Ann. Parasitol. Hum. Comp.,
1989, 64, nº 6, pp. 433-442.

ULTRASTRUCTURE OF THE FLAME BULBS OF THE MONOPISTHOCOTYLEAN MONOGENEA *LOIMOSINA WILSONI* (LOIMOIDAE) AND *CALCEOSTOMA HERCULANEA* (CALCEOSTOMATIDAE)

K. ROHDE*, J.-L. JUSTINE**, N. WATSON*

SUMMARY. The flame bulbs of *Loimosina* and *Calceostoma* consist of a row of external and a row of internal longitudinal ribs connected by a « membrane » apparently of extracellular matrix, and two cytoplasmic cords connected by a septate junction. Internal ribs are continuous with the terminal cell, external ribs and cytoplasmic cords with the proximal canal cell. Both species have many internal leptotriches, in the first, some external leptotriches were also seen. Internal leptotriches arise from the perikaryon of the terminal cell or the internal ribs. The flame bulbs closely resemble those of the Monogenea Polyopisthocotylea examined and of *Gyrodactylus*, *Dactylogyrus* and an unidentified ancyrocephaline, i. e. evidence available indicates that all Monogenea share one type of flame bulb, also found in aspidogastreans and digeneans, but different from that of gyrocotylids, amphilinids and cestoideans. *Udonella* has a different type of flame bulb and, therefore, is unlikely to be a monogenean.

Key-words: *Loimosina*, *Calceostoma*. *Udonella*. Monogenea. Platyhelminthes. Protonephridia. Ultrastructure. Phylogeny.

Ultrastructure des cellules-flammes des Monogènes Monopisthocotylea *Loimosina wilsoni* (Loimoidae) et *Calceostoma herculanea* (Calceostomatidae).

RÉSUMÉ. Les bulbes des cellules-flammes de *Loimosina* et *Calceostoma* sont constitués d'une rangée externe et d'une rangée interne de côtes connectées par une « membrane » apparemment constituée par la matrice extracellulaire, et de deux cordes cytoplasmiques connectées par une jonction septée. Les côtes internes sont en continuité avec la cellule terminale, les côtes externes et les cordes cytoplasmiques sont en continuité avec la cellule proximale du canal. Les deux espèces ont de nombreuses leptotriches internes ; chez *Loimosina*, quelques leptotriches externes ont aussi été vues. Les bulbes des cellules-flammes ressemblent beaucoup à ceux des Monogènes Polyopisthocotylea examinés, et à ceux de *Gyrodactylus*, de *Dactylogyrus* et d'un Ancyrocephalinae indéterminé. Les données disponibles indiquent donc que tous les Monogènes possèdent un seul type de bulbe de cellule-flamme, qui existe aussi chez les Aspidogastres et les Digènes, mais qui est différent de celui des Gyrocotylides, Amphilinides et Cestodes. *Udonella* a un type différent de bulbe de cellule-flamme et n'est donc probablement pas un Monogène.

Mots-clés : *Loimosina*. *Calceostoma*. *Udonella*. Monogènes. Plathelminthes. Protonéphridies. Ultrastructure. Phylogénie.

* *Department of Zoology, University of New England, Armidale, N. S. W. 2351, Australia.*
** *Laboratoire des Vers, Muséum National d'Histoire Naturelle, 61, rue Buffon, 75231 Paris Cedex 05, France.*

Accepté le 18 juillet 1989.

Introduction

A recent study of the ultrastructure of flame bulbs and protonephridial capillaries of *Udonella* showed that this genus differs from the polyopisthocotylean Monogenea examined to date in several features (Rohde *et al.*, 1989). A comparison with monopisthocotylean Monogenea, in which *Udonella* is sometimes included, could not be made, since not a single electron-microscopic study of the protonephridial system of such monogeneans had been made. In the meantime, flame bulbs and protonephridial capillaries of *Gyrodactylus* were examined and found to be similar to those of Polyopisthocotylea (Rohde, in press). *Gyrodactylus*, in several respects, is intermediate between the Polyopisthocotylea and Monopisthocotylea (Lambert, 1980; Justine *et al.*, 1985) and studies of « typical » Monopisthocotylea are necessary to show whether all Monogenea share a similar type of protonephridial system and whether *Udonella* is a monogenean or not. The phylogenetic status of *Udonella* is of particular interest since it has been claimed to be the sister group of all other major groups of parasitic Platyhelminthes, the Neodermata of Ehlers (1984, 1985) which correspond to the Cercomeridea of Brooks, O'Grady and Glen (1985) (Brooks *et al.*, 1985).

