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DIVERGENT PROTONEPHRIDIAL ARCHITECTURE WITHIN THE KALYPTORHYNCHIA (PLATYHELMINTHES) AND IMPLICATIONS FOR THE PHYLOGENY OF THE RHABDOCOELA

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Abstract. Transmission electron microscopy of the protonephridial filtration structures in nine species of platyhelminths from five families in Kalyptorhynchia revealed three different types of architecture. Type A, found in representatives of Schizorhynchia and in two species of Cicerinidae (Eukalyptorhynchia), shows a terminal perikaryon associated with each flame bulb, and the bulb is connected to the proximal canal with a septate junction. Type B, found in two other species of Cicerinidae, also has a junction between the flame bulb and the proximal canal but does not have a nucleus in close association with the flame. Type C, found in the representatives of two other families of Eukalyptorhynchia, has no terminal perikaryon and the flame bulb is continuous with the proximal canal without the intervention of a junction. Type A may be considered the most plesiomorphic arrangement described for any rhabdocoel taxon while Type C is highly derived. Type C resembles that found in other orders of Rhabdocoela, namely Typhloplanida, Dalyelliida and Temnocephalida. The distribution of flame bulb types within Kalyptorhynchia, in conjunction with spermiogenesis data, suggests that the Cicerinidae is paraphyletic and that a subtaxon including the genera *Toia*, *Nannorhynchides* and (probably) *Pocillorhynchus* forms a sister group with the Schizorhynchia, while a subtaxon including at least the genera *Cicerina* and *Ptyalorhynchus* forms a sister group with the remaining Eukalyptorhynchia.

Key words: flame cells, protonephridia, Eukalyptorhynchia, Schizorhynchia, Turbellaria

INTRODUCTION

Ultrastructural studies of the terminal regions of protonephridia have contributed significantly to debate about phylogenetic relationships within the phylum Platyhelminthes (see reviews by ROHDE, 1990, 1991; ROHDE *et al.,* 1995). Species assigned to the large taxon Rhabdocoela (exclusive of the parasitic Neodermata) have in common a flame bulb with a weir or filtration apparatus formed from a single row of longitudinal ribs that are supported by bundles of microtubules. This is clearly a derived condition within the phylum and a synapomorphy for those taxa. Moreover, in contrast to non-rhabdocoels, the first several rhabdocoels studied possess flame bulbs with a further modification – lack of a terminal perikaryon near the flame, and no separation between the cytoplasmic cylinder around the flame and the proximal canal (REISINGER, 1923; WILLIAMS, 1981; ROHDE, 1987; ROHDE *et al.*, 1987a,b, 1988). With light microscopic studies, REISINGER (1923) demonstrated that in this kind of arrangement, a single nucleus was associated with a number of flames and that this nucleus was some distance along the capillary removed from the flames. More recently, however, several rhabdocoel taxa that do have a terminal perikaryon have been studied: *Syndisyrinx punicea* (Hickman, 1956) and *Pterastericola pellucida* Jondelius, 1989, have several flames per perikaryon (ROHDE *et al.,* 1992), while the kalyptorhynch *Baltoplana magna* Karling, 1949, and possibly *Luriculus australiensis* Faubel, Rohde & Watson, 1994, have a single flame per perikaryon (ROHDE & WATSON, 1994; ROHDE *et al.,* 1993).

Kalyptorhynchia is one of four recognised divisions of the (non-neodermatan) Rhabdocoela, the others being Dalyelliida, Typhloplanida and Temnocephalida (see system in Cannon, 1986). The arrangement found in the kalyptorhynch *B. magna* (one flame per terminal perikaryon) resembles the situation in orders outside the rhabdocoels. It may, therefore, represent the least derived state within the Rhabdocoela. It differs markedly from the arrangement seen in all examined species of Temnocephalida and Typhloplanida, and from most examined species of the Dalyelliida. This study was undertaken to determine protonephridial architecture in a range of kalyptorhynchs from other families in both sub-orders (Eukalyptorhynchia and Schizorhynchia) to contribute to the understanding of relationships within Kalyptorhynchia and within the rhabdocoels in general.

MATERIAL AND METHODS

The following table provides taxonomic and collection data on the species studied.

Species	*Origin*
EUKALYPTORHYNCHIA	
Cicerinidae	
Cicerina remanei Meixner, 1928	Belgian sandy beach (Mariakerke, July 1995)
Ptyalorhynchus coecus Ax, 1951	Belgian sandy beach (Bredene, July 1995)
Toia calceformis Brunet, 1973	Belgian sandy beach (Mariakerke, September, 1987)
Nannorhynchides herdlaensis (Karling, 1956)	Marine algae, 10-12m (Kristineberg Sweden, 1988)
Gnathorhynchidae	
Ancistrorhynchus ischnurus L'Hardy, 1963	French sandy beach (Roscoff, June 1996)
Polycystididae	
Gyratrix hermaphroditus Ehrenberg, 1831	Brackish sands (Stockholm Sweden, August 1995)

SCHIZORHYNCHIA	
Schizorhynchidae	
Thylacorhynchus conglobatus Meixner, 1928	Belgian sandy beach (Mariakerke, July 1995)
T. pyriferus Karling, 1950	Fjord sandy bottom at 30cms, Kristineberg Sweden, August 1995
Diascorhynchidae	
Diascorhynchus rubrus Boaden, 1963	Belgian sandy beach (Bredene, July 1995)

All were fixed in glutaraldehyde, post-fixed in OsO_4, dehydrated in ethanol and embedded in Spurr or Epon/Araldite resin. Ultrathin serial sections were cut through at least half the body of two individuals of the species *C. remanei*, *D. rubrus* and *T. calceformis*, and of one individual of each of the remaining species. Sections were collected on Formvar-coated parallel-bar grids (75p), stained with uranyl acetate and lead citrate and examined with a JEOL 1200EX transmission electron microscope (TEM). In most cases, strategic photographs were taken through at least two longitudinally and two transversely oriented flame regions for each species, with many additional (but often oblique) flames examined for confirmation of character consistency.

RESULTS

Schizorhynchia

The three species examined in this division are from different families (Schizorhynchidae and Diascorhynchidae), but all showed essentially the same organisation of the terminal regions. Features of the congeners *T. conglobatus* and *T. pyriferus* were identical, so only the former is illustrated. Flame cells were nucleated, and the nucleus was located close to the flame bulb (Figs 1, 3, 5, 50A), although its exact position varied between basal and baso-lateral, even within each individual. Cilia of the flame had rootlets (Figs 1, 5, 7) with cross striations (Fig. 1). The filtration apparatus consisted of a single row of longitudinal ribs containing bundles of microtubules, with extracellular material in slits between the ribs (Fig. 11). Near the top of the flame, the ribs fused into a distal cylinder (Fig. 10), and this cylinder was joined to the proximal canal cell which overlapped it, by a septate junction adjacent to the lumen (Figs 1, 2, 4, 6, 9). Tips of cilia projected a short distance into the capillary (Figs 6, 8). No nucleus of the proximal canal cell was located nearby the flames, and capillaries were formed by an entire cellular cylinder. In *T. conglobatus*, microtubules of the ribs were surrounded with dense material (Figs 7, 10, 11), and dense material also lay beneath the cell membrane along the distal tube (Figs 6, 9). A compact ring of similar material surrounded the most distal part of the tube in *D. rubrus* (Fig. 2), and the proximal canal regions were also lined with a layer of dense cytoplasm that formed microvillar projections (Fig. 4). Such dense cytoplasm and projections were absent from the capillaries of the two *Thylacorhynchus* spp.

