

ULTRASTRUCTURE OF THE FLAME BULBS AND PROTONEPHRIDIAL CAPILLARIES OF *RUGOGASTER HYDROLAGI* (PLATYHELMINTHES, TREMATODA, ASPIDOGASTREA)

N. A. WATSON, K. ROHDE

SUMMARY

The ultrastructure of the terminal apparatus and protonephridial capillaries of *R. hydrolagi* is described. The weir of the flame bulb consists of alternating rows of ribs connected by a membrane of extracellular matrix. Internal ribs, internal leptotriches and cilia of the flame are continuous with the terminal cell, the perikaryon of which lies basal to and alongside the weir. External ribs, external leptotriches and a pair of cytoplasmic cords along the weir are continuous with the proximal canal cell. Distally,

some ciliary membranes extend beyond the end of the microtubules but do not enter the cytoplasm. The distal half of the flame is surrounded by cytoplasm of the proximal canal cell with many electron dense inclusions. The lumen of proximal capillaries is relatively smooth, but in more distal capillaries the lumen surface is enlarged by increasing numbers of lamellae, and lateral flames are present. The phylogenetic position of Rugogastridae is discussed.

RÉSUMÉ : Ultrastructure des flammes vibratiles et des capillaires protonéphridiaux de *Rugogaster hydrolagi* (Platyhelminthes, Trematoda, Aspidogastrea).

L'ultrastructure de l'appareil terminal et des capillaires protonéphridiaux de *Rugogaster hydrolagi* est décrite. Le filtre de la cellule-flamme consiste en des rangées alternées de côtes connectées par une membrane de matrice extracellulaire. Les côtes internes, les leptotriches internes et les cils de la flamme sont en continuité avec la cellule terminale, dont le péricaryon est situé à la base et le long du filtre. Les côtes externes, les leptotriches externes et une paire de cordes cytoplasmiques qui longent le filtre sont en continuité avec la cellule proximale du canal. Distalement, quel-

ques membranes ciliaires s'étendent au delà de l'extrémité des microtubules mais ne pénètrent pas dans le cytoplasme. La moitié distale de la flamme est entourée par le cytoplasme de la cellule proximale du canal, contenant de nombreuses inclusions denses aux électrons. La lumière des capillaires proximaux est relativement lisse, mais dans les capillaires plus distaux la surface interne du canal est augmentée par un nombre croissant de lamelles et des flammes latérales sont présentes. La position phylogénétique des Rugogastridae est discutée.

INTRODUCTION

Ultrastructural studies have made major contributions to our understanding of the phylogeny of Platyhelminthes (Ehlers, 1985; Rohde, 1990) and studies of protonephridia have led to the recognition of at least two major lineages within the phylum (review by Rohde, 1991). *Rugogaster hydrolagi* is the only known member of the family Rugogastridae (Schell, 1973). The adult from *Hydrolagus colliei* in Pacific North America, and hatched larvae were described by Schell, and the species has recently been reported from chimaerid hosts off the coast of Southeastern Australia (Rohde *et al.*, 1992). No intermediate host has yet been found. *Rugogaster* is of particular interest because

it appears to have some characters known only from the Digenea but not the other Aspidogastrea, *i. e.* a bifurcate intestine and a ventral sucker in the adult stage. The ultrastructure of vitellogenesis, the tegument and spermatogenesis of the species have been studied to date (Rohde and Watson, 1991 b, in press, Watson and Rohde, in press), and this paper describes the ultrastructure of protonephridia of *R. hydrolagi*.

MATERIALS AND METHODS

Specimens of *Rugogaster hydrolagi* were collected from the rectal glands of chimaerid hosts during an expedition of the CSIRO Research vessel, FRV « Southern Surveyor », off the north eastern coast of Tasmania during June-July, 1991. Some were fixed for 2 h in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer, made with filtered seawater, at pH 7.3, 4° C. After 4 washings in buffer, they were stored in buffer at 4° C for 21 days, post-

Department of Zoology, University of New England, Armidale, NSW, 2351, Australia.

Accepté le : 2 mars 1992.

fixed in 1 % OsO₄ in buffer for 1 h at room temperature, dehydrated in ethanol, embedded in Spurr resin and polymerized at 65° C for 20 h. Other specimens were held in the fixative at 4° C for 21 days and then buffer washed and further processed by the same schedule. Ultrathin sections were stained with saturated uranyl acetate and lead citrate and examined under a Jeol 1200 Ex electron microscope at 60 kV.