This paper describes the ultrastructure of the flame bulbs of two monopisthocotyleans, *Loimosina* and *Calceostoma*. The ultrastructure of the protonephridia of two other species, *Dactylogyrus* and Ancyrocephalinae gen., is described in another paper (Rohde *et al.*, in press).

Materials and Methods

Loimosina wilsoni Manter, 1944 was collected from *Sphyrna lewini* (Cuvier, Griffith and Smith, 1834), *Calceostoma herculanea* Euzet and Vala, 1976 from *Umbrina canariensis* (Valenciennes, 1843) caught near Dakar, Senegal, West Africa. Worms were fixed for 1 hour in cold 2.5 % glutaraldehyde in 0.1 M sodium-cacodylate buffer containing 0.1 M saccharose and 0.2 mM $CaCl_2$, at pH 7.2, rinsed in buffer, postfixed for 1 hour in cold 1 % OsO_4 in the same buffer, dehydrated and embedded in Epon. Specimens were sectioned, stained with saturated uranyl acetate in 50 % ethanol and Reynolds lead citrate, and examined under a Jeol 1200 EX at 60 kV.

Results

Loimosina. Longitudinal sections of the flame bulb are illustrated in *figs. 1-4*, cross-sections in *figs. 5-8*. The flame bulb is formed by a terminal cell and a proximal canal cell. The perikaryon with the nucleus of the terminal cell is located

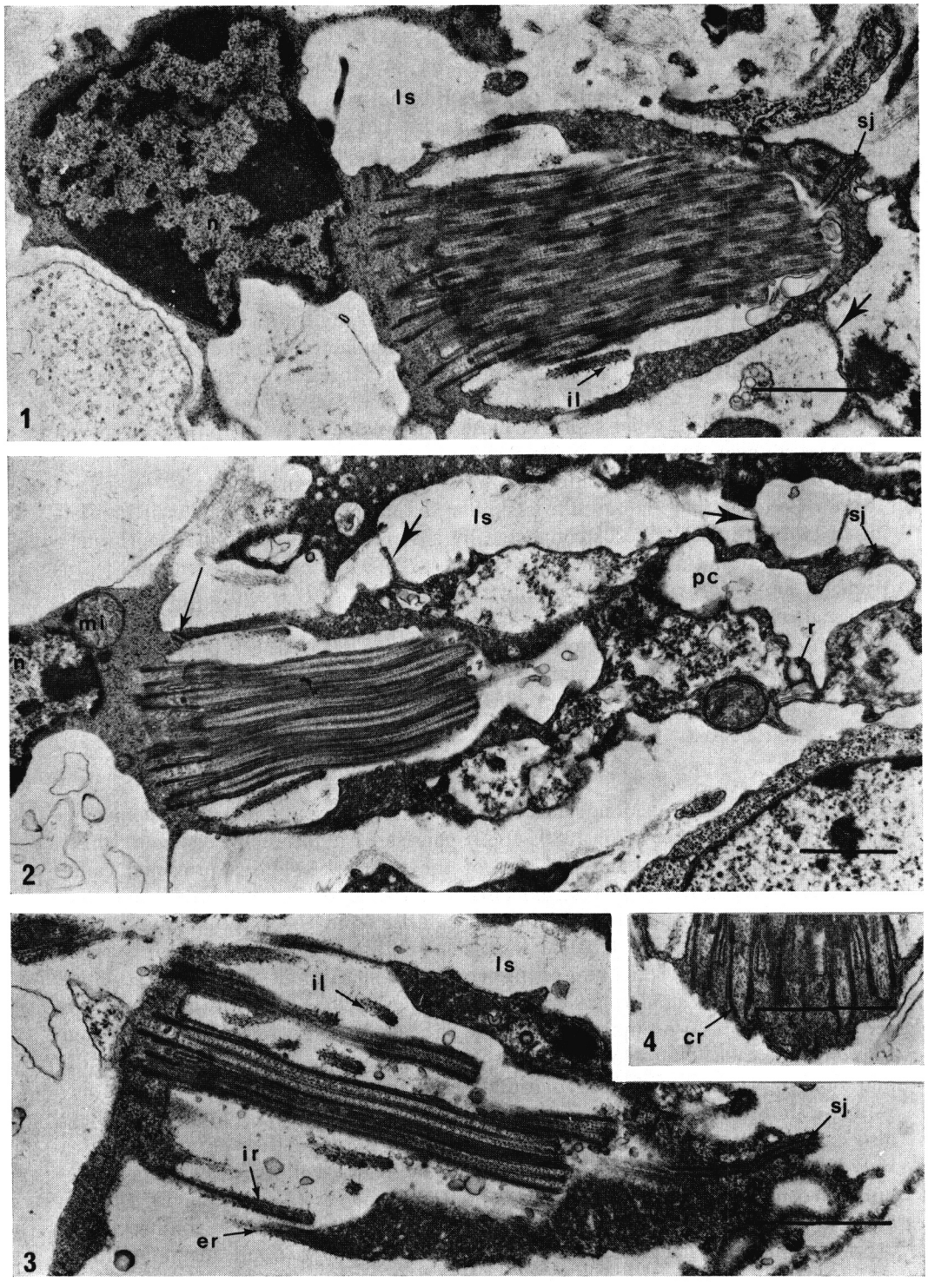
basal to the basal bodies of the cilia *(figs. 1, 2)* or partly lateral to the flame *(fig. 8)*, and contains large mitochondria *(fig. 2)*. Internal ribs of the weir arise from the perikaryon of the terminal cell *(figs. 1-3)*, external ribs are continuations of the distal cytoplasm (the proximal canal cell) *(figs. 1-3)*. A septate junction extends along the weir *(fig. 7, 8* in oblique sections e. g., *fig. 8*, sometimes appearing double), and the distal part of the flame bulb *(figs. 1, 3, 5, 6)*, connecting parts of the same cell, i. e. the proximal canal cell. Internal leptotriches arise from the cytoplasm of the perikaryon *(figs. 1-3)*, or the internal ribs *(fig. 7)* and there are few external leptotriches arising from the external ribs *(fig. 7)*. The flame bulb is suspended in a large liquid filled space *(figs. 1-3, 7, 8)* by means of cytoplasmic bridges extending from the distal cytoplasm of the flame bulb to cytoplasm lining the space distally *(fig. 2)*. The space contains many fibres in its outer parts *(fig. 8)*. Ciliary rootlets of cilia forming the flame are very short *(figs. 1-4)*. The surface area of the protonephridial capillary close to the flame bulb is enlarged by means of spaces connected to its lumen *(fig. 2)*.