Eukalyptorhynchia

Cicerinidae: Four species were examined from this family *Cicerina remanei* and *Ptyalorhynchus coecus* had very similar protonephridial organisation. The perikaryon of the flame bulb was adjacent to the flame (Figs 12, 16) and the filtration apparatus was formed by a single row of ribs supported by bundles of microtubules (Figs 12, 13, 16) with extracellular matrix between the ribs (Figs 13, 16). At the distal end the ribs fused into a tube in which the microtubules continued (Figs 14, 17), and there was a septate junction between this distal tube and the proximal canal (Fig. 50A). They differed in that the proximal canal cell enwrapped the lumen, with a junction between the two adjacent edges, in *P. coecus* but not in *C. remanei*. In *C. remanei*, therefore, the lumen was surrounded by an unbroken cylinder. More distal capillaries of *C. remanei* were similarly without junctions (Fig. 15). Dense material lining the distal tube of the terminal cell was more pronounced in *P. coecus* (Figs 16-18) than in *C. remanei* (Fig. 14). In both species the tips of the flame cilia projected slightly into the proximal canal lumen (Figs 14, 19).

The other two species studied, *Toia calceformis* and *Nannorhynchides herdlaensis,* resembled one another closely but differed from the two cicerinids described above. In *T. calceformis* and *N. herdlaensis* no terminal perikaryon was found despite examination of several sets of serial sections through transverse and longitudinally positioned flames. This was unexpected, since the cytoplasm of the terminal bulb surrounding the flame was clearly separated from the proximal canal by a septate junction (Figs 29, 31, 35, 37, Fig. 50B). In both species, the proximal canal also had a long junction to itself (Figs 29, 30, 35, 36), and the cilia of the flame projected a considerable distance into this lumen (Figs 29, 31, 35). Ribs supported by microtubules were much less regular than in the other cicerinids (Figs 23-28). In *N. herdlaensis* there were also a few internal leptotriches (Fig. 33) and the slits between ribs appeared to be formed both by finger-like interdigitations of the column surrounding the flame and as discrete longitudinal slits (Fig. 34). Cilia of the flames had cross-striated rootlets (Figs 20-22, 32). Lateral flames were present in some capillaries of both species (see Fig. 30 for *T. calceformis),* and there were junctions in capillary walls. No nearby nuclei were identified.

Gnathorhynchidae and Polycystididae: The representatives of these two families, *A. ischnurus* and *G. hermaphroditus* had the same basic arrangement of protonephridial terminal structures as one another, but the arrangement differed from those described above. The filtration apparatus was formed by a single row of longitudinal ribs supported by microtubules (Figs 38-41, 46-48) and these ribs fused into a tube in the distal region of the flame (Figs 42-44, 49, 50C). The slits between the ribs were covered with extracellular material (Figs 40, 47). The arrangement of slits was more regular in *G. hermaphroditus* than in *A. ischnurus*. There was no terminal nucleus and no junction between the flame region and the proximal canal (Figs 42-44, 46); that is the cytoplasmic tube surrounding the top of the flame was continuous with the proximal canal region. In *G. hermaphroditus* but not in *A. ischnurus* dense material lined the inner plasma membrane of the cytoplasmic tube between the end of the ribs and the beginning of the canal region where the cilia terminated (Fig. 44). There was no junction within the wall of the proximal canal (Fig. 43).

DISCUSSION

The protonephridial flame bulbs and capillaries of four other kalyptorhynch species have previously been examined in varying degrees of detail: *Baltoplana magna* (ROHDE & WATSON, 1994), *Gyratrix* sp. and *Odontorhynchus* sp. (ROHDE *et al.*, 1987a) and *Rhinolasius* sp. (ROHDE *et al.,* 1988). All, as well as those reported in the present study, have a filtration apparatus of longitudinal slits between a single row of cytoplasmic ribs that are supported by bundles of microtubules. This architecture is characteristic of all (non-neodermatan) rhabdocoels that have been examined from all subtaxa (see review by ROHDE, 1991 and earlier references therein; LUMBSCH *et al.,* 1995; ROHDE *et al.,* 1992; WILLIAMS, 1994). It has not been found outside of the Rhabdocoela with the single exception of the prolecithophoran *Archimonotresis limophila* (see EHLERS, 1989), but other prolecithophorans studied do not share this arrangement (EHLERS & SOPOTT-EHLERS, 1997, WATSON & ROHDE personal observations).

The results presented here together with the previous studies of other kalyptorhynchs, reveal three different arrangements of protonephridial components within Kalyptorhynchia, illustrated in Fig. 50. Type A: two species of Cicerinidae (*C. remanei* and *P. coecus*) and all examined schizorhynchs (*B. magna*, *Thylacorhynchus* spp and *D. rubrus*) from three different families have a terminal perikaryon and a septate junction between the terminal cell which forms the filter region, and the adjacent proximal canal. The canal is an entire cylinder in the schizorhynchs and in *C. remanei*, but a junction is present in *P. coecus*. Type B: *T. calceformis* and *N. herdlaensis* resemble Type A but no nucleus of the terminal «cell» could be located. Serial sections were followed completely through six individual flame cells of *T. calceformis* and one of *N. herdlaensis* but no nucleus was found. We cannot, however, rule out the possibility that a perikaryon is located at a considerable distance from the flame bulb, possibly connected to it by a very thin cytoplasmic strand, since it is hard to imagine how such an active cell could function without a nucleus. Both species have an extensive junction along the proximal canal, joining the edges of the enwrapping cell, as well as the pronounced septate junction between the flame/filter region and the proximal canal. Type C: species examined from the eukalyptorhynch families Gnathorhynchidae, Polycystididae and Koinocystidae have protonephridia without a terminal perikaryon, no junction separating the filter region from the proximal canal and no junction within the wall of the proximal canal. This corresponds to the arrangement found in all other rhabdocoels that have been studied with the exceptions of the dalyelliids *Syndisyrinx punicea*, *Pterastericola pellucida* and *Luriculus australiensis* (ROHDE *et al.,* 1992; ROHDE *et al.,* 1993). Type C may be considered the most derived state since it lacks the terminal perikaryon found in all other flatworm groups as well as all outgroups with protonephridia (see BARTOLOMAEUS & AX, 1992). Type A represents the most plesiomorphic condition within Rhabdocoela, having major elements in common with outgroups within the Platyhelminthes. Light microscopy has shown that in the Type C state in typhloplanids, a number of flame bulbs that lack nuclei, together with their proximal canal regions, join smaller branches and then the main longitudinal protonephridial ducts, where the few nuclei are located (REISINGER, 1923).

