Ultrastructure of the heart and pericardium of an aplacophoran mollusc (Neomeniomorpha): evidence for ultrafiltration of blood

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SUMMARY

Transmission electron microscopy of the auricular wall in an undescribed mesopsammic mollusc of the genus *Meiomenia* (Class Aplacophora, Subclass Neomeniomorpha) has revealed podocytes interposed between the haemocoel and the pericardial coelom. These cells comprise the auricular epicardium and overlie a loose inner myocardium. Podocytes characterize sites of blood ultrafiltration in the Polyplacophora and most conchiferan molluscan classes, and are here described from the Aplacophora for the first time. This evidence for ultrafiltration suggests an excretory role for the neomenioid aplacophoran pericardial coelom and supports coelomic ultrafiltration of blood as a plesiomorphic molluscan trait.

1. INTRODUCTION

Views regarding the evolutionary origin of the Phylum Mollusca have centred around the question of coelomate against acoelomate ancestry, with proposed sister groups ranging from spiralian eucoelomate phyla (Götting 1980; Wingstrand 1985; Ghiselin 1988; Eernisse *et al.* 1992; Scheltema 1993) to the Acoelomorpha (Salvini-Plawen 1985; Willmer 1990). Naturally, the interpretation of origin, fine structure and function of the molluscan coelom has been central to this debate, but the lack of data on associated characters for the smaller classes has impeded evolutionary and phylogenetic analyses of molluscan taxa. In critical need of further study is the Class Aplacophora, considered to be among the earliest lineages of the phylum.

It has been shown for most molluscan classes that the mesodermally derived coelom is restricted to three organs: the gonad, pericardium and kidneys (Raven 1966; Moor 1983). These coelomic derivatives remain in communication with one another to varying degrees in adult members of different molluscan taxa (for review see Martin 1983). This organ system has received considerable attention from the functional perspective with regard to its role in excretion and circulation (for review see Martin (1983) and Andrews (1988)). As has been demonstrated experimentally in most conchiferan classes, urine is formed initially by ultrafiltration of blood through the heart wall, into the pericardial cavity (Gastropoda (Harrison (1962); Little (1965); Andrews & Taylor (1988)); Cephalopoda (Harrison & Martin (1965); Martin & Aldrich (1970)); Bivalvia (Jones & Peggs (1983), Hevert (1984))). The blood ultrafiltrate is then passed to the kidney lumen by way of renopericardial ducts, where it is modified by absorption and secretion before being released from the body (for review see Martin 1983).

The fine structural details of this system have been presented in several reports, with some general features of the molluscan excretory system emerging, at least as related to the larger (more diverse) classes. The ultrafiltration of blood is associated with podocytes, which are generally found interposed between the haemocoel and coelomic cavities in the metanephridial systems of coelomates (Kümmel 1973; Farquhar 1981; Ruppert & Smith 1988; Bartolomaeus & Ax 1992). In molluscs, podocytes are found in the heart wall or associated structures of all classes examined to date, as described by Pirie & George (1979: Bivalvia), Økland (1980: Polyplacophora), Andrews (1981: Gastropoda), Schipp & Hevert (1981: Cephalopoda) and Reynolds (1990: Scaphopoda). Podocytes possess numerous branches (or pedicels) between which uniform spaces (or ultrafiltration slits) provide a pathway for an ultrafiltrate of the blood to pass from the haemocoel to the pericardial cavity. The basal lamina, underlying the podocytes and ultrafiltration slits, has been demonstrated to be the functional ultrafilter (Andrews 1981; Farquhar 1981; Morse 1987).

Unlike the larger molluscan classes, no excretory process involving coelomic derivatives has been demonstrated experimentally in the Aplacophora, nor have fine structural studies provided insights to physiological function of the aplacophoran pericardial coelom (Salvini-Plawen 1985, 1988). The only experimental data on excretion in the Aplacophora are those of Baba (1940), who demonstrated that amoebocytes release

wastes through the midgut epithelium into the midgut lumen. Excretion through the epidermal papillae has been conjectured from histological sections (see Salvini-Plawen 1985). The absence of data on pericardial function in the aplacophoran groups has precluded the confirmation of symplesiomorphic molluscan traits associated with the circulatory and excretory systems. This deficiency has hindered speculation on the original nature of the molluscan excretory system (see, for example, Salvini-Plawen 1985; Haszprunar 1992), and accommodated the view that ultrafiltration of blood to the pericardial coelom may have been secondarily derived within the Mollusca (Salvini-Plawen 1985).

