope, PSF and PM can be confused for each other where both occur; they have similar thicknesses and both lie close to the midgut's contents. However, PM is a less ordered and less substantial material. Moreover, it contains a periodicity that corresponds to the spacing of midgut microvilli (Fig. 8), as reported for other arthropods (e.g., Harper and Hopkins, 1997).

Discussion

In *G. oceanicus,* funnel cuticle is formed, together with other cuticular layers (general body surface, foregut lining), during pre- and postmolt. Produced from the highly folded surface of posterior foregut epithelium, the funnel reaches its final length by epithelial unfolding. As the epithelium unfolds, it retracts partially from its cuticular covering, creating the funnel's cellular (ASF) and acellular (PSF) regions. At ecdysis it is shed with the rest of the foregut lining then replaced by pre- and postmolt synthesis of the next molt cycle. Until this replacement, the funnel serves as a conduit for indigestible materials into the midgut, where they are enclosed by PM that is secreted there.

The funnel and PM are thus different entities in *Gammarus oceanicus,* as they also appear to be in the amphipods *Corophium volutator* and *Parathemisto gaudichaudi* (see Icely and Nott, 1975; Sheader and Evans, 1975) but possibly not in *Caprella equilibra* and *Cyamus boopis,* in which species the funnel is suggested to be continuous with PM (Keith, 1974).

Several amphipod species have been reported to egest faecal pellets in which a compacted mass of waste particles is enveloped by a tube of PM (Peters, 1968; Georgi, 1969; Lautenschlager *et al.,* 1978; Hansen and Peters, 1997/98); some of these reports identify the presence of chitin in PM. Peritrophic membrane synthesis in these species clearly occurs much more frequently than molt-related synthesis of new cuticle, a situation that is compatible with funnel and PM being separate entities. In contrast, it is not immediately apparent how continuity of funnel and