The two species with Type B protonephridia, placed by conventional classification into the family Cicerinidae (see CANNON, 1986), nevertheless have synapomorphies which separate them from other cicerinids (DE VOCHT, 1992), such as eyes with lenses and sperm without two incorporated axonemes. DE VOCHT (1992) linked a third genus, *Pocillorhynchus,* with *Toia* and *Nannorhynchides* because it has lenses in the eyes (2 lens elements in *Pocillorhynchus,* 1 in *T. calceformis* and 3 in *N. herdlaensis*) as well as other synapomorphies of the proboscis bulb musculature and glands. The four cicerinids studied here are all clearly distinguished from other eukalyptorhynchs in possessing the less derived type of protonephridia with a distinct junction between terminal and canal cells, and in this regard they closely resemble the schizorhynchs. However, *T. calceformis* and *N. herdlaensis* also share with schizorhynch taxa a reduction in the number of incorporated axonemes in the sperm brought about by a comparable process during spermiogenesis (WATSON in press). This contrasts with the presence of two fully incorporated axonemes in the sperm of *C. remanei, P. coecus* and all other examined eukalyptorhynchs (see L'HARDY, 1988; DE VOCHT, 1992; personal observations). It therefore appears likely that Cicerinidae in its conventional composition is paraphyletic, and that a subset of that family including the genera *Toia*, *Nannorhynchides* and probably *Pocillorhynchus* forms a sister group to the schizorhynch kalyptorhynchs. A further subset of species probably forms a sister group with the remainder of the Eukalyptorhynchia.

The species that have been examined from three other eukalyptorhynch families have a protonephridial architecture resembling that found in typhloplanids, temnocephalids and the majority of dalyelliids, *i.e.* a highly derived arrangement. However, if the Kalyptorhynchia is a monophyletic taxon with the apomorphies of a muscular proboscis and fused axonemes in the spermatozoon, then Type C protonephridia may have evolved at least twice (separately within the eukalyptorhynchs and in other rhabdocoel taxa). An alternative scenario homologising the derived protonephridia of these eukalyptorhynchs with those found in other rhabdocoels would require the assumption of loss of the proboscis in the other rhabdocoel taxa, or the development of a proboscis in three separate lineages.

Clearly, ultrastructural studies of protonephridia can make an important contribution to elucidation of phylogenetic relationships within the Platyhelminthes and especially within the Rhabdocoela. There are still many families within the four main rhabdocoel taxa (Kalyptorhynchia, Typhloplanida, Dalyelliida and Temnocephalida) where no species have been studied in this regard. Such further studies are needed to clarify the distribution of protonephridial types and the likely relationships between them.

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REFERENCES

BARTOLOMAEUS, T. & P. AX (1992) – Protonephridia and Metanephridia – their relation within the Bilateria. *Zeitschrift für zoologische Systematik und Evolutionsforschung*, **30**: 21-45.

CANNON, L.R.G. (1986) – Turbellaria of the World. A Guide to Families and Genera. Queensland Museum, Brisbane: 131pp.

DE VOCHT (1992) – Anatomy and ultrastructure of the proboscis in Eukalyptorhynchia (Platyhelminthes, Rhabdocoela). PhD Thesis, Limburgs Universitair Centrum, Belgium. 260 pages.

EHLERS, U. (1989) – The protonephridium of *Archimonotresis limophila* Meixner (Plathelminthes, Prolecithophora). *Microfauna Marina*, **5**: 261-275.

EHLERS, U. & B. SOPOTT-EHLERS (1997) – Ultrastructure of protonephridial structures within the Prolecithophora (Plathelminthes). *Microfauna Marina*, **11**: 291-315.

L'HARDY, J.-P. (1988) – Sperm morphology in Kalyptorhynchia (Platyhelminthes, Rhabdocoela). *Fortschritte der Zoologie*, **36**: 303-307.

LUMBSCH, M., U. EHLERS & B. SOPOTT-EHLERS (1995) – Proximal regions of the protonephridial system in *Pseudograffilla arenicola* (Plathelminthes, Rhabdocoela): ultrastructural observations. *Microfauna Marina*, **10**: 67-78.

REISINGER, E. (1923) – Untersuchungen über Bau und Funktion des Excretionsapparates bei rhabdocölen Turbellarien. 2. Über die Terminalorgane und das Kanalsystem einiger bekannter Typhloplaniden. *Zoologischer Anzeiger*, **56**: 205-224.

ROHDE, K. (1987) – Ultrastructure of the flame cells and protonephridial capillaries of *Craspedella* and *Didymorchis* (Platyhelminthes, Rhabdocoela). *Zoomorphology*, **106**: 346-351.

ROHDE, K. (1990) – Phylogeny of Platyhelminthes, with special reference to parasitic groups. *International Journal for Parasitology*, **20**: 979-1007.

ROHDE, K. (1991) – The evolution of protonephridia of the Platyhelminthes. *Hydrobiologia*, **227**: 315-321.

ROHDE, K., L.R.G. CANNON & N. WATSON (1987a) – Ultrastructure of epidermis, spermatozoa and flame cells of *Gyratrix* and *Odontorhynchus* (Rhabdocoela, Kalyptorhynchia). *Journal of Submicroscopic Cytology*, **19**: 585-594.

ROHDE, K., L.R.G. CANNON & N. WATSON (1988) – Ultrastructure of the flame bulbs and protonephridial capillaries of *Gieysztoria* sp. (Rhabdocoela Dalyelliida), *Rhinolasius* sp. (Rhabdocoela Kalyptorhynchia) and *Actinodactylella blanchardi* (Rhabdocoela Temnocephalida). *Journal of Submicroscopic Cytology and Pathology*, **20**: 605-612.

ROHDE, K., A.M. JOHNSON, P.R. BAVERSTOCK & N.A. WATSON (1995) – Aspects of the phylogeny of Platyhelminthes based on 18S ribosomal DNA and protonephridial ultrastructure. *Hydrobiologia*, **305**: 27-35.

ROHDE, K. & N.A. WATSON (1994) – Ultrastructure of the terminal parts of the protonephridial system of *Baltoplana magna* (Platyhelminthes, Kalyptorhynchia, Schizorhynchia, Karkinorhynchidae). *Malaysian Journal of Science*, **15A**: 13-18.

ROHDE, K., N. WATSON & L.R.G. CANNON (1987b) – Ultrastructure of the protonephridia of *Mesostoma* sp. (Rhabdocoela, 'Typhloplanoida'). *Journal of Submicroscopic Cytology*, **19**: 107-112.

ROHDE, K., N.A. WATSON & A. FAUBEL (1993) – Ultrastructure of the epidermis and protonephridium of an undescribed species of Luridae (Platyhelminthes: Rhabdocoela). *Australian Journal of Zoology*, **41**: 415-421.

ROHDE, K., N.A. WATSON & U. JONDELIUS (1992) – Ultrastructure of the protonephridia of *Syndisyrinx punicea* (Hickman, 1956) (Rhadocoela, Umagillidae) and *Pterastericola pellucida* (Jondelius, 1989) (Rhabdocoela, Pterastericolidae). *Australian Journal of Zoology*, **40**: 385-399.

WATSON, N.A. (in press) – Spermiogenesis and eyes with lenses in two kalyptorhynch flatworm species, *Toia calceformis* and *Nannorhynchides herdlaensis* (Eukalyptorhynchia, Platyhelminthes). *Invertebrate Biology.*

WILLIAMS, J.B. (1981) – The protonephridial system of *Temnocephala novaezealandiae*: structure of the flame cells and main vessels. *Australian Journal of Zoology*, **29**: 131-146.

WILLIAMS, J.B. (1994) – Ultrastructural observations on *Temnocephala minor* (platyhelminthes, Temnocephaloidea), including notes on endocytosis. *New Zealand Journal of Zoology*, **21**: 195-208.