Diagrams of the flame bulb of *Loimosina* are given in *fig. 14*.

Calceostoma. The flame bulb is similar to that of *Loimosina*, its weir consisting of two cytoplasmic cords connected by a septate junction and « membrane » connected external and internal ribs *(figs. 11, 12)*. There are many internal leptotriches *(figs. 9-12)*, rudimentary ciliary rootlets *(figs. 9, 11)*, and a nucleus of the terminal cell partly basal, partly lateral to the flame *(fig. 11)*. Distally, the septate junction extends into the proximal canal cell *(fig. 13)*, whose cytoplasm close to the lumen is densely granular.

Discussion

The flame bulbs of Monogenea Polyopisthocotylea examined *(Polystomoides, Gotocotyla*, see Rohde, 1975, 1980, 1982) consist of a terminal cell from which internal leptotriches and internal ribs of the weir arise, and a proximal canal cell which contributes the external ribs and two cytoplasmic cords connected by a septate junction. External leptotriches arise from the external ribs. Protonephridial capillaries also have at least one septate junction, and their surface area is increased by a reticulum of interconnected spaces and/or lamellae (Rohde, 1973, 1975, 1980). *Gyrodactylus* which, according to some authors (for references see Rohde, in press) occupies an intermediate position between the Monogenea Polyopisthocotylea and Monopisthocotylea, has an almost identical flame bulb and similar protonephridial capillaries (Rohde, in press). In the present paper, we have shown that two monopisthocotyleans belonging to two different families, also have identical flame bulbs. Minor differences between these species and the Polyopisthocotylea are the larger number of external leptotriches in the flame bulbs of *Gotocotyla* (see Rohde, 1980) and *Polystomoides* (see Rohde, 1973), and long, though thin, ciliary rootlets in the latter species. The flame bulbs of the other two monopisthocotyleans examined, i. e. *Dactylogyrus* and an unidentified