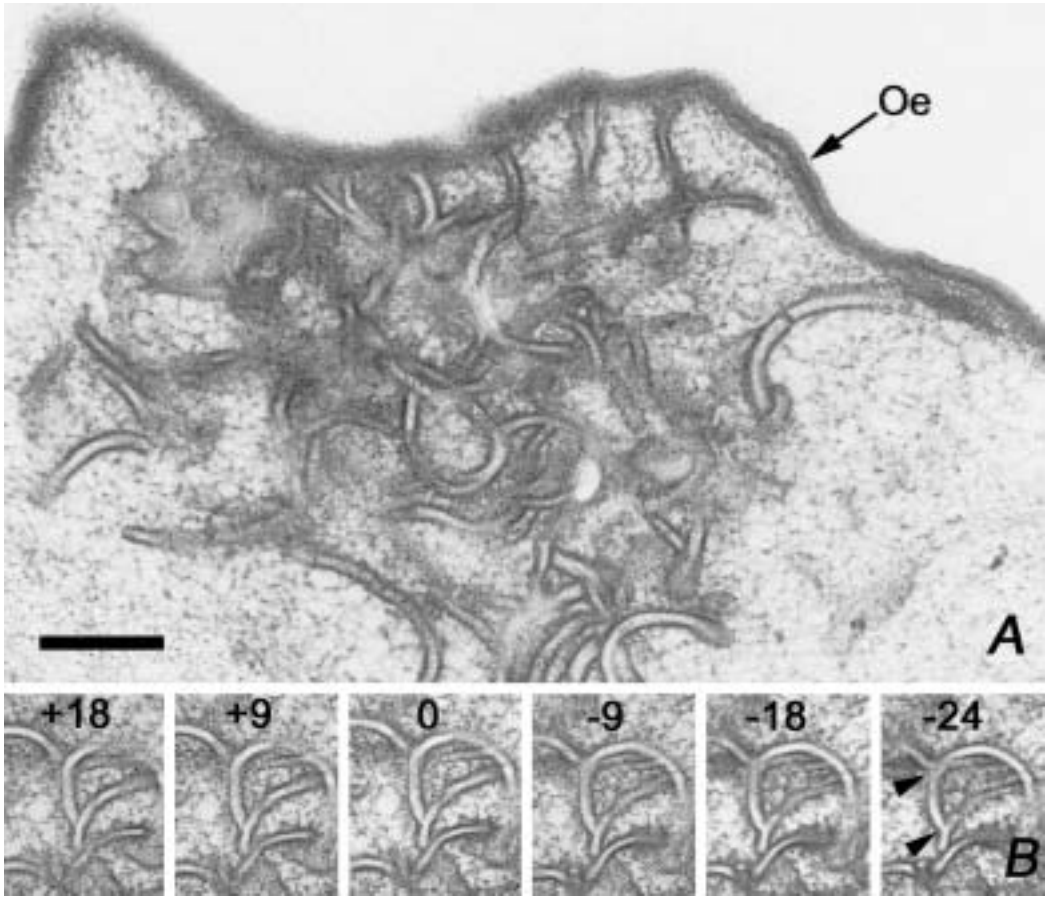

Fig. 6. A, Cluster of displaced chitin macrofibrils in procuticle of inner layer of funnel posterior segment. Oe, multilaminate outer epicuticle. Scale bar represents 200 nm. B, Goniometric series showing that apparent branching of macrofibrils (arrowheads) develops from overlapping of macrofibrils. The section was tilted, at the angles indicated, in an axis parallel to the the figure's vertical sides. Scale bar of A represents 185 nm in B.

PM could permit production of the latter more frequently than molt-related funnel replacement. The situation in those species for which continuity of funnel and PM has been suggested should be reexamined.

The organization of the PSF as two cuticular layers in direct contact and not separated by intervening epithelia and haemocoel is unusual. The only comparable structure described in the literature is the insect wing (Richards, 1951). Although a diagram of the branchiopod *Ophryoxus gracilis* Sars (see Martin, 1992) suggests that epidermal cells might not extend into the ventral carapace edge, such cells are present here (personal communication, Prof. G. Fryer). In the developing insect wing, epithelial cells "may die, leaving the two closely appressed cuticles seemingly cemented together" (Richards, 1951: 243). Although the two cuticular layers of the PSF in *Gammarus* are rarely seen to be separate, except at its posterior terminus, there is no evidence to suggest that the layers' close association is sustained by anything other than the manner in which the contact develops. In the absence of any force sufficient to separate the two layers, they remain as generally seen.

Close contact between inner and outer cuticular layers in the posterior segment of the funnel exists partly because similar lengths of these layers contribute to the PSF. The two epithelial layers are unlikely to create, in premolt, precisely the same lengths of cuticle, even if their apical surfaces were not extensively folded. However, any discrepancy between the lengths would only be apparent after epithelial retraction and funnel unfolding

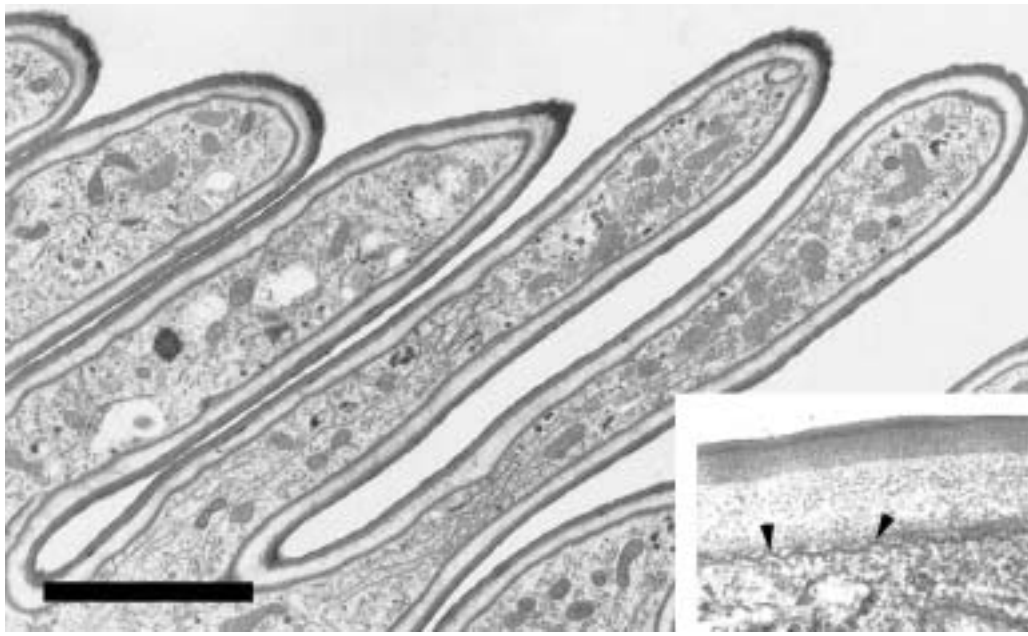

Fig. 7. Section through new funnel inner cuticle forming at the surface of the FG epithelium in premolt individual. The epithelial apical membrane is highly folded; it bears small rounded projections, some of which have electron-dense tips (inset, arrowheads). Scale bar represents 5 μm (550 nm in inset).

approach completion. Inspection of Fig. 1B reveals unequal lengths of the two cuticular layers; the outer layer is still attached to its underlying epithelium but, anterior to the point where the two layers become associated, a folded length of inner layer is seen to have detached from its epithelium. Neither of the two consequences of this situation—unfolding of the fold (accompanied by breakage of the much thinner outer layer?) or retention of the fold—would be likely to compromise the integrity and function of the funnel.