Legends to the figures (see pages 149-158)

Figs 1-4. – TEM of flame cells of *Diascorhynchus rubrus*. C, canal cell; F, cilia of the flame; J, junction; TCN, terminal cell nucleus. Fig. 1. – Longitudinal section showing basal nucleus, long roots of the cilia and microtubules extending deeply below the ribs. Fig. 2. – Distal region of the flame showing microtubules in the ribs (arrowhead), septate junction between the terminal cell and the canal cell, and dense region at the tip of the terminal cell (large arrow). Fig. 3. – Two close flame cells, each with a somewhat lateral nucleus. Fig. 4. – Oblique transverse section at the distal tip of a flame cell. Note microtubules (arrowhead) in the terminal cell, junction with the canal cell, dense region at the tip of the terminal cell (large arrow) and canal lumen lined with dense cytoplasm with microvillus-like projections into the lumen. Scales 0.5μm (Figs 2, 4), 1μm (Fig. 1), 2μm (Fig. 3)

Figs 5-11. – TEM of 2 flame cells of *Thylacorhynchus conglobatus*. Figs 5-7. – Longitudinal section of one cell, Figs 8-11 Transverse section of second cell. C, canal cell; E, extracellular matrix; F, cilia of the flame; J, junction; TCN, terminal cell nucleus; R, rib. Fig. 5. – Longitudinal section showing lateral nucleus, rootlets of the cilia (arrow) and dense material in the ribs (arrowhead). Fig. 6. – Distal region of the flame showing microtubules in the ribs surrounded by dense material (arrowhead), continuation of dense material beyond the tips of the microtubules (double arrowhead), septate junction between terminal and canal cells and cilia projecting into the lumen of the canal cell. Fig. 7. – Basal end of the flame bulb showing basal bodies and rootlets (arrow) together with microtubules that are continuous with the ribs (arrowhead). Fig. 8. – Distal tip of the flame where cilia project into the lumen of the proximal canal cell. Fig. 9. – Less distal region where mainly only dense material lines the terminal cell, and a continuous septate junction joins terminal and canal cells. Fig. 10. – Still less distal. All but one of the slits has terminated leaving a cytoplasmic cylinder containing microtubules in dense material (arrowhead) surrounding the cilia of the flame. Fig. 11. – Basal level of the flame showing basal bodies of some cilia, terminal plates of other cilia, ribs supported by microtubules surrounded by dense material (arrowhead) and joined by extracellular matrix in the slits. Scales 0.2µm (Figs 6-11), 1µm (Fig. 5).

Figs 12-15. – TEM of flame cells and capillaries of *Cicerina remanei*. C, canal cell; E, extracellular matrix; F, cilia of the flame; J, junction; TCN, terminal cell nucleus; R, rib. Fig. 12. – Mid-level transverse section showing the nucleus and ribs supported by microtubules (arrowhead) surounding the flame. Fig. 13. – Ribs supported by microtubules (arrowhead) are joined by extracellular matrix across the intervening slits. Fig. 14. – Two flames, the top one at its tip where cilia project into the lumen of the canal cell. Note septate junction joining terminal and canal cells. Fig. 15. – Extensive region of expanded protonephridial capillaries (arrows) just beneath the sub-epidermal muscle layers. Scales 0.5µm

Figs 16-19. – TEM of the one flame bulb of *Ptyalorhynchus coecus*. C, canal cell; E, extracellular matrix; F, cilia of the flame; J, junction; R, rib; TCN, terminal cell nucleus. Fig. 16. – Mid-flame level showing terminal cell nucleus, ribs and extracellular matrix, and microtubules in ribs (bottom arrowhead) and in the cellular column above the termination of ribs (top arrowhead). Fig. 17. – Higher up the flame where some cilia have terminated, the flame is surrounded by a cellular column still containing bundles of microtubules, and the canal cell partly surrounds the terminal cell and is joined to it by a septate junction. Fig. 18. – Few cilia remain (double arrowheads) at the tip of the flame and the terminal cytoplasmic column (TC) with microtubules still visible (arrowhead) is no longer closed. The canal cell surrounds the tip of the terminal cell and shows a septate junction to itself. Fig. 19. – Only one cilium from the flame remains (double arrowhead), surrounded by the canal cytoplasm which is joined to itself by a septate junction. Scales 0.5µm.

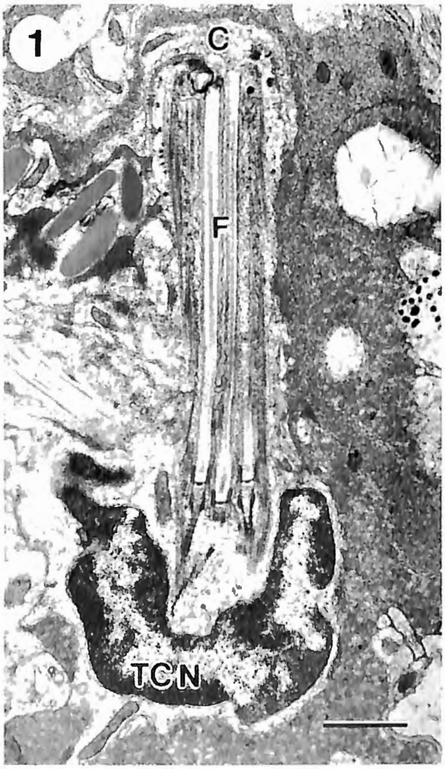
Figs 20-29. – TEM showing part of a series through the one flame bulb of *Toia calceformis* from base (Fig. 20) to capillary (Fig. 29). At first rootlets only are visible (arrows), followed by basal bodies (BB) and microtubules (arrowheads). Ribs (R) with extracellular matrix (E) between them are few and rather irregular along the cytoplasmic column of the terminal cell (TC). Fig. 29 shows that the canal cell, with a junction to itself, enwraps the higher parts of the flame cell column and is joined to it by a septate junction. There is no nucleus closely associated with the flame nor with any nearby region of the capillary. Scales 0.2µm.

Figs 30-31. – TEM of parts of flame cells and capillaries of *Toia calceformis*. Fig. 30. – A single cilium in the narrow lumen of a capillary cell (C) which has a long junction (J) from the lumen to the outer cell membrane. Note long rootlets (arrows) of cilia of a lateral flame, and microtubules in capillary cell cytoplasm (arrowhead). Fig. 31. – The top of a flame cell where the terminal region is joined by a junction (J) to the capillary (C). Ribs (R) and microtubules (arrowhead) are still visible in the terminal cytoplasm. No nucleus was found associated with the flame nor with any nearby region of the capillary. Scales 0.5µm (Fig. 30), 0.2µm (Fig. 31).

Figs. 32-37. – TEM showing part of a series through a single flame bulb and capillary of *Nannorhynchides herdlaensis*. Fig. 32. – Long rootlets (arrows) in the basal cytoplasm accompanied by microtubules (arrowheads). Fig. 33. – Oblique view through the flame showing cilia, microtubules in the wall (arrowhead), ribs (R) with extracellular matrix (E) between them, and one of several internal leptotriches (L). Fig. 34. – Oblique section through the flame wall showing ribs (R) and extracellular matrix (E). Note that in one case (on the left) the extracellular material goes completely around a rib tip, suggesting that this «rib» is an interdigitation with the distal part of the column. Fig. 35. – Cilia of the flame (F) extend beyond the ribs into the adjacent capillary. There is a junction between the terminal and capillary regions (J on right) and another within the capillary itself (J on left). Fig. 36. – Enlargement from Fig. 35 of the junction (J) within the capillary cell (C). Fig. – 37. A more tangential section through the junction region to the right in Fig. 35. Note cilia of the flame (F), capillary cytoplasm (C), junction (J) and terminal cell cytoplasm (TC). No nucleus was found associated with the flame nor with any nearby region of the capillary. Scales 0.5µm.