ls
sj
n
il
1
ls
sj
pc
mi
r
n
2
il
ls
ir
er
sj
3
cr
4

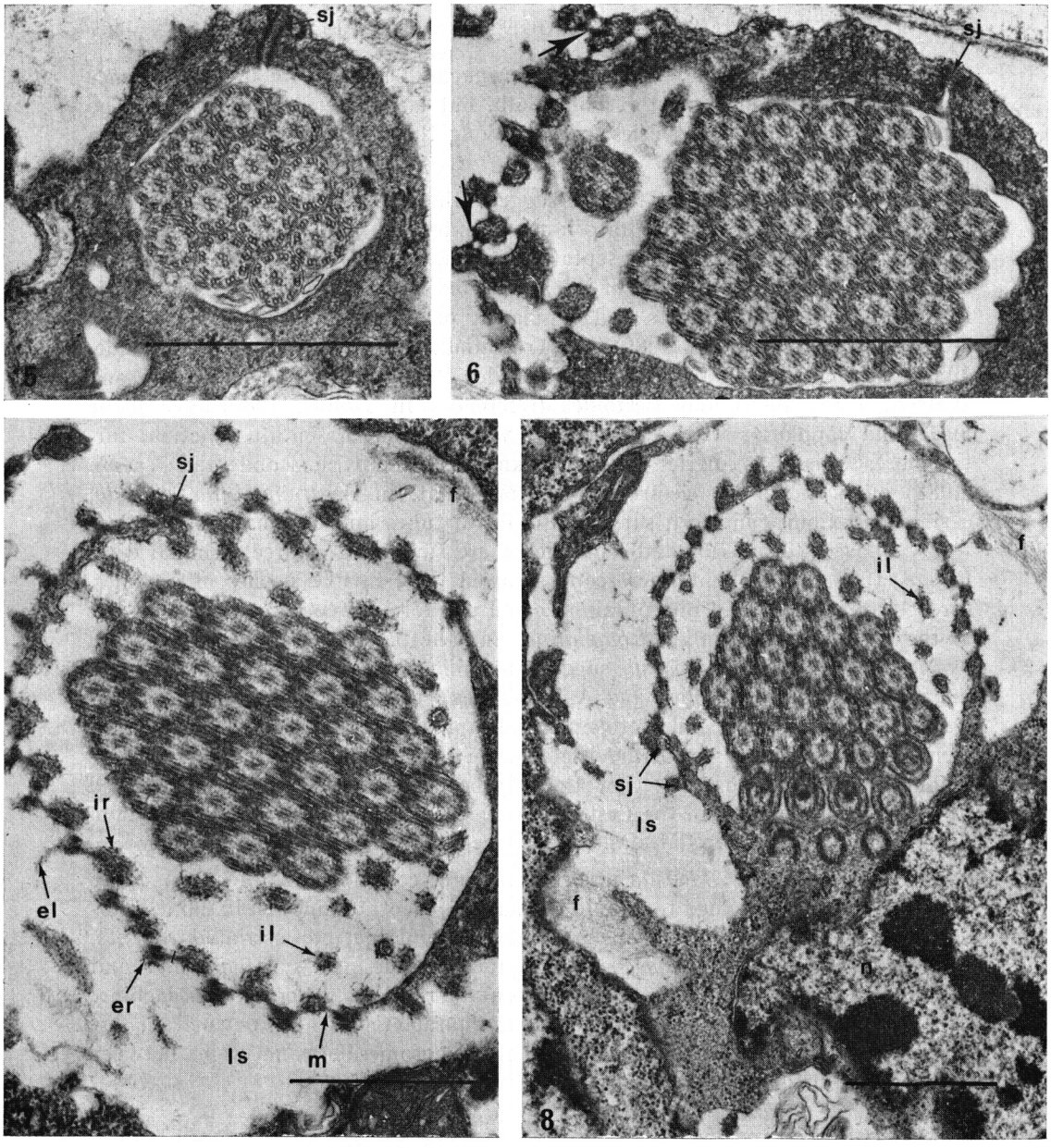
ancyrocephaline, resemble those of *Loimosina* and *Calceostoma* in the presence of a septate junction along the flame bulb, but lack external leptotriches. The septate junction in these species extends along the capillaries (Rohde *et al.*, in press).

Thus, evidence available to date suggests that all Monogenea have a similar type of flame bulb, with a septate junction extending along it. Protonephridial capillaries also have at least one septate junction, and their surface area is increased by lamellae and/or reticula of interconnected spaces. The same characteristic is found in the flame bulbs and capillaries of the Trematoda Aspidogastrea (Rohde, 1970, 1971, 1972, 1989) and Trematoda Digenea (references in Rohde, 1989). In contrast, Gyrocotylidea (Xylande, 1987), Amphilinidea (Rohde and Watson, 1987, 1988) and Cestoidea (references in Rohde, 1989) have flame bulbs and capillaries with a continuous wall, without a septate junction, furthermore, walls of capillaries have short knob-like microvilli. Rohde and Watson (1988) have shown by electron microscopy that, in *Austramphilina elongata*, a single proximal canal cell supplies the distal tubes and external ribs of three weirs, whose internal ribs and leptotriches are supplied by three terminal cells. Bugge (1902), using light microscopy, showed that, in five species of cestodes, three terminal cells and one proximal canal cell form three flame bulbs, similar to the weir formation in *Austramphilina*, and the pattern of flame bulbs found by Xylander (1987) in *Gyrocotyle* suggests a similar formation. Hence, all cestodes (including the Gyrocotylidea and Amphilinidea), not only have a similar ultrastructure of flame bulbs and protonephridial capillaries, but also a similar development of flame bulbs, supporting the view of their monophyletic origin. These findings do not support the view that Gyrocotylidea are more closely related to the Monogenea than to the Cestoidea (e. g. Llewellyn, 1986). Development of the flame bulbs of trematodes and monogeneans has not yet been examined.