RESULTS

The weir of the protonephridial system is formed by interdigitations of two cells, the terminal cell and the proximal canal cell (Figs. 1-3). The terminal cell gives rise to a tight bundle of cilia, the flame (Figs. 1, 3), and contributes an internal row of ribs to the weir (Figs. 1-3). Cilia have small cross-striated rootlets (Fig. 1), and are held together tightly by a matrix between their external membranes (Figs. 3, 4). Axonemal microtubules are mostly oriented in the same direction (Fig. 3). In a region close to the basal body, cilia have no central pair of microtubules and the peripheral doublets are incomplete (Figs. 3, 4). Further from the basal body, central doublets appear and the peripheral doublets are reinforced by additional spokes arising between pairs of doublets and also to the outside of the circle, between the *a* and *b* tubules (Figs. 3, 4). The cilia are then normal for the remainder of their length until microtubules diminish, and cilia terminate at different levels (Figs. 1, 5). Membranes from some of the cilia continue on after the microtubules have terminated, forming a network which is closely associated with the cytoplasm of the canal cell (Figs. 1, 7). Large quantities of floccular material found within the confines of these membranes appears to be the same as that seen between cilia at the base of the flame (Figs. 1, 3-5).

Internal leptotriches arise from the basal cytoplasm of the terminal cell and also from the internal ribs (Figs. 1, 2). The nucleus of the terminal cell lies basal to and alongside the weir (Fig. 2) and the cytoplasm is rich in mitochondria (Figs. 1, 2). The canal cell contributes a row of external

ribs to the weir (Figs. 1-3) and two cytoplasmic cords joined by a septate junction that extend along the weir (Fig. 2). External leptotriches arise from the external ribs (Figs. 1-3). Both internal and external ribs are strengthened by microfilaments, a few microtubules were seen only in the internal ribs, and « membranes » of extracellular matrix between the ribs are anchored in electron-dense regions in the margins of the ribs (Fig. 3).

Distal to the weir, the cytoplasm of the canal cell wraps tightly around the flame (Figs. 1, 5) and is joined by a septate junction (Fig. 5) while more distally, the lumen widens (Figs. 1, 7). At the tip of the flame, the proximal canal bends and rapidly becomes convoluted so that several spaces of the lumen appear in one section of a single canal cell (Fig. 7). The cytoplasm of the proximal canal cell in this region contains mitochondria and a large number of electron dense inclusions of various sizes, some surrounded by a membrane (vesicles) and others apparently without a boundary membrane. There are also vacuoles with loose, scattered contents, Golgi apparatus, microtubules, some coated vesicles and a large number of small uncoated (pinocytotic?) vesicles. Surface lamellae in the proximal canal are scarce (Fig. 7) and the nucleus of the canal cell does not lie close to the flame. Areas of the proximal canal form folds which enclose « spaces » of various sizes (Figs. 1, 5, 7). More distal from the terminal complex, the capillary remains convoluted, a septate junction persists, dense inclusions are replaced by myelin bodies and there are large numbers of small vesicles and some coated vesicles (Fig. 8). More vacuoles are present in this region than in the more proximal region of the canal, occasional lamellar processes into the lumen become more numerous distally, and the canal cell nucleus is visible (Fig. 6). The capillary complex is surrounded by a layer of fibrous matrix which extends throughout the animal, enveloping muscle bundles, organ systems and individual parenchyma cells (Figs. 6, 8).

Figures 9 and 10 show a different region of the proto-

Fig. 1-4 — Sections (1 — longitudinal, 2 to 4 — transverse) through the flame bulb of *Rugogaster hydrolagi*. Fig. 1. Proximal canal cell (p) with prominent dense inclusions (d) contributes external ribs (er), which alternate with internal ribs (ir) from the terminal cell (t), to the weir apparatus. Note small rootlets (r) of cilia (c), internal (i) and external (e) leptotriches, floccular material (fl) between cilia at their base and tip regions, reinforced region of cilia (open arrowhead), terminating cilia (solid arrowheads), mitochondria (m), golgi apparatus (g) and invaginations of the cell membrane into the cytoplasm of the canal cell (arrows) widening to larger spaces in the cell. Fig. 2. At the level of the weir, note terminal cell (t) with nucleus (n), cytoplasmic cords (cc) with septate junction (s) between them, cilia (c) of the flame, and internal (i) and external (e) leptotriches. Fig. 3. At the base of the flame, note basal bodies (b) of cilia, terminal cell (t) continuous with the internal row of ribs (ir), microfilaments (f) in both internal and external (er) ribs, microtubules (mt) in internal ribs, and a membrane (me) between the rows, basal regions of cilia without central element and with incomplete microtubule pairs (solid arrowhead), floccular material (fl) between the bases of cilia, reinforced region of cilia (open arrowhead), common orientation and tight association of ciliary membranes. Fig. 4. Enlargement of cilia at the base of the flame, showing incomplete microtubule doublets (arrows) and additional spokes between microtubule doublets within the ring, and to the outside of the ring between a and b tubules (arrowheads). Also note floccular material (fl) between cilia at the base of the flame.