Figs 38-43. – Part of a series of serial sections through two adjacent flame cells of *Ancistrorhynchus ischnurus*. BB, basal bodies; F, flame; C, capillary cytoplasm; R, ribs; arrow, rootlets; arrowhead, microtubules. The top cell of the two is depicted from the basal cytoplasm with rootlets in Fig. 38 through to the diminished flame surrounded by a cytoplasmic column without ribs in Fig. 43. The lower cell is depicted from a level of the flame just above basal bodies (surrounding ribs contain bundles of microtubules with extracellular matrix between them), to the level of the disappearance of the cilia and a collapsed lumen (arrow) in the capillary in Fig. 43. There is no nucleus associated with the flame nor with any nearby region of the capillary. Scales 0.5µm.

Figs 44-49. – TEM of flame cells of *Gyratrix hermaphroditus*. Figs 44-46 show longitudinal sections of the one cell. C, capillary cytoplasm; F, cilia of the flame; R, ribs; T, cytoplasmic tube. In this species the cytoplasm is continuous from below the cilial rootlets (arrow in Fig. 45) through the ribs with bundles of microtubules (arrowheads) (Figs 46-48), to the cytoplasmic tube still with microtubules (in Figs 44 and 49), to the capillary beyond the tip of the flame. There is no nucleus associated with the flame nor with any nearby region of the capillary. Scales 0.2µm (Fig. 44), 0.5µm (Figs 45-49).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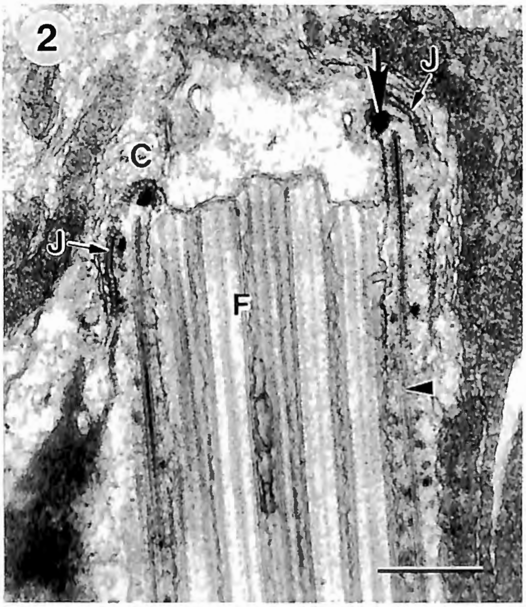
C
F
TCN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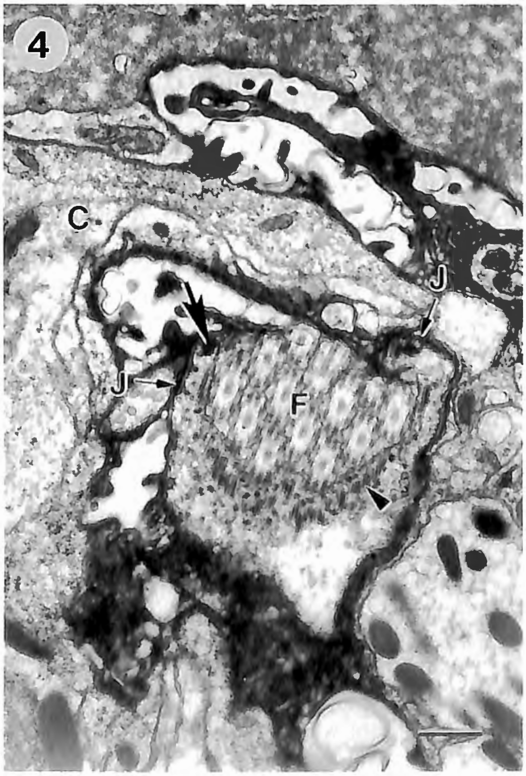
C
J
F


3
F
TCN
F
TCN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C
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J
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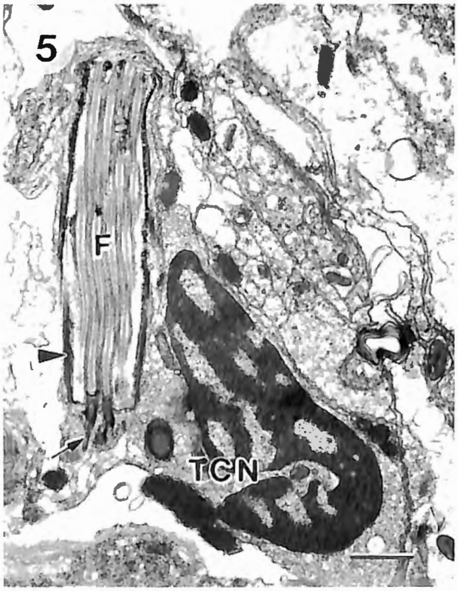
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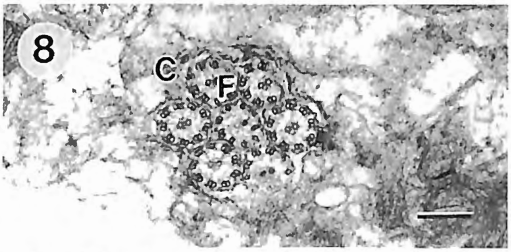
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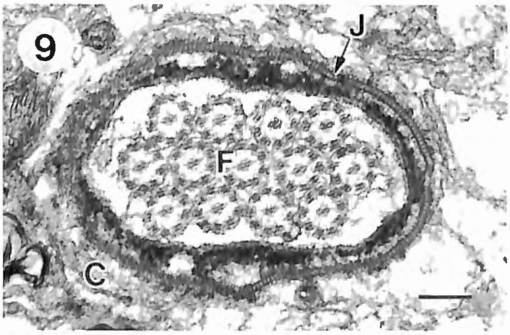
F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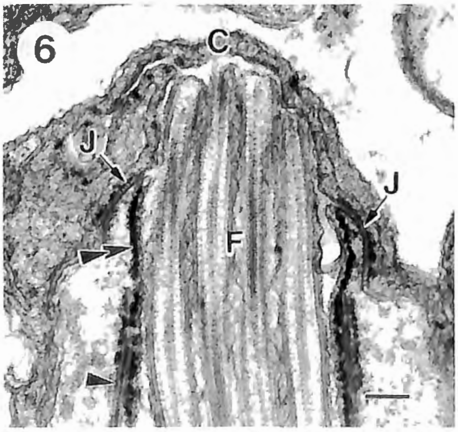
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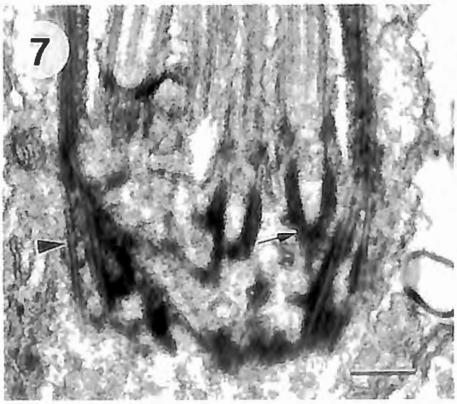
C
J
J
F