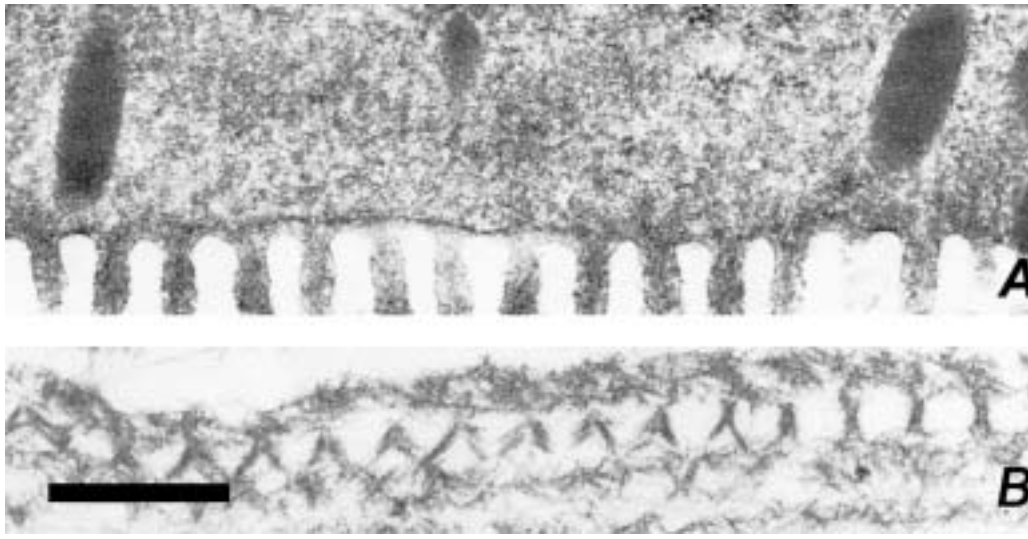

Fig. 8. Sections of midgut epithelial surface (A) and peritrophic membrane (B), aligned to demonstrate correspondence between spacing of midgut microvilli and of periodicity within peritrophic membrane. Scale bar represents 250 nm in both micrographs.

Chitin crystallites of arthropod integument are consistently described as straight, rodlike objects (Neville, 1975, 1993), so the presence of short lengths of curved macrofibrils in PSF is unexpected. The curved arcs seen in oblique sections of arthropod integument are almost universally accepted to be illusory; they are generated in the section by the aligned assemblages of short, straight lengths of chitin rods arranged, within successive horizontal laminae, at consistently changing orientations to each other, as first proposed by Bouligand (1965). Many of the macrofibril fragments in *Gammarus* PSF have radii of curvature so small that it is difficult to reconcile the apparent shape of the whole macrofibril with conventional views of cuticle organization. However, in a more recent discussion of other artifacts produced in cuticle sections (Bouligand, 1986), it has been suggested how rodlike objects might be distorted by sectioning such that they become curved at both ends by two passes of the microtome knife. This almost certainly is the origin of the curvatures of the macrofibril fragments of Fig. 6. A close similarity between fragment length and section thickness is not to be anticipated, given the different orientations of macrofibrils in the unsectioned cuticle. Lengths of the shortest fragments, presumably derived from macrofibrils lying more nearly perpendicular to the block face, are, however, similar to section thickness (pale gold, about 100 nm).

Acknowledgements

I thank Andrew Wear (UNBSJ) and Dr. C. V. L. Powell (Electron Microscopy Unit, Saint John Regional Hospital) for technical assistance, Wilfred Morris (UNBSJ) for advice on graphics manipulation, two anonymous reviewers for their constructive comments, and the University of New Brunswick for financial support.

Literature Cited

Bouligand, Y. 1965. Sur une architecture torsadée répandue dans de nombreuses cuticules d'Arthropodes.—Comptes rendus de l'Academie des Sciences Paris 261: 3665–3668.

———. 1986. Theory of microtomy artefacts in arthropod cuticle.—Tissue and Cell 18: 621–643.

Bozzola, J. J., and L. D. Russell. 1992. Electron Microscopy. Jones and Bartlett, Boston.