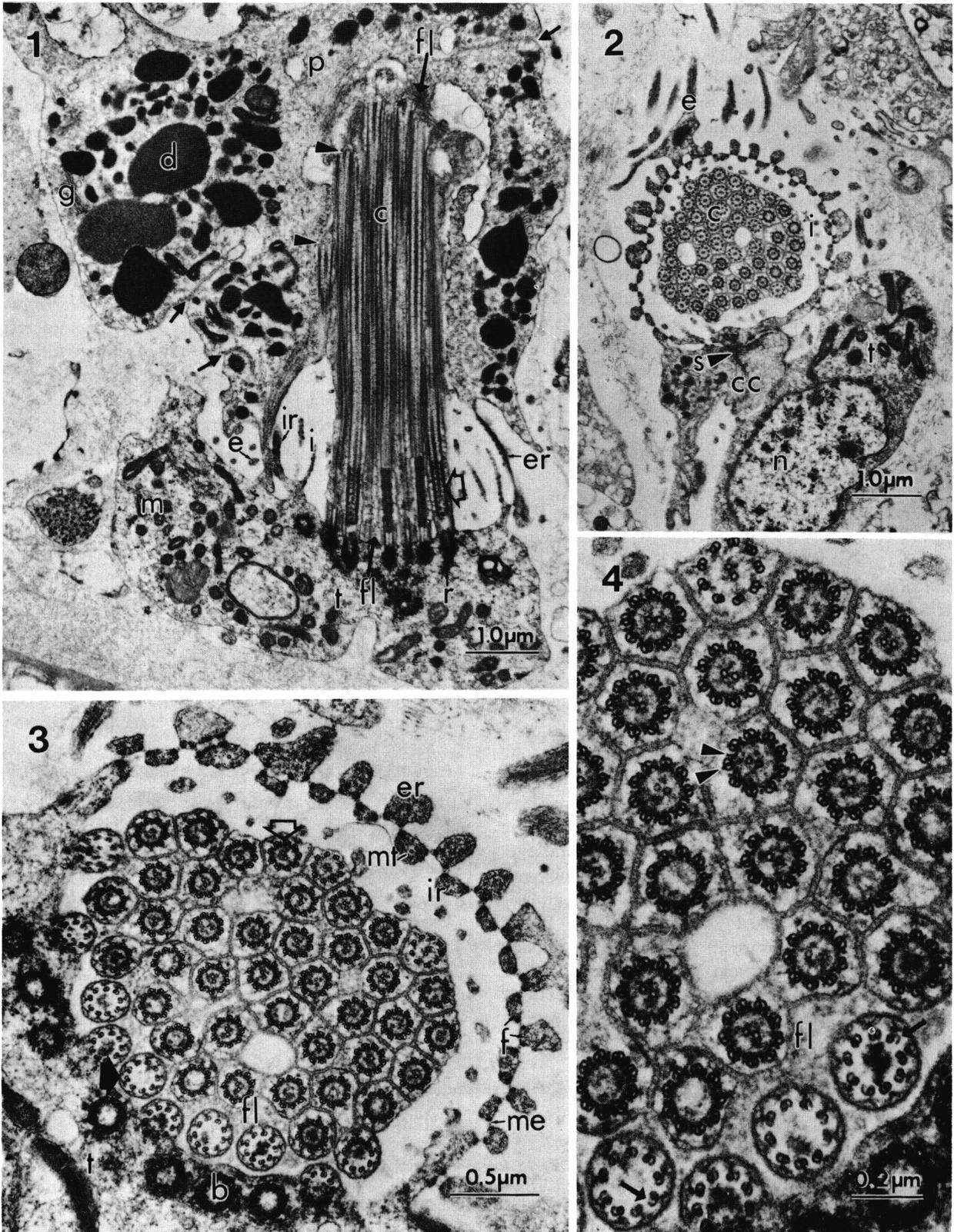


FIG. 1 to 4.

nephridial canal system, where the lumen is more heavily lamellated and a few cilia of one or several lateral flames are visible (Fig. 9). The canal cell perikaryon is joined to the canal by a narrow stalk of cytoplasm. The canal cytoplasm contains many invaginations by the fibrous matrix of the extracellular network and the basal lamina (Fig. 10). The cytoplasm contains microtubules, mitochondria and many vesicles (Fig. 10), and a septate junction is present (Fig. 9).

In larger ducts lateral flames are also present and the wall of the lumen is extensively lamellated (Fig. 11). The outer surface of the canal cell is deeply lobed with invaginations of extracellular matrix on a thin basal lamina that is more conspicuous in the narrow spaces between cellular processes. Numerous dark regions beneath the plasma membrane link the extracellular matrix to the canal cell cytoplasm which contains mitochondria, microtubules, Golgi apparatus and small vacuoles.