10
F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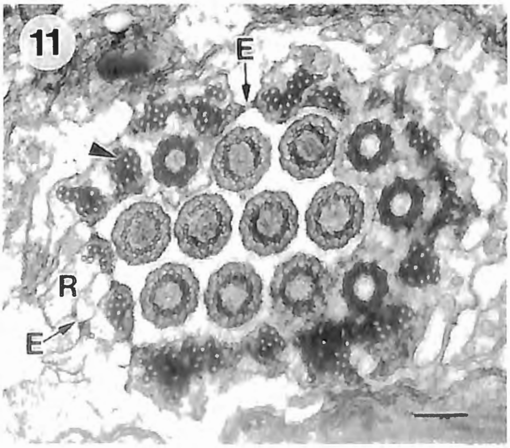
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E
R
E


12
TCN
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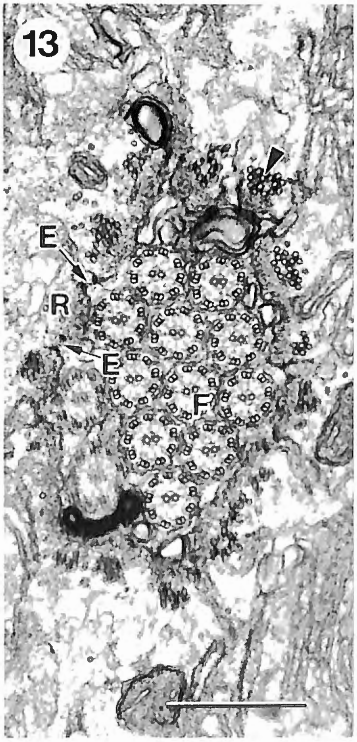
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E
R
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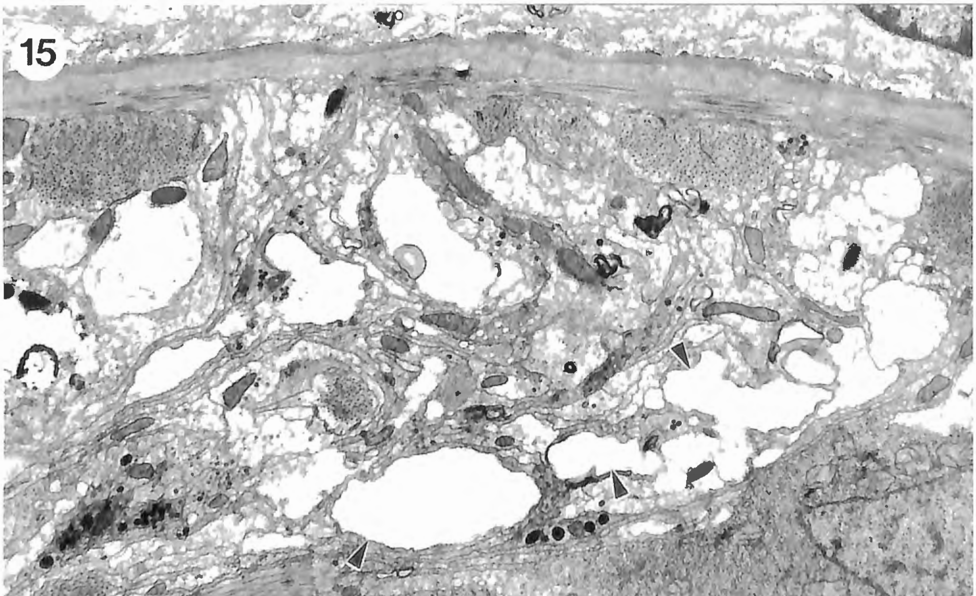
F


14
C
F
J
F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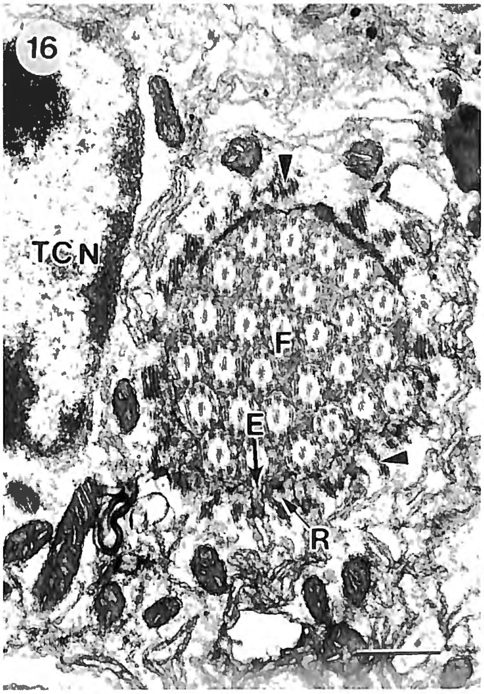
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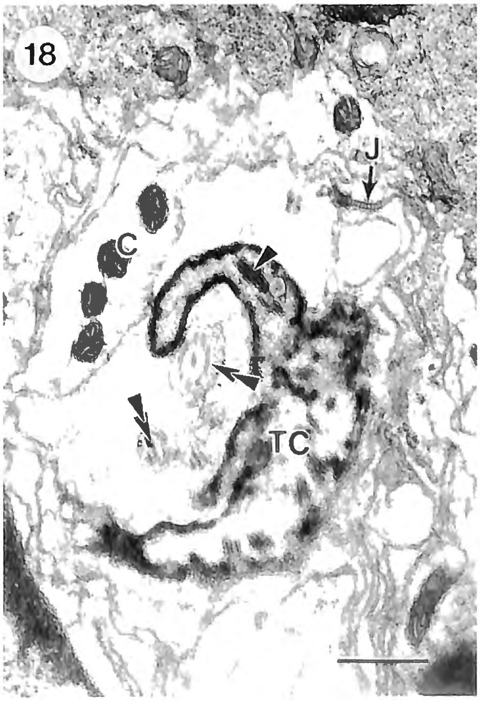
TCN
F
E
R


17
C
J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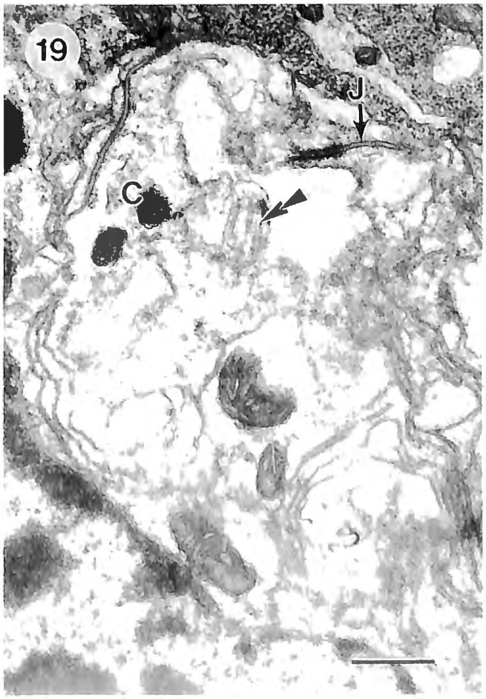
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C