Coleman, C. O. 1991. Comparative fore-gut morphology of Antarctic Amphipoda (Crustacea) adapted to different food sources.—Hydrobiologia 233: 1–9.

———. 1992. Foregut morphology of Amphipoda (Crustacea). An example of its relevance for systematics.—Ophelia 36: 135–150.

de Jong, L., and B. Casanova. 1997. Comparative morphology of the foregut of three *Eucopia* species (Crustacea, Mysidacea, Lophogastrida).—Journal of Natural History 31: 389–402.

Georgi, R. 1969. Feinstruktur peritrophischer Membranen von Crustaceen.—Zeitschrift für Morphologie der Tiere 65: 225–273.

Halcrow, K. 1996. Dynamics of shape and location in epidermal cells of *Gammarus oceanicus* (Amphipoda).—Journal of Crustacean Biology 16: 35–44.

———, and C. V. L. Powell. 1992. Ultrastructural diversity in the pore canal systems of amphipod crustaceans.—Tissue and Cell 24: 417–436.

Hansen, U., and W. Peters. 1997/98. Structure and permeability of the peritrophic membranes of some small crustaceans.—Zoologischer Anzeiger 236: 103–108.

Harper, M. S., and T. L. Hopkins. 1997. Peritrophic membrane structure and secretion in European corn borer larvae (*Ostrinia nubilalis*).—Tissue and Cell 29: 463–475.

Icely, J. D., and J. A. Nott. 1984. On the morphology and fine structure of the alimentary canal of *Corophium volutator* (Pallas) (Crustacea: Amphipoda).—Philosophical Transactions of the Royal Society of London B 306: 49–78.

Kanneworff, E., and W. Nicolaisen. 1969. The stomach (foregut) of the amphipod *Bathyporeia sarsi* Watkin.—Ophelia 6: 211–229.

Keith, D. E. 1974. A comparative study of the digestive tracts of *Caprella equilibra* Say and *Cyamus boopis* Lütken (Amphipoda, Caprellidea).—Crustaceana 26: 127–134.

Kiernan, J. A. 1992. Histological and Histochemical Methods: Theory and Practice. Second edition. New York, Pergamon Press.

Kobusch, W. 1998. The foregut of the Mysida (Crustacea, Peracarida) and its phylogenetic significance.—Philosophical Transactions of the Royal Society of London B 353: 559–581.

Lautenschlager, K. P., N. K. Kaushik, and J. B. Robinson. 1978. The peritrophic membrane and faecal pellets of *Gammarus lacustris limnaeus* Smith.—Freshwater Biology 8: 207–211.

Martin, A. L. 1964. The alimentary canal of *Marinogammarus obtusatus* (Crustacea, Amphipoda).—Proceedings of the Zoological Society of London 143: 525–544.

Martin, J. W. 1992. Branchiopoda. Pp. 25–224 *in* F. W. Harrison and A. G. Humes, eds. Microscopic Anatomy of Invertebrates. Vol. 9. Wiley-Liss, New York.

Metillo, E. B., and D. A. Ritz. 1994. Comparative foregut functional morphology of three co-occurring mysids (Crustacea: Mysidacea) for south-eastern Tasmania.—Journal of the Marine Biological Association of the United Kingdom 74: 323–336.

Neville, A. C. 1975. Biology of the Arthropod Cuticle. Springer-Verlag, New York.

———. 1993. Biology of Fibrous Composites. Cambridge University Press, Cambridge.

Peters, W. 1968. Vorkommen, Zusammensetzung und Feinstruktur peritrophischer Membranen im Tierreich.—Zeitschrift für Morphologie der Tiere 62: 9–57.

Pignot-Paintrand, I., and C. Bressac. 1992. Rapid three-dimensional reconstruction at the light microscope level and a technique for re-embedding the same semithin sections for electron microscopic examination.—Biotechnic and Histochemistry 67: 55–57.

Richards, A. G. 1951. The Integument of Arthropods. University of Minnesota Press, Minneapolis.

Sheader, M., and F. Evans. 1975. Feeding and gut structure of *Parathemisto gaudichaudi* (Guerin) (Amphipoda, Hyperiidea).—Journal of the Marine Biological Association of the United Kingdom 55: 641–656.

Spence, K. 1991. Structure and physiology of the peritrophic membrane. Pp.78–93 *in* K. Binnington and A. Retnakaran, eds. Physiology of the Insect Epidermis. CSIRO, Australia.

RECEIVED: 3 June 2000.
ACCEPTED: 10 January 2001.