DISCUSSION

As a useful phenetic system, Gibson and Chinabut, 1984, recognize two orders of the subclass Aspidogastrea, the Aspidogastrida with the single family Aspidogastridae and the Stichocotyliida with the families Stichocotylidae, Multicalycidae and Rugogastridae, but lack of knowledge concerning the biology of Stichocotyliida hampers understanding of their phylogenetic relationships. Gibson (1987) points out that Aspidogastrea have few features which could be considered synapomorphies other than atrophy of the oral sucker, hypertrophy of the posterior sucker, the marginal bodies (present only in Aspidogastrida and the multicalycid stichocotylidans) and the monocaecal gut (bifid in *Rugogaster*). He considers that the Aspidogastrea may be paraphyletic. While some members of the Aspidogastridae have been studied in detail at the ultrastructural level (e. g. Rohde, 1970; Bailey and Tompkin, 1971; Halton and Lyness, 1971; Rohde, 1971; Rohde, 1972; Bakker and Diegenbach, 1973; Bakker and Diegenbach, 1974; Frederickson, 1978; Ip and Desser, 1984; Rohde, 1989; Rohde and Watson, 1990; Rohde and Watson, 1991 a; Rohde *et al.*, 1991; Watson and Rohde, 1991), Rohde (1990) pointed out that ultrastructure and life cycles of species

of the three families of Stichocotyliida are not known, and their relationship with the Aspidogastridae therefore remains obscure. Ultrastructural studies of species of all families of Aspidogastrea are therefore essential for a better understanding of the phylogeny of the group.

Rohde and Watson (1991 b in press) and Watson and Rohde (in press) investigated the ultrastructure of vitellogenesis, the tegument, ventral sucker and rugae, and spermatogenesis, respectively in *Rugogaster hydrolagi*. Spermatogenesis occurs in the standard manner as for most trematodes and does not reveal any synapomorphies for Aspidogastrea. Studies of the tegument, sucker and rugae, however, demonstrate the presence of surface microtubercles in common with Aspidogastridae, and development of rugae from the ventral sucker. Thus microtubercles represent a synapomorphy of the Aspidogastridae and Rugogastridae, and the ventral sucker plus rugae must be considered to be homologous with the ventral disc of Aspidogastridae. Both develop from a ventral sucker, which persists in *Rugogaster* adults but not in adults of Aspidogastridae.

The protonephridial system of *R. hydrolagi*, as described in this paper, corresponds in general arrangement to that found in all Monogenea-Trematoda examined to date (Rohde, 1991 and references therein). Thus, the weir of the flame bulb is formed by two alternating rows of ribs, one row originating from the terminal cell which has a nucleus close to the base of the flame; two cytoplasmic cords joined by a septate junction and originating from the proximal canal cell extend along the weir; surface area of some protonephridial capillaries is increased by the presence of lamellae or a reticulum of interconnected spaces. Cestoda, on the other hand, although also possessing a flame bulb formed from the interdigitations of a terminal and a canal cell, do not have cytoplasmic cords along the flame bulb, nor septate junctions in the capillary wall, and the surface area of capillaries is increased by short microvilli rather than lamellae. *R. hydrolagi* also has internal and external leptotriches, which are common in Monogenea-Trematoda, whereas Cestoda have internal leptotriches but few or no external leptotriches.

In *R. hydrolagi*, it appears that additional intracellular spaces are formed by folding of the proximal canal cell

FIG. 5-7. — Transverse sections through proximal canal cell of *Rugogaster hydrolagi*. Fig. 5. Above the level of the weir, note proximal canal cell (p) wraps around the flame and is joined by a septate junction (s). Also note dense inclusions (d) and invagination of cell membrane enclosing a space in the cell (arrow). Fig. 6. More distal from the flame, note nucleus (n) of capillary cell and fibrous matrix (fm) surrounding the convoluted capillary. Fig. 7. At the tip of the flame, note few cilia remaining, ciliary membranes (cm) in a network, closely associated with canal cell cytoplasm but not seen to enter it, floccular material (fl) between membranes, septate junctions (s), invaginations of cell membrane into cytoplasm (arrow) widening to a space, and vesicles (v), coated vesicles (cv), dense inclusions (d) and large vacuoles (va) within cytoplasm of proximal canal cell.