Protonephridia of *Udonella* resemble those of cestodes but differ from those of all Monogenea examined to date in the lack of a septate junction in flame bulbs and capillaries. Cilia of its flame also have very large, cross-striated rootlets, whereas *Gyrodactylus*, *Dactylogyrus*, Ancyrocephalinae gen. sp., *Loimosina* and *Calceostoma* have either very small, rudimentary rootlets, or rootlets are lacking. In addition, the flame bulb of *Udonella* has characteristics not known from any other platyhelminth, i. e. a large number of desmosome-like connections between the weir and the surrounding tissue.

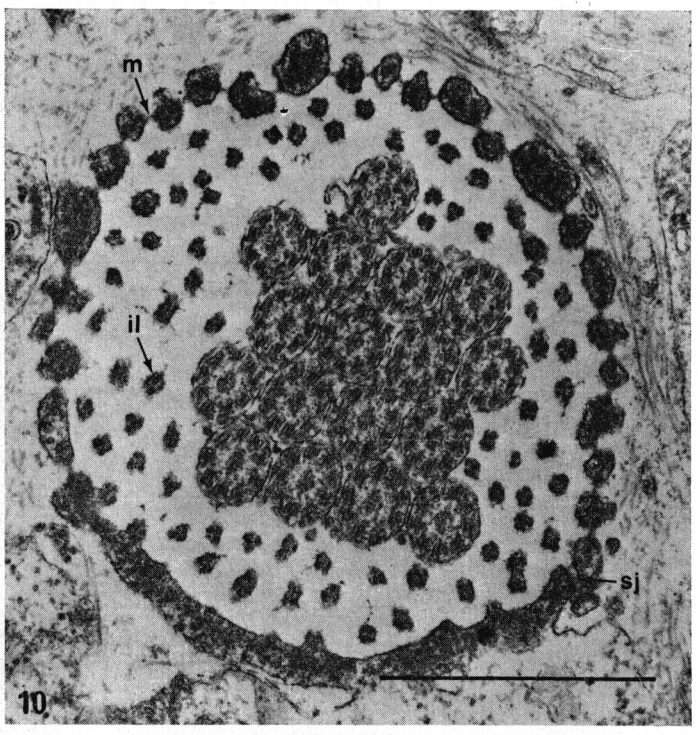
←

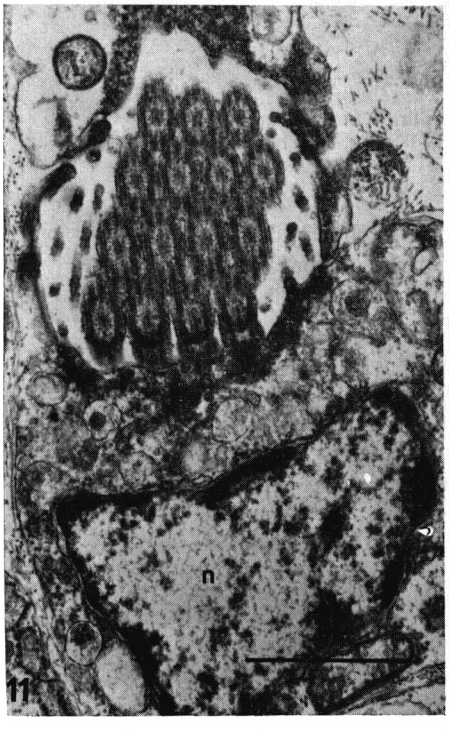
FIGS. 1-4. — Longitudinal sections through flame bulb of *Loimosina*. Note septate junction (sj) along flame bulb in *figs. 1 and 3*, weakly developed ciliary rootlets (cr) in *fig. 4*, space apparently filled with liquid (ls) around flame bulb in *figs. 1-3*, cytoplasmic bridges crossing the space (large arrows in *figs. 1 and 2*), and junction between external rib (or cytoplasmic cord?) and perikaryon of terminal cell (long arrow in *fig. 2*). er, external rib (rod); il, internal leptotrich; ir, internal rib (rod); m, mitochondrion; pc, protonephridial capillary; r, reticulum of spaces in wall of capillary connected to lumen. Scale bars 1 μm.