This paper presents the first ultrastructural detail of the heart and pericardium in a neomenioid (Subclass Neomeniomorpha; = Class Solenogastres, *sensu* Salvini-Plawen) aplacophoran mollusc, an undescribed member of the genus *Meiomenia*. This study provides the first direct evidence for ultrafiltration of blood across the heart wall into the pericardial coelom of the Class Aplacophora, and thus supports coelomic ultrafiltration as a plesiomorphic molluscan trait.

2. MATERIALS AND METHODS

Specimens of *Meiomenia* sp., approximately 150 μm × 1 mm in size, were collected by dredging subtidal shell hash from two sites 6 miles due east of Fort Pierce, Florida (27° 29.13′ N, 80° 11.65′ W, and 27° 26.43′ N, 80° 14.15′ W). Meiofauna were removed from the sediment samples by an elution process, and live neomenioid aplacophorans were sorted out and processed for electron microscopy. Before fixation, 7.5% magnesium chloride was added drop by drop to the seawater in which the animals were held until they were relaxed; the specimens were then fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate at 7.4 pH, adjusted to 1250 mosm† with 0.1 M NaCl and 0.27 M sucrose. Decalcification was achieved by using a 1:1 mixture of 10% EDTA and primary fixative. After a 0.2 M sodium cacodylate buffer rinse, specimens were post-fixed in 1% osmium tetroxide in a 0.2 M sodium cacodylate and 0.3 M NaCl buffer solution. Specimens were dehydrated in an ethanol series and transferred to propylene oxide before embedding in Epon 812 resin. Grey–silver sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined in a JEOL 100-B, Philips EM-300 or a JEOL 1200EX II transmission electron microscope.

3. RESULTS

Cross-sectional views of the posterior region of *Meiomenia* sp. show the relative positions of the auricle, pericardium, gametoducts and copulatory spicule sacs (figure 1). The single auricle is thin walled, located dorsally, and lies within a spacious pericardial coelom. The upper and lower gametoducts form a U-shaped pathway between the pericardial coelom and the exterior; the upper gametoduct, confluent with the pericardial cavity more posteriorly, joins the lower gametoduct in the region of the heart (figure 1).

The auricle is an invagination of the dorsal wall of the pericardium, i.e. the pericardial epithelium is continuous with that of the auricular epicardium. The most ventral cells of this epicardium closely appose cells of the ventral floor of the pericardial cavity, dividing the pericardial cavity and isolating the lateral auricular walls (figure 1). The auricular epicardium is comprised of several flattened cells (figure 2), each cell extending from the dorsal to the ventral wall of the pericardial cavity. These cell bodies are thrown into folds, each enclosing a portion of haemocoel (figures 2 and 3) above the underlying myocardium (figure 2).

High magnification of the cellular extensions (figures 3 and 4) shows them to be podocytes, the pedicels forming intervening ultrafiltration slits. The minimum slit width observed is approximately 22 nm, and in some instances this gap appears to be bridged by electron-opaque strands or slit diaphragms (figure 4). The pedicels (minimum observed width = 80 nm, height = 30 nm) are underlain by a basal lamina (figures 3 and 4) that is continuous with that surrounding the muscle cells of the auricular myocardium (figure 2), and therefore completely lines the haemocoel.

In the myocardial musculature (figure 2), dense bodies are scattered among the filaments, whereas mitochondria and nuclei are located peripherally. There is little evidence of intercellular junctions, and muscle cells are often completely separated (figure 2).

The pericardial cavity is an extensive space that is divided for part of its length into two longitudinal ducts by the apposition of the ventral auricular epicardium and ventral pericardial wall (figure 1). Ciliated pericardial epithelium forms a tract of cilia that runs longitudinally along the dorsal pericardial wall on each side of the heart (figure 1); the cilia have a typical 9+2 microtubule complement (figure 5). The pericardium has extensive smooth musculature oriented circumferentially around the pericardial cavity (figures 5 and 6). As in the auricular myocardium, thick (approximately 30 nm) and thin (approximately 10 nm) myofilaments show no clear organization with respect to one another. The myofilaments show some alignment of Z-material only when in the contracted state (figure 6). The pleated cell membranes of the contracted muscle cells (figure 6) are created in part by attachment plaques which join the muscle cell membrane with myofilaments; the resulting sarcomeral length is approximately 2 μm. Dense bodies are frequently observed among myofilaments. The sarcoplasmic reticulum is poorly developed, with only a few smooth subsarcolemmal cisternae observed. Nuclei and mitochondria are located peripherally (figure 6).