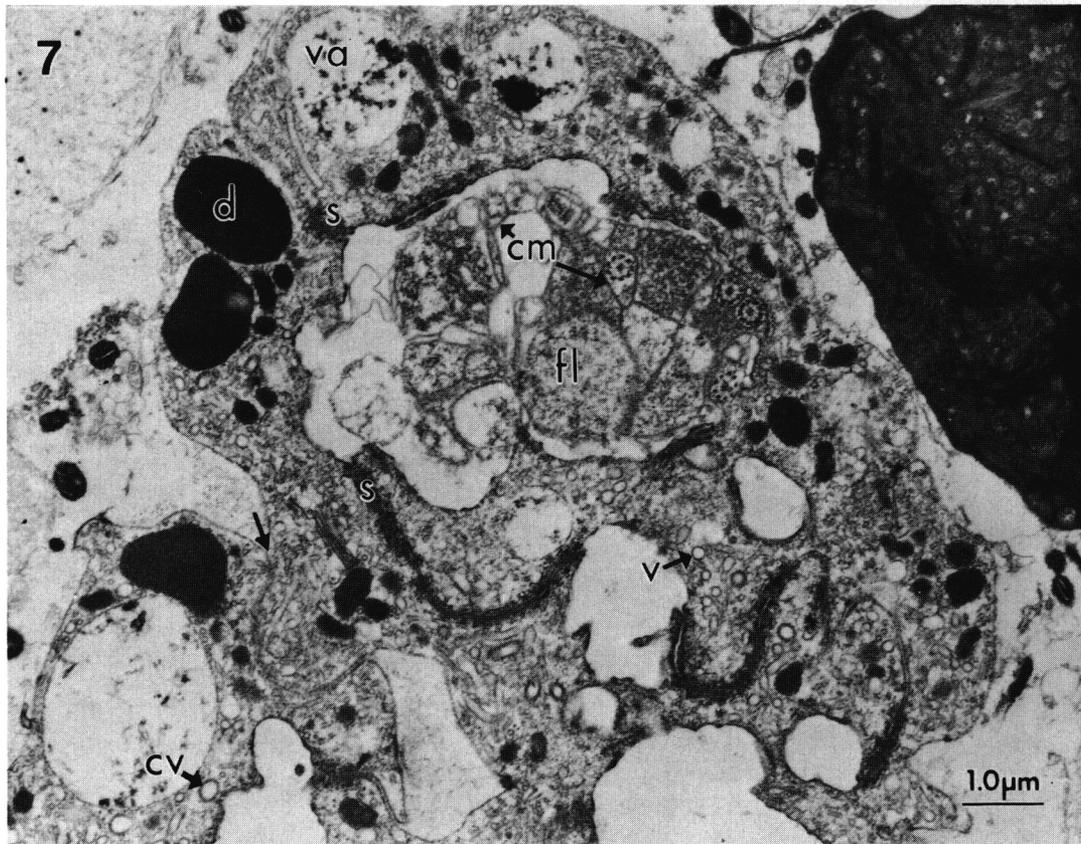
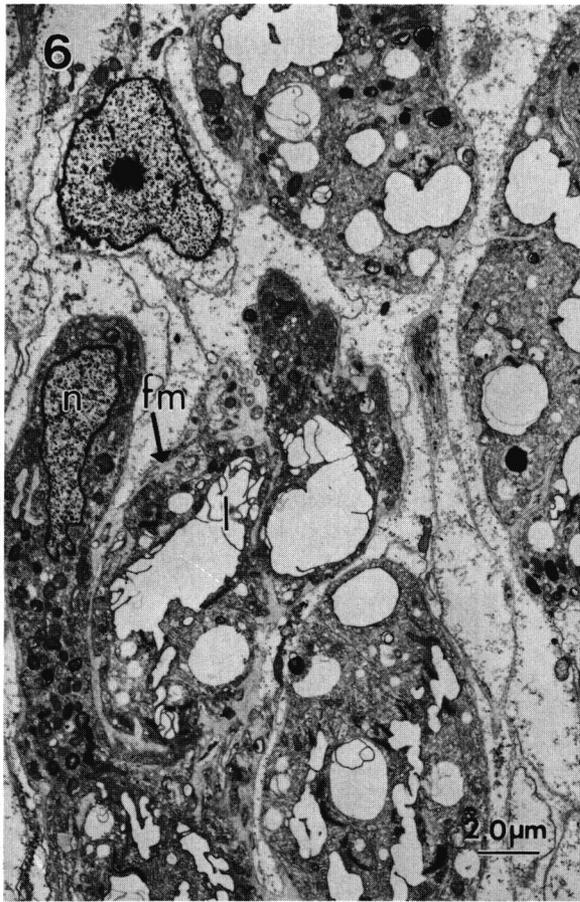
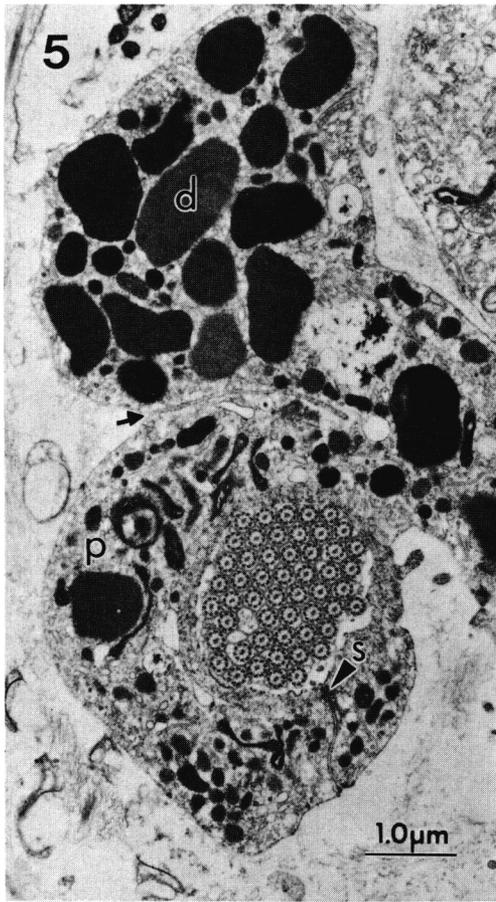


FIG. 5 to 7.