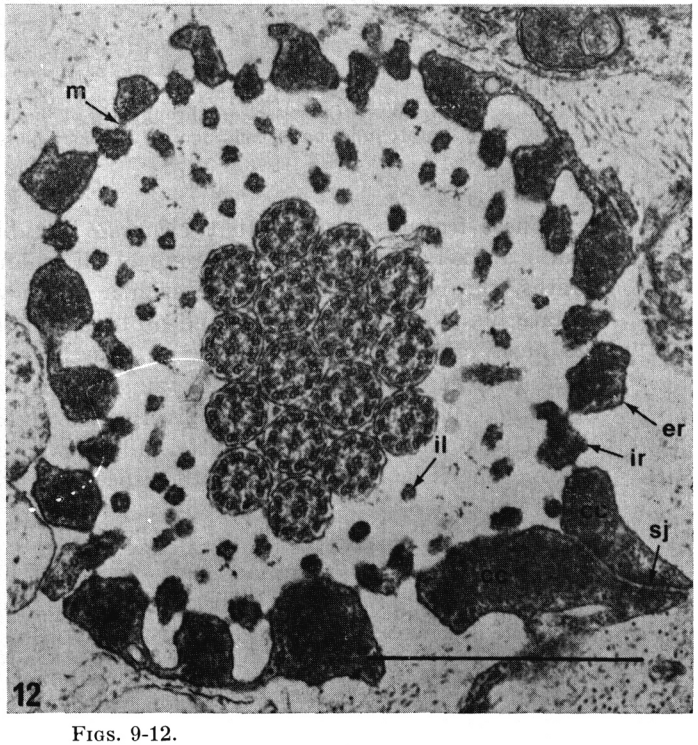


FIGS. 5-8. — Cross-sections through flame bulb of *Loimosina*. Note septate junction (sj) along thick cytoplasmic wall of distal part of flame bulb *(figs. 5, 6)* and along weir *(figs. 7, 8)*, external (er)and internal (ir) ribs (rods) of weir *(figs. 7, 8)*, numerous internal (il) and few external leptotriches (el) *(figs. 7, 8)*. Also note fibres (f) in periphery of liquid filled space (ls) surrounding flame bulb *(figs. 7, 8)*. « Membrane » (m) between ribs of weir sometimes double (large arrows in *fig. 6)* n, nucleus of terminal cell. Scale bars 1 μm.

FIGS. 9-12. — Longitudinal *(fig. 9)*, oblique *(fig. 11)* and cross-sections *(figs. 10, 12)* through flame bulb of *Calceostoma*. Note alternating internal (ir) and external (er) longitudinal ribs of weir, connected by a « membrane » (m), cytoplasmic cords (cc) connected by a septate junction (sj), and internal leptotriches (il). Scale bars 1 μm.







FIGS. 9-12.

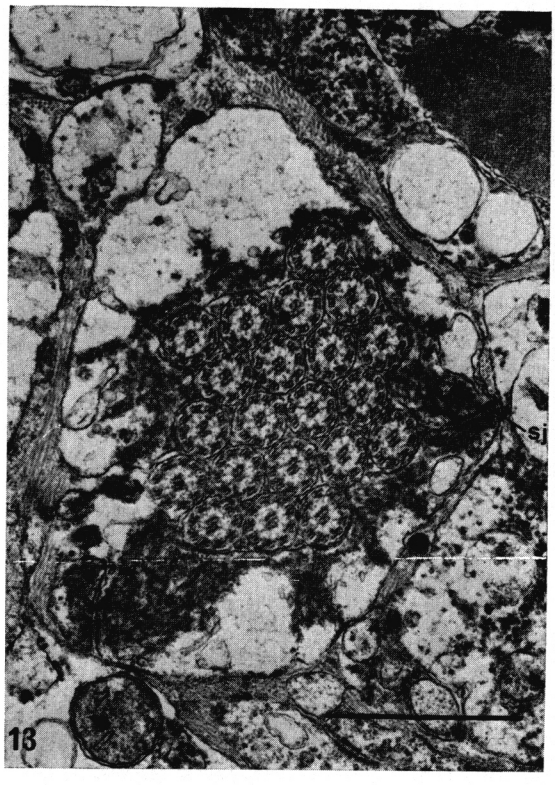


FIG. 13. — Cross-section through tip of flame bulb of *Calceostoma*. Note septate junction (sj) and dense cytoplasm close to flame. Scale bar 1 µm.