4. DISCUSSION

In the Aplacophora, the lumina of the gonads, pericardium and gametoducts are interconnected, and the heart is contained within a spacious pericardial cavity. The cylindrical invagination of the dorsal pericardial wall forming the heart (Pruvot 1891; Heath 1911, 1918) is often divided by a constriction into paired or fused posterior auricles connected by a muscular auriculo-ventricular pore to the anterior ventricle (Salvini-Plawen 1985; Scheltema & Kuzirian

† One osmole contains one mole of osmotically active particles.

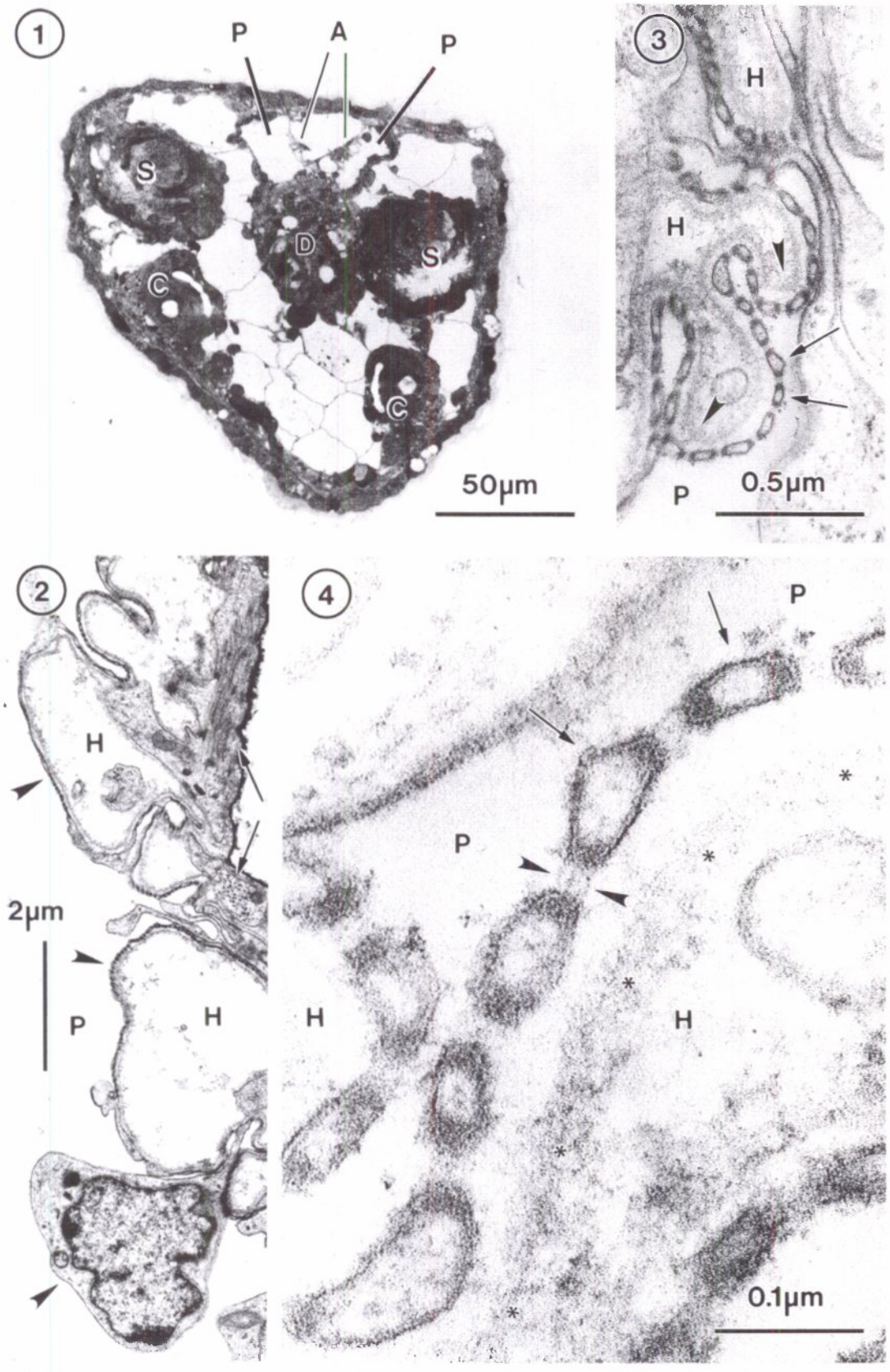

Figure 1. Light micrograph of a cross section through the posterior region of *Meiomenia* sp. D, digestive tract; C, copulatory spicule sacs; S, junction of upper and lower gametoducts; P, pericardial coelom (lines pass through dorsal walls of pericardium, where ciliated tracts are found); A, auricular walls.