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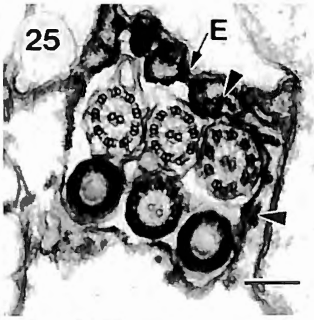
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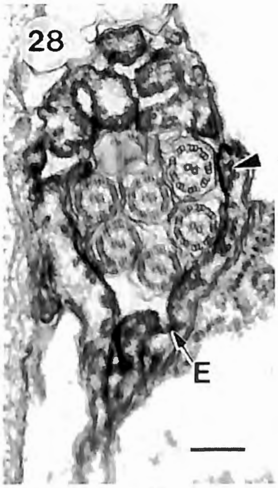
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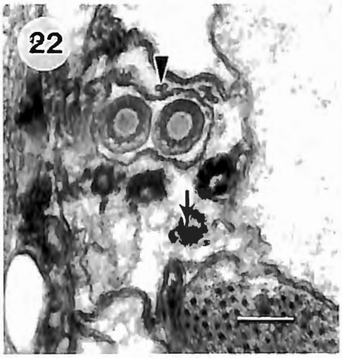
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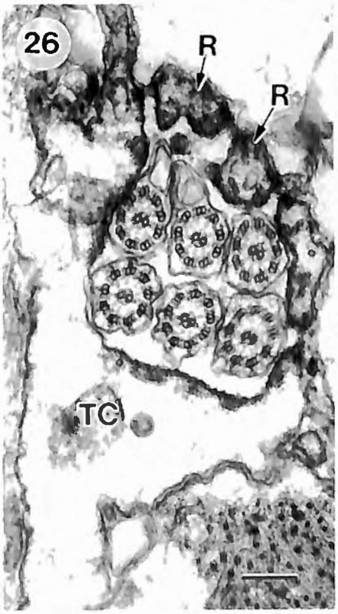
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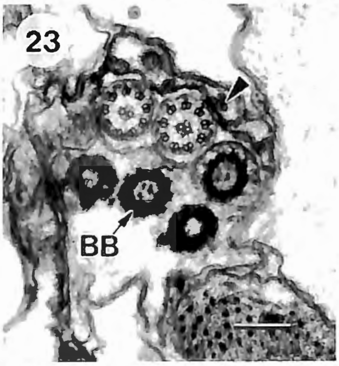
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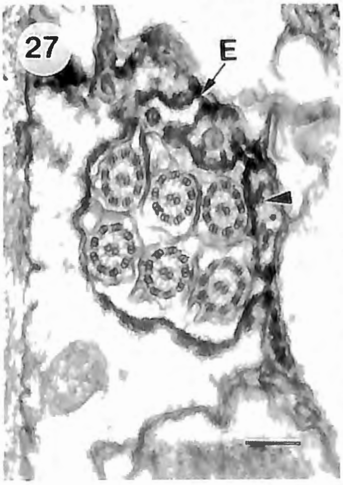
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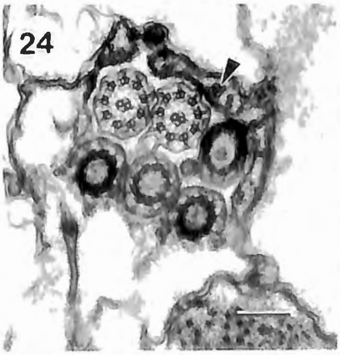
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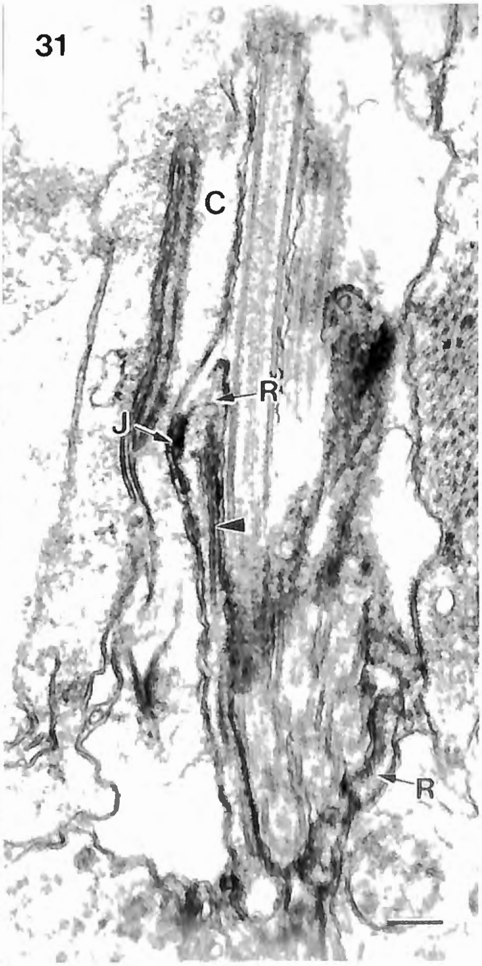
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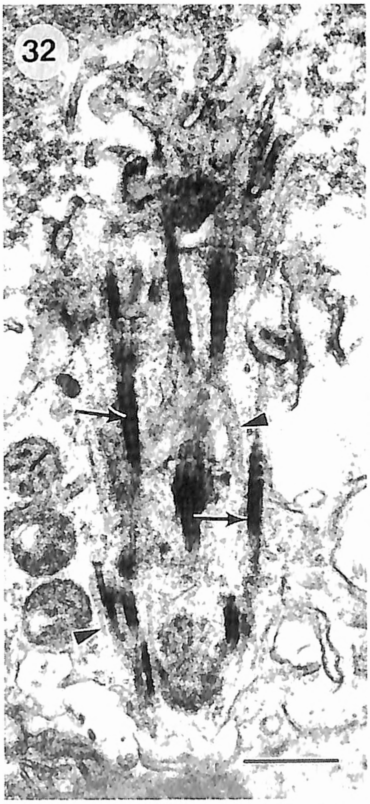
C
R
J
R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33
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R
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34
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R
R
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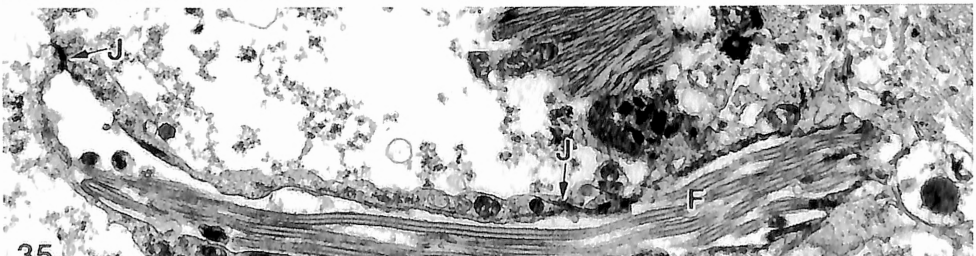
F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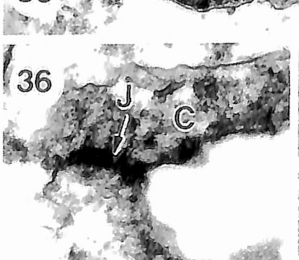
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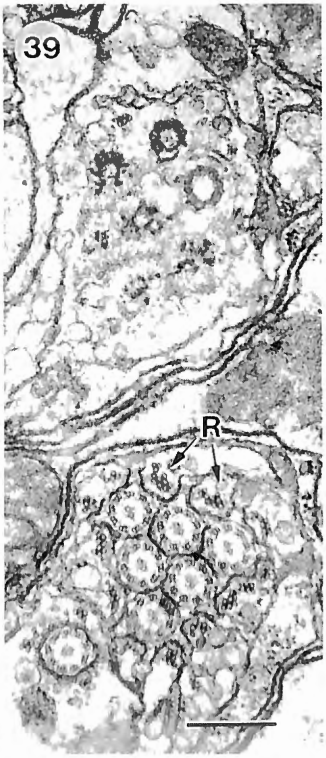
J
C