These features indicate that *Udonella* is unlikely to be a monogenean. It is also unlikely to be the sister group of all major groups of parasitic platyhelminths, the Neodermata, because such a group would be expected to have a septate junction in flame bulbs and protonephridial capillaries, in common with the most original neodermatans, i. e. the trematodes and monogeneans. The sperm pattern of *Udonella*, corresponding to « pattern 2 » of the Monogenea (Justine *et al.*, 1985) may be due to convergent evolution, as pointed out by Justine *et al.* (1985) and Justine and Mattei (1987), who emphasized the need to study spermiogenesis in *Udonella* (and other species in which only the sperm structure is known).

ACKNOWLEDGMENTS. — Financial support was given by the Australian Research Council (ARC) and the University of New England. The first stages of preparing the material for electron microscopy were done at the Département de Biologie Animale, Faculté des Sciences, Université de Dakar, Sénégal. We wish to thank P. R. Garlick for making facilities at the Electron Microscope Unit, University of New England, available to us, and Mrs V. Watt for typing the paper. Z. Enoch printed the electron micrographs.

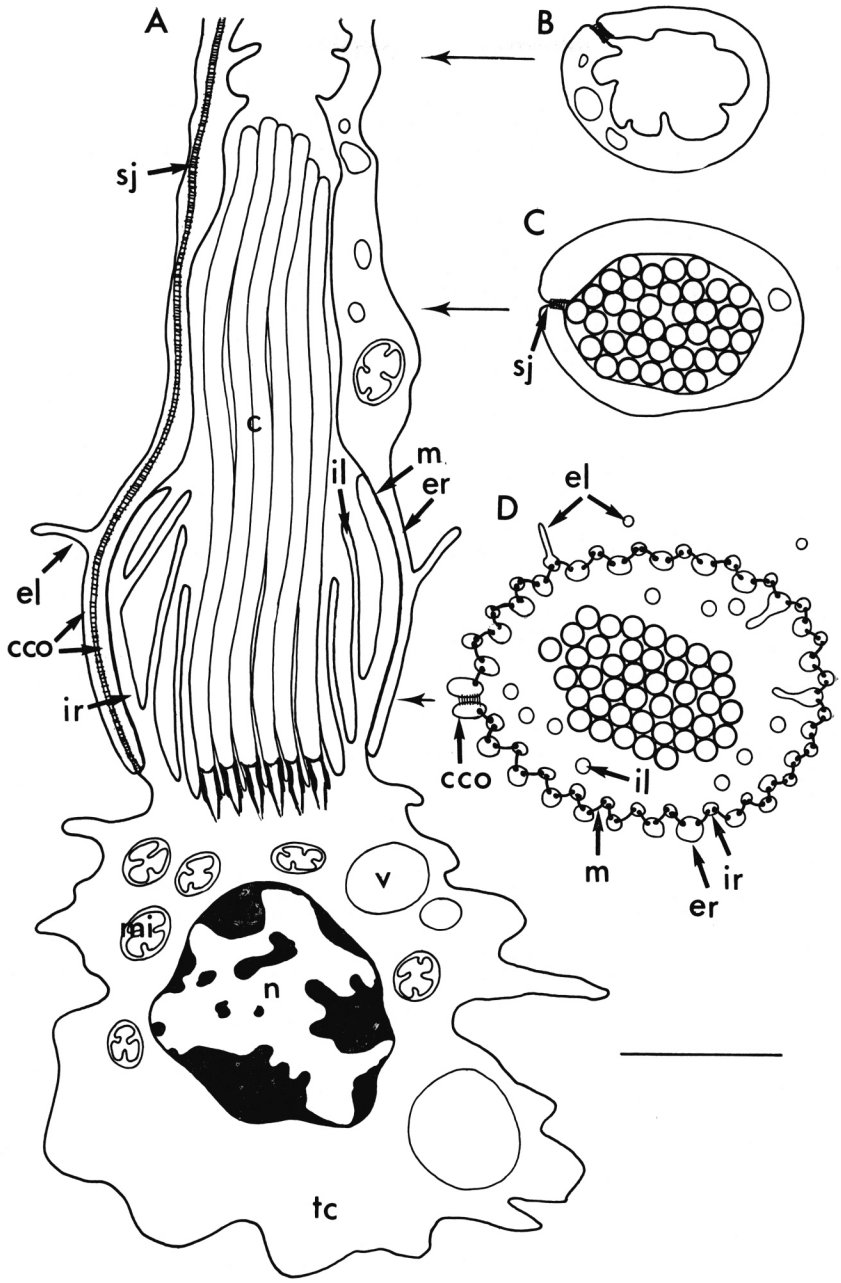


FIG. 14. — Diagrams of flame bulb of *Loimosina*. A. Longitudinal section. B. Cross-section through capillary. C. Cross-section through tip of flame. D. Cross-section through weir. c, cilium; cco, cytoplasmic cord; el, external leptotrich ; er, external rib; il, internal leptotrich; ir, internal rib; m, « membrane »; mi, mitochondrion ; n, nucleus of terminal cell; sj, septate junction; tc, terminal cell; v, vacuole. Scale bar 1 μm.