Figure 2. Transmission electron micrograph of the auricular wall. Note the folded epicardial cell or podocyte (arrowheads) and underlying myocardial cells (arrows). H, haemocoel; P, pericardial coelom.

Figure 3. Transmission electron micrograph of the auricular wall, showing a portion of the podocyte cell body with pedicels (arrows). H, haemocoel; P, pericardial coelom; arrowheads, basal lamina.

Figure 4. Pedicels (arrows) and ultrafiltration slits of the podocytes. Note the electron-opaque strands, or slit diaphragms, between pedicels (arrowheads). H, haemocoel; P, pericardial coelom; asterisk, basal lamina.

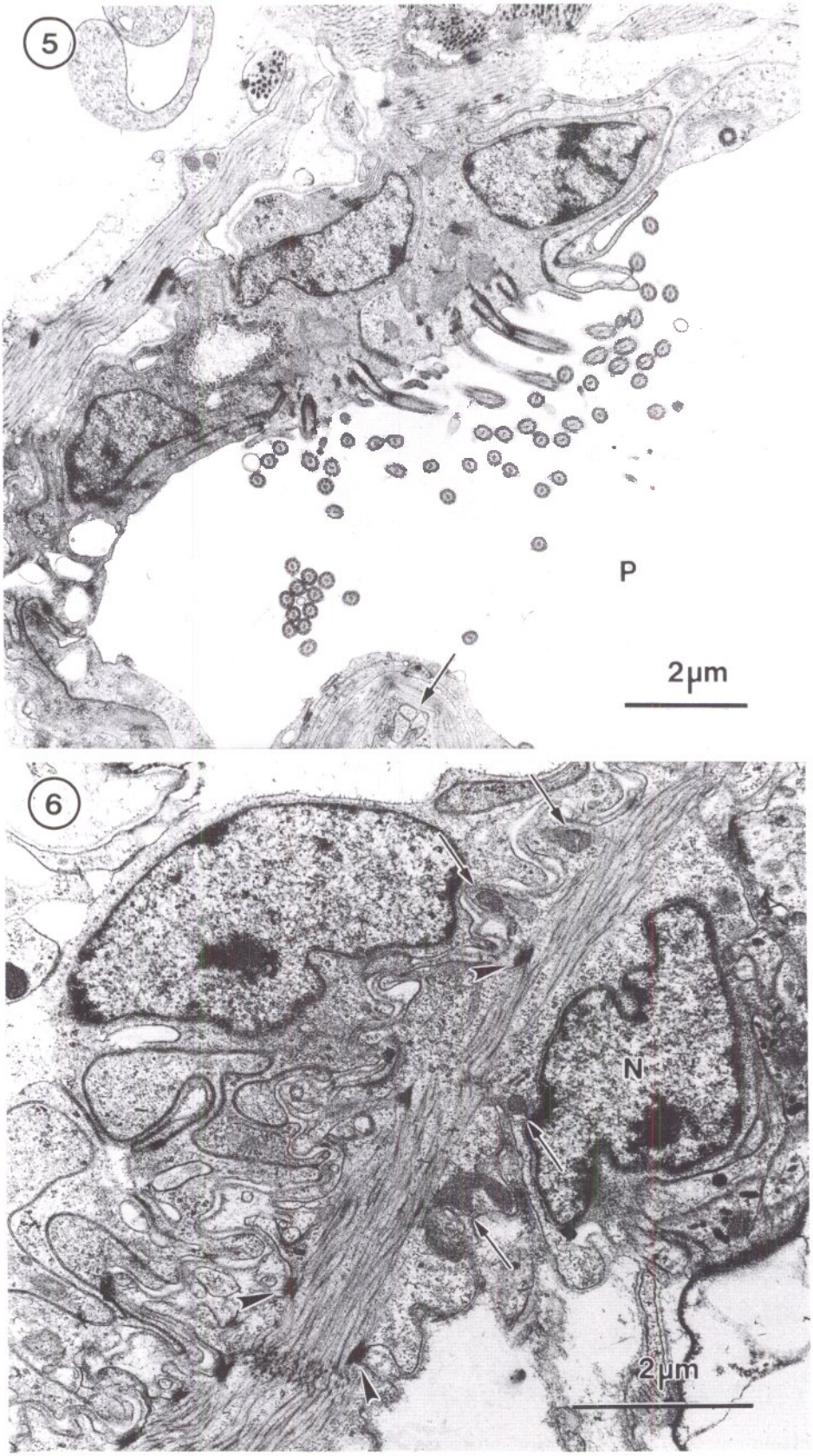

Figure 5. Ciliary tract of the dorsal pericardial wall in *Meiomenia* sp. (see also figure 1). P, pericardial coelom; arrow, pericardial musculature with adjacent nerve cells.