37
J
C
TC
F
J


38
F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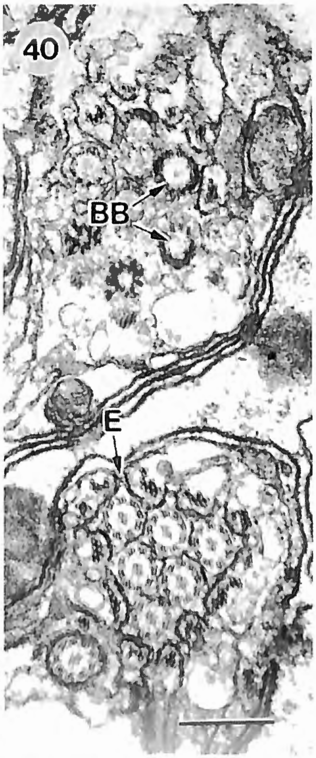
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BB
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41
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42


43
C


44
C
F
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45
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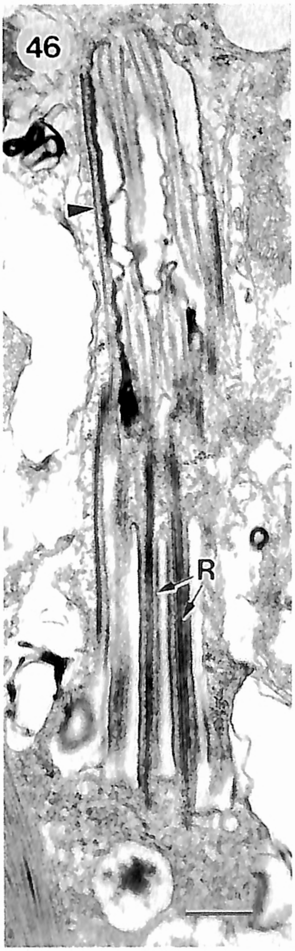
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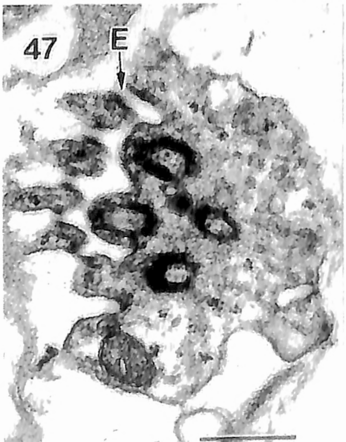
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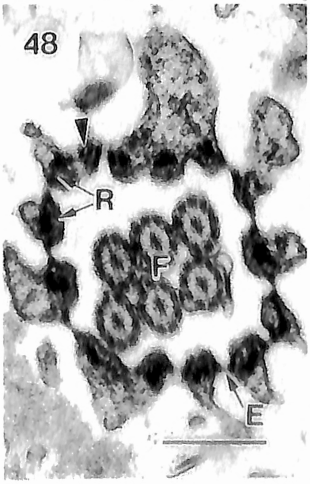
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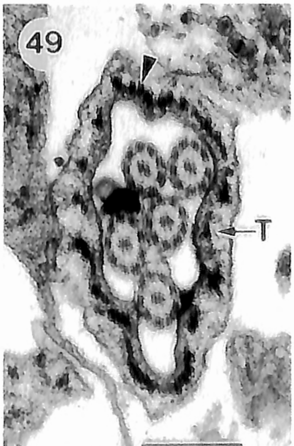
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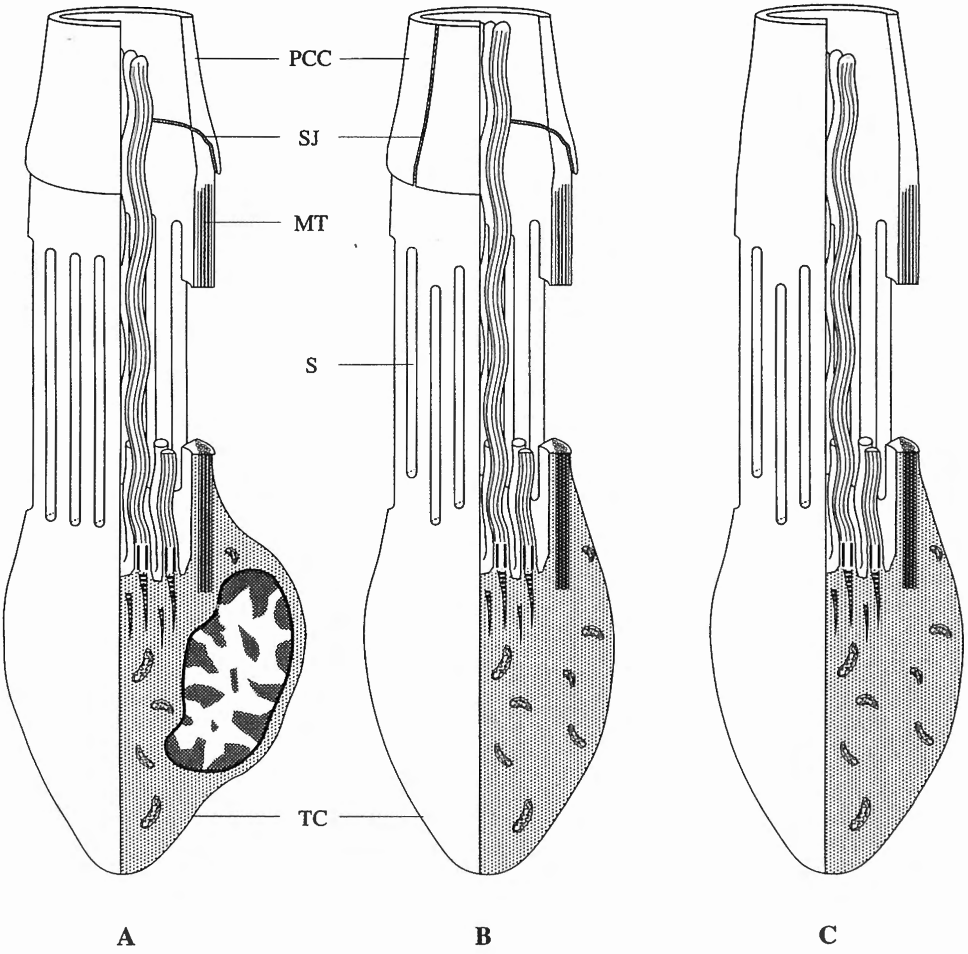

Fig. 50. – Diagrammatic representation of the terminal protonephridial filtration regions in kalyptorhynch turbellarians. Type A found in *Ptyalorhynchus coecus, Cicerina remanei, Thylacorhynchus* spp. and *Diascorhynchus rubrus*. In *P. coecus*, the canal cell enwrapped the lumen and a junction was thus formed between the adjacent edges. Type B found in the two cicerinids *Toia calceformis* and *Nannorhynchides herdlaensis*. No nucleus could be located for the «terminal cell». Type C found in the gnathorhynchid *Ancistrorhynchus ischnurus* and the polycystid *Gyratrix hermaphroditus*. In Type C the filtration region is continuous with the proximal canal and no nuclei are found in the vicinity.

MT, microtubules, PCC, proximal canal cell; S, filtration slits; SJ, septate junction; TC, terminal cell.