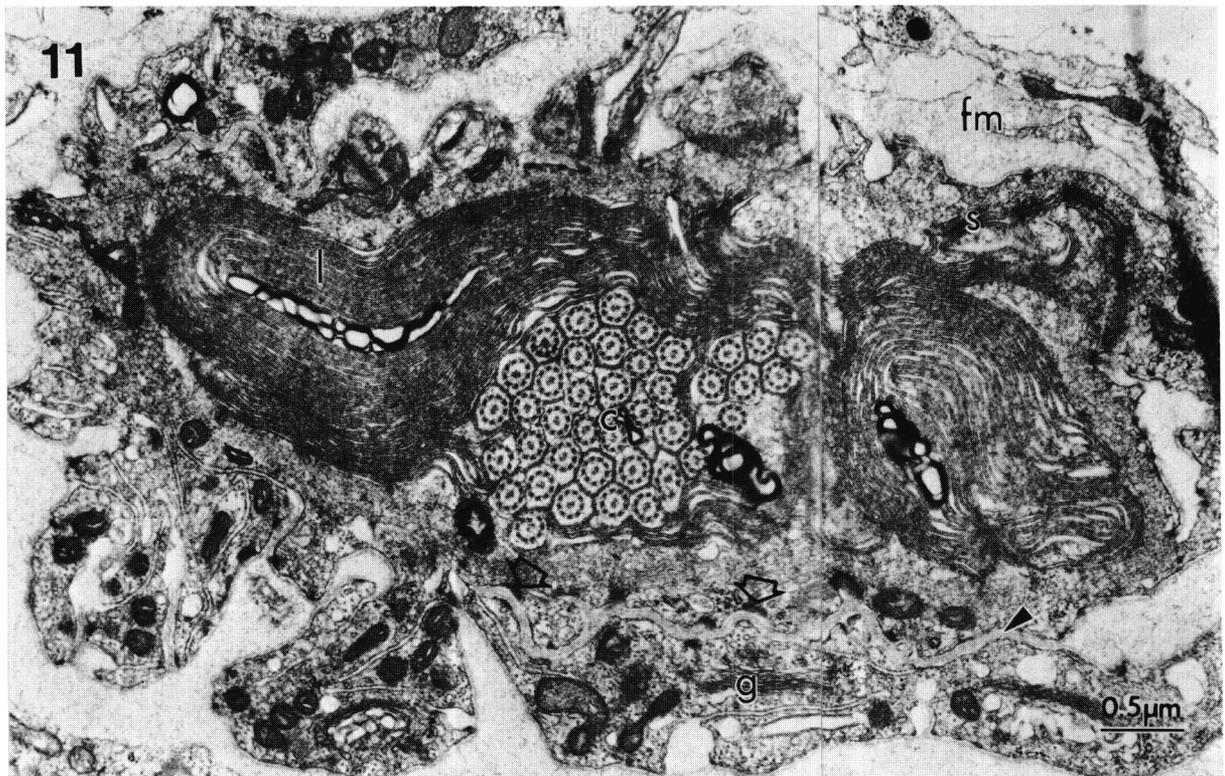
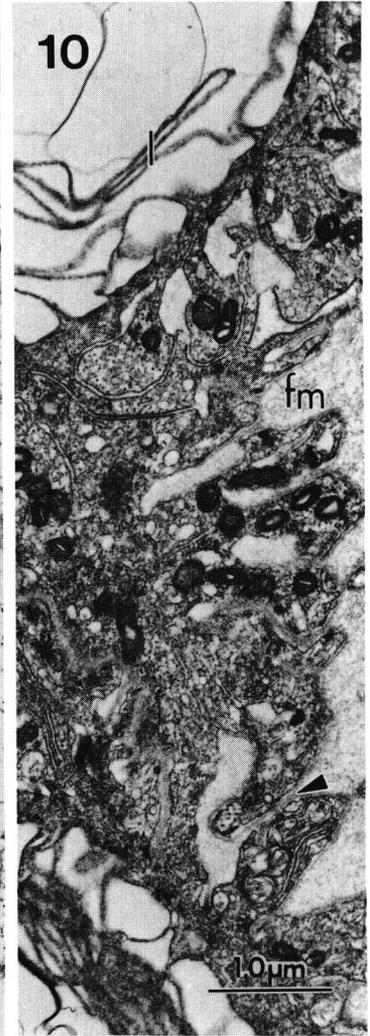


FIG. 8 to 11.

cytoplasm. It is possible that septate junctions then form to isolate these « spaces » from the extracellular environment, thus creating the appearance of several canal lumens as described in the results.