Figure 6. Musculature of the pericardium. N, nucleus; arrowheads, attachment plaques; arrows, peripheral mitochondria.

1991; Scheltema *et al.* 1993). The anatomy of the heart and pericardium in the meiofaunal *Meiomenia* species of this study is similar to that of most other neomenioid aplacophorans.

The arrangement of auricular and pericardial tissues in this *Meiomenia* species appears similar to that described in other molluscan classes: basal lamina lines the haemocoel, with which muscle cells are associated (a myocardium), and these are overlain by a coelomic epithelium (an epicardium) that includes podocytes (Økland 1980; see review by Wingstrand 1985). The surface area of the podocyte layer is increased by the folding of the cell bodies, enveloping haemocoelic spaces that are partitioned from the auricular lumen by the myocardial musculature, as seen, for instance, in prosobranchs (Andrews 1981, 1985) and bivalves (Meyhöfer *et al.* 1985). However, the level of organization of these anatomical structures is generally less complex in *Meiomenia* sp. than in the larger molluscan classes. For example, the slit diaphragms are not highly organized as in some gastropods (see, for example, Boer & Sminia 1976), and the podocytes are not developed into a secondary level of organization as in the pericardial glands of bivalves or branchial hearts of cephalopods (see, for example, Schipp & Hevert 1981; Meyhöfer *et al.* 1985), structures which increase the surface area over which ultrafiltration of blood can occur. Instead, the putative filtration site in *Meiomenia* sp. is reminiscent of the level of organization described in the Polyplacophora (Økland 1980, 1981; M. P. Morse, unpublished results) and Scaphopoda (Reynolds 1990). It is difficult at this stage, however, to interpret these similarities. Although Økland (1980) described the contractile pericardium and relatively poor development of podocytes, pedicels and myocardium as primitive, Reynolds (1990) suggested that the reduced scaphopod heart and pericardium is related to the derived, larger-scale reorganization of the scaphopod body form, which also entailed the loss of ctenidia. Lacking comparative data, it is difficult to surmise at this stage whether the simple form of heart and pericardial structures in this *Meiomenia* species is representative of neomenioid aplacophorans, or is instead a consequence of adaptation to the mesopsammic habitat.

Fine structural characteristics of the auricular myocardium and pericardial musculature in *Meiomenia* sp. are similar to those described for *Lepidopleurus asellus* and *Tonicella marmorea* by Økland (1980), although a greater development of the sarcoplasmic reticulum was observed in the chiton musculature. Although the ultrafiltration slit width of the epicardial podocytes is the same, pedicel size is considerably smaller in *Meiomenia* sp. than in either of the polypalcophoran species examined by Økland (1980).

Given the numerous studies linking podocytes of the molluscan heart wall with ultrafiltration of blood, the presence of epicardial podocytes in *Meiomenia* sp. is strong evidence for blood ultrafiltration into the neomenioid pericardial coelom. This information helps to resolve the question of the functional role of the aplacophoran pericardium, and contributes to the discussion on the origin of the molluscan excretory system. Ultrafiltration of blood into the pericardial cavity is supported as a plesiomorphic character of the Mollusca, and as such argues against a secondary acquisition of an excretory role for the molluscan coelom. Confirmation that these features are shared in all molluscan classes awaits morphological or physiological evidence of ultrafiltration through the heart wall in the Monoplacophora. However, even if podocytes are absent from the monoplacophorans, or from the aplacophoran subclass Chaetodermomorpha (= Class Caudofoveata, *sensu* Salvini-Plawen), the known occurrence of podocytes amongst molluscan classes would still support the plesiomorphy of coelomic ultrafiltration as the most parsimonious reconstruction of this character's evolution, based on recent hypotheses of molluscan phylogeny (see, for example, Salvini-Plawen 1988; Scheltema 1993). This evidence is consistent with a coelomate origin for the Mollusca, with ultrafiltration of hemolymph serving as a source of coelomic fluid. Fine structural or physiological evidence for modification of this ultrafiltrate by secretion or reabsorption is presently lacking for the Neomeniomorpha, and needs to be addressed in future studies.

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