RÉFÉRENCES

BROOKS D. R., O'GRADY R. T., GLEN D. R. : The phylogeny of the Cercomeria Brooks, 1982 (Platyhelminthes). *Proc. Helminth. Soc. Wash.*, 1985, *52*, 1-20.

BUGGE G. : Zur Kenntnis des Excretionsgefäss-Systems der Cestoden und Trematoden. *Zool. Jahrb. Abt. Morph.*, 1902, *16*, 177-234.

EHLERS U. : Phylogenetisches System der Plathelminthes. *Verh. Naturwiss. Ver. (N. F.)*, 1984, *27*, 291-294.

EHLERS U. : Das phylogenetische System der Plathelminthes. Fischer, Stuttgart, New York, 1985.

JUSTINE J.-L., LAMBERT A., MATTEI X. : Spermatozoon ultrastructure and phylogenetic relationships in the monogeneans (Platyhelminthes). *Int. J. Parasitol.*, 1985, *15*, 601-608.

JUSTINE J.-L., MATTEI X. : Phylogenetic relationships between the families Capsalidae and Dionchidae (Platyhelminthes, Monogenea, Monopisthocotylea) indicated by the comparative ultrastructural study of spermiogenesis. *Zool. Scr.*, 1987, *16*, 111-116.

LAMBERT A. : Oncomiracidiums et phylogenèse des Monogenea (Plathelminthes). Deuxième partie: structures argyrophiles des oncomiracidiums et phylogenèse des Monogenea. *Ann. Parasitol. Hum. Comp.*, 1980, *55*, 281-325.

LLEWELLYN J. : Phylogenetic inference from platyhelminth life-cycle stages. *In: Parasitology. Quo Vadit.* Proceedings of the Sixth International Congress of Parasitology. M. J. HOWELL, ed., Aust. Acad. Sci., Canberra, 1986, 281-289.

ROHDE K. : Ultrastructure of the flame cells of *Multicotyle purvisi* Dawes. *Naturwiss.*, 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. *Adv. Parasitol.*, 1972, *10*, 77-151.

ROHDE K. : Ultrastructure of the protonephridial system of *Polystomoides malayi* Rohde and *P. renschi* Rohde (Monogenea: Polystomatidae). *Int. J. Parasitol.*, 1973, *3*, 329-333.

ROHDE K. : Fine structure of the Monogenea, especially *Polystomoides* Ward *Adv. Parasitol.*, 1975, *13*, 1-33.

ROHDE K. : Some aspects of the ultrastructure of *Gotocotyla secunda* and *Hexostoma euthynni*. *Angew. Parasitol.*, 1980, *21*, 32-48.

ROHDE K. : The flame cells of a monogenean and an aspidogastrean, not composed of two interdigitating cells. *Zool. Anz.*, 1982, *209*, 311-314.

ROHDE K. : Ultrastructure of the protonephridial system of *Lobatostoma manteri* (Trematoda, Aspidogastrea). *J. Submicr. Cyt. Pathol.* 1989, *21*, 599-610.

ROHDE K. : Ultrastructure of the protonephridial system of *Gyrodactylus* (Monogenea, Gyrodactylidae). *Zool. Anz.* (in press).

ROHDE K., WATSON N. : Ultrastructure of the protonephridial system of larval *Austramphilina elongata* (Platyhelminthes, Amphilinidea). *J. Submicr. Cyt. Pathol.*, 1987, *19*, 113-118.

ROHDE K., WATSON N. : Development of the protonephridia of *Austramphilina elongata*. *Parasitol. Res.*, 1988, *74*, 255-261.

ROHDE K., WATSON N., ROUBAL F. : Ultrastructure of flame bulbs, sense receptors, tegument and sperm of *Udonella* (Platyhelminthes) and the phylogenetic position of the genus. *Zool. Anz.*, 1989.

ROHDE K., WATSON N., ROUBAL F. : Ultrastructure of the protonephridial system and sperm of *Dactylogyrus* spp. and an unidentified ancyrocephaline (Monogenea: Dactylogyridae). *Int. J. Parasitol.* (in press).

XYLANDER W. : Ultrastructure of the lycophora larva of *Gyrocotyle urna* (Cestoda, Gyrocotylidea). III. The protonephridial system. *Zoomorph.*, 1987, *107*, 88-95.