Tight association and common orientation of cilia within the flame, as seen in *R. hydrolagi*, are visible in micrographs of many other platyhelminth species (e. g. Lumsden and Hildreth, 1983; Xylander, 1987; McCullough and Fairweather, 1991) and are postulated to belong to the ground pattern of the Neodermata (see Ehlers, 1985; Ehlers and Sopott-Ehlers, 1986). However, the basal regions of the cilia of *Rugogaster* appear unusual in having reinforcements of microtubular doublets.

In *Rugogaster*, some ciliary membranes of the cilia forming the flame continue distally after the microtubules have terminated, closely associated with but not entering the distal cytoplasm of the canal cell. Unusual features of the distal end of the cilia forming the flame were also seen in another aspidogastrid, *Multicotyle purvisi*. In adults of this species, ciliary membranes extend into the distal cytoplasm of the canal cell, thus anchoring the tip of the flame in the cytoplasm (Rohde, 1971, 1972). According to unpublished observations larval *Multicotyle* also has extended distal ciliary membranes. On the other hand, the aspidogastrid *Lobatostoma manteri* lacks them (Rohde, 1989). Hence it seems that there is tendency for continuation of ciliary membranes beyond the distal end of the flame in the Aspidogastrea, but such structures are not characteristic of all the species in the group.

In conclusion, despite possession of multiple testes, bifid gut, anterior Laurer's canal and oviduct looping around one of the gut caeca not found in any other aspidogastreae, the amphistomatous larva (Schell, 1973) and our findings outlined above confirm the placement of *Rugogaster* in the Trematoda, Aspidogastrea.

Acknowledgements. — This work was supported by grants from the Australian Research Council and the University of New England, Armidale. The specimens were collected and fixed by Craig HAYWARD. We thank the Australian Antarctic Division for allowing him to accompany the expedition of FRV « Southern Surveyor », during June-July, 1991. Peter GARLICK made facilities of the Electron Microscopic Unit, UNE available to us. Rick PORTER developed and Zoltan ENOCH printed the electron micrographs.

REFERENCES

Bailey H. H., Tompkin S. S. : Ultrastructure of the integument of *Aspidogaster conchicola*. *J. Parasitol.*, 1971, 57, 848-854.
 Bakker K. E., Diegenbach P. C. : The ultrastructure of spermatozoa of *Aspidogaster conchicola* Baer, 1826 (Aspidogastridae, Trematoda). *Neth. J. Zool.*, 1973, 23, 345-346.
 Bakker K. E., Diegenbach P. C. : The structure of the opisthaptor of *Aspidogaster conchicola* Baer, 1826 (Aspidogastridae, Trematoda). *Neth. J. Zool.*, 1974, 24, 162-170.
 Ehlers U. : Das phylogenetische System der Plathelminthes, 1985. *Gustav Fischer*, Stuttgart, 317 p.
 Ehlers U., Sopott-Ehlers, B. : Vergleichende Ultrastruktur von Protonephridien : ein Beitrag zur Stammesgeschichte der Plathelminthen. Comparative ultrastructure of protonephridia: a contribution to the phylogeny of the Plathelminthes. *Verh. Dtsch. Zool. Ges.*, 1986, 79, 168-169.
 Frederickson D. W. : The fine structure and phylogenetic position of the cotylocidium larva of *Cotylogaster occidentalis* Nickerson 1902 (Trematoda: Aspidogastridae). *J. Parasitol.*, 1978, 64, 961-976.
 Gibson D. I. : Questions in digenean systematics and evolution. *Parasitology*, 1987, 95, 429-460.
 Gibson D. I., Chinabut S. : *Rohdella siamensis* gen. et sp. nov. (Aspidogastridae: Rohdellinae subfam. nov.) from freshwater fishes in Thailand, with a reorganization of the classification of the subclass Aspidogastrea. *Parasitology*, 1984, 88, 383-393.
 Halton D. W., Lyness R. A. W. : Ultrastructure of the tegument and associated structures of *Aspidogaster conchicola* (Trematoda: Aspidogastrea). *J. Parasitol.*, 1971, 57, 1198-1210.
 Ip H. S., Desser S. S. : Transmission electron microscopy of the tegumentary sense organs of *Cotylogaster occidentalis* (Trematoda: Aspidogastrea). *J. Parasitol.*, 1984, 70, 563-575.
 Lumsden R. D., Hildreth M. B. : The fine structure of adult tape-worms. In: Biology of the Eucestoda (Vol. 1), Arme C., Pappas P. W. (eds.), *Academic Press*, London, p. 177-233.
 McCullough J. S., Fairweather I. : Ultrastructure of the excretory system of *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphylidea). *Parasitol. Res.*, 1991, 77, 157-160.
 Rohde K. : Ultrastructure of the flame cells of *Multicotyle purvisi* Dawes. *Naturwissenschaften*, 1970, 57, 398.
 Rohde K. : Untersuchungen an *Multicotyle purvisi* Dawes, 1941 (Trematoda, Aspidogastrea). VIII. Elektronenmikroskopischer Bau des Exkretionssystems. *Int. J. Parasitol.*, 1971, 1, 275-286.
 Rohde K. : The Aspidogastrea, especially *Multicotyle purvisi* Dawes, 1941. *Advan. Parasitol.*, 1972, 10, 77-151.
 Rohde K. : Ultrastructure of the protonephridial system of *Lobatostoma manteri* (Trematoda, Aspidogastrea). *J. Submicrosc. Cytol. Pathol.*, 1989, 21, 599-610.
 Rohde K. : Phylogeny of Platyhelminthes, with special reference to parasitic groups. *Int. J. Parasitol.*, 1990, 20, 979-1007.
 Rohde K. : The evolution of protonephridia of the Platyhelminthes. *Hydrobiologia*, 1991, 227, 315-321.

FIG. 8-11. — Proximal (Fig. 8) and more distal (Figs. 9-11) protonephridial capillaries. Fig. 8. Note septate junction (s) and vacuoles with condensing contents (arrowheads). Fig. 9. Note nucleus (n) of capillary, septate junction (s) in wall, increasing numbers of lamellae (l) and a few cilia (c) of one or several lateral flames. Fig. 10. From a similar region to Fig. 9, note lamellae (l) in lumen, basal lamina (arrowhead) and fibrous matrix (fm) surrounding canal cell with many evaginations into it. Fig. 11. Heavily lamellated region of capillary, note cilia (c) of lateral flame, septate junction (s), basal lamina (arrowhead) and fibrous matrix (fm) surrounding canal cell, dense regions (open arrowheads) linking the canal cell to the extracellular matrix, and Golgi apparatus (g).

- Rohde K., Heap M., Hayward C. J., Graham K. : *Calicotyle australiensis* n. sp. and *Calicotyle* sp. (Monogenea, Monopisthocotylea) from the rectum and rectal glands and *Rugogaster hydrolagi* Schell, 1973 (Trematoda, Aspidogastrea) from the rectal glands of holocephalans off the coast of southeastern Australia. *Systematic Parasitology*, 1992, 21 : 69-79.
- Rohde K., Watson N. : Ultrastructure of pigmented photoreceptor of larval *Multicotyle purvisi* (Trematoda, Aspidogastrea). *Parasitol. Res.*, 1991 a, 77, 485-490.
- Rohde K., Watson N. A. : Vitellogenesis of *Rugogaster hydrolagi* (Trematoda, Aspidogastrea). *Ann. Parasitol. Hum. Comp.*, 1991 b, 66 : 273-280.
- Rohde K., Watson N. A. : Ultrastructure of tegument, ventral sucker and rugae of *Rugogaster hydrolagi* (Trematoda, Aspidogastrea). *Int. J. Parasitol.* in press.
- Rohde K., Watson N. A., Cribb T. : Ultrastructure of sperm and spermatogenesis of *Lobatostoma manteri* (Trematoda, Aspidogastrea). *Int. J. Parasitol.*, 1991, 21, 409-419.
- Rohde K., Watson : Paired multiciliate receptor complexes in larval *Multicotyle purvisi* (Trematoda, Aspidogastrea). *Parasitol. Res.*, 1990, 76, 597-601.
- Schell S. C. : *Rugogaster hydrolagi* gen. et sp. n. (Trematoda: Aspidobothrea: Rugogastridae fam. n.) from the ratfish, *Hydrolagus colliei* (Lay and Bennett, 1839). *J. Parasitol.*, 1973, 59, 803-805.
- Watson N. A., Rohde K. : Ultrastructure of spermatogenesis and sperm of *Multicotyle purvisi* (Trematode, Aspidogastrea). *Zoomorphology*, 1991, 110, 347-356.
- Watson N. A., Rohde K. : Ultrastructure of sperm and spermatogenesis of *Rugogaster hydrolagi*, Schell 1973 (Platyhelminthes, Trematoda, Aspidogastrea, Rugogastridae). *Parasitol. Res.* in press.
- Xylander W. E. R. : Ultrastructure of the lycophora larva of *Gyrocotyle urna* (Cestoda, Gyrocotylidae). III. The protonephridial system. *Zoomorphology*, 1987, 107, 88-95.