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The protonephridium of *Moniezia expansa* (Cestoda: Cyclophyllidea), with comments on the protonephridium concept *

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Abstract Transmission electron microscopic observations have been made on the protonephridium of the cestode *Moniezia expansa*. The flame bulb of *M. expansa* has a filtration apparatus constructed as a "weir" and consisting of ribs (rods) which are outgrowths of the terminal cell as well as the proximal canal cells. The ribs are connected by a "membrane" of extracellular substance through which filtration is believed to occur. Pores connect the protonephridial lumen to the intercellular space of connective tissue (parenchyma) and are located at the junction of a flame cell and a proximal canal cell. The cytoplasmic cords of protonephridial capillaries do not contain a septate junction. The surface area of the capillaries and other protonephridial ducts lining the lumen is enlarged by many small bead-like microvilli. From the present study of *M. expansa* and from those of protonephridia in other invertebrates, it is suggested that the concept of defining a protonephridium as having a closed inner end is improper. We suggest its definition should be modified as follows: a protonephridium is a flame cell system consisting of flame cells, canal cells and nephropore cells and that the flame bulb functions as a filtration apparatus with or without slits (pores) opening to the intercellular space of connective tissue (parenchyma) [*Acta Zoologica Sinica* 50 (4): 638 - 644, 2004].

Key words Platyhelminthes, Cestode, *Moniezia expansa*, Excretory/osmoregulatory system, Protonephridium, Protonephridium concept

扩张莫尼茨绦虫 (绦虫纲: 圆叶目) 的原肾管及原肾管概念的评述 *

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摘 要 本研究应用透射电子显微镜研究了扩张莫尼茨绦虫原肾管的细胞学特征, 莫尼茨绦虫原肾管的焰茎球为一个过滤器结构, 类似于"挡河坝"样构造, 此构造由端细胞和近管细胞外突形成的肋条 (或称杆) 相互交错排列而成。肋条之间由细胞外物质构成的"膜"结构连接, 过滤作用通过该"膜"发生。焰细胞与近管细胞交界处有裂缝或孔与细胞外的结缔组织 (实质组织) 相通; 原肾管的毛细排泄管细胞质索之间没有隔状联结; 毛细排泄管及排泄管的管腔内有大量珠状微绒毛突起以增加表面积。从扩张莫尼茨绦虫及其它一些无脊椎动物原肾管的研究结果表明, 原原肾管概念将焰细胞作为封闭的盲端已不再合适, 需要进行修订, 建议修订为: 原肾管是一种焰细胞系统, 通常由焰细胞、管细胞和肾孔细胞组成, 焰茎球作为过滤装置与周围的结缔组织 (实质组织) 有或没有裂缝 (孔) 相通 [动物学报 50 (4): 638 - 644, 2004]。

关键词 扁形动物 绦虫 扩张莫尼茨绦虫 排泄/渗透调节系统 原肾管 原肾管概念

The protonephridial system is the common type of excretory and/or osmoregulatory system of acoelomates including platyhelminths, some pseudocoelomates and certain larvae and adults of coelomates and is based on the flame cell. The flame cells are connected by small convoluted tubules to larger, collect-

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ing ducts which join to form a muscular bladder that opens externally via an excretory pore. The major function of the protonephridial system seems to be the regulation of body fluid composition, including maintenance of water content, and in the excretion of waste. It plays a pivotal role in maintaining homeostasis. The concept of the protonephridium just described is traditionally perceived as with one end closed system. Thus, the tubules are closed on the inner end and excretory waste is concentrated from a fluid that must first enter the tubules by being transported across flame cells (Hickman et al., 2001). In an earlier study, Howells (1969) found that in the nephridial system of the cyclophyllidean tapeworm *Moniezia expansa* pores, which connect the nephridial lumen to the intercellular space of connective tissue, exist at the junction of a flame cell and a nephridial duct. Howells suggested that those pores might be considered nephrostomes and that the system is, therefore, not protonephridial as was originally envisaged. The main aim of the present study was to ascertain the type of excretory and/or osmoregulatory system of *M. expansa* using transmission electron microscopy and, secondly, to add comments on the protonephridium concept of invertebrates in general.

1 Materials and methods

Adult specimens of *M. expansa* were collected from the intestine of sheep slaughtered at Lurgan abattoir, near Belfast, UK. Worms were placed in warm 0.9% physiological saline and maintained at 35 - 37 ℃ in a vacuum flask for transport to the laboratory. They were quickly cut into small (approximately 0.5 cm) pieces, fixed immediately in ice-cold 4% glutaraldehyde in 0.1 mol cacodylate-HCl buffer containing 3% (w/v) sucrose, pH 7.4, for 4 - 5 h, followed by two 0.5 h washes in cacodylate buffer and stored for 24 h in cacodylate buffer prior to post-fixation in 1% aqueous osmium tetroxide for 2 h. Specimens were then dehydrated through an ethanol series and orientated appropriately prior to embedment in Epon 812 resin. Ultrathin sections were cut and collected on uncoated 200-mesh copper grids, stained with alcoholic uranyl acetate (15 min) and aqueous lead citrate (8 min), and examined using a JEOL 100-CX electron microscope operated at 100 kV.

2 Results

The protonephridium of *M. expansa* consists essentially of flame cells, capillaries and convoluted ducts which include primary collecting-ducts, longitudinal ducts and transverse ducts. Their main cytological features are as follows.

2.1 The flame cell

The flame cell is a composite and irregularly-shaped structure (Plate Ⅰ: 2 - 4). The nucleus is relative large, its shape varying from ovoid to irregular in section, and its content is sometimes densely granular andheterochromatic. The cytoplasm is rich in mitochrondria and possesses some small membrane-bounded vesicles, short lengths of granular endoplasmic reticulum and numerous unattached ribosomes. The flame cell is cup-shaped and bears a tuft of some 89 hexagonally arranged cilia forming the "flame" that extends from the inner surface of the cup into the capillary. The cilia develop from basal bodies whose ultrastructre resembles that of a centriole; branched rootlet fibres secure the basal bodies in the cytoplasm of the flame cell. Each cilium has a typical 9 + 2 axial filament complex and these are arranged so that alternate rows of cilia undulate successively in opposing directions. The periphery of the flame cell surface is developed into a short velum and from its rim a whorl of incurving external ribs are produced. Sixty-two such ribs are found in this whorl (Plate Ⅰ: 7, 8). Inside the whorl from the ribs arise numerous internal leptotriches, giving each flame-cell rib a longitudinal comb-like appearance. Within each of the ribs there are several microtubules and, similarly, within each of the leptotriches there is at least one central supporting microtubule (Plate Ⅰ:8).

2.2 Capillary

The lumen of the capillary is intercellular but extends through a single row of cells, which are also called proximal canal cells, and the end cell of each capillary is developed into a protonephridial funnel that is in close association with the flame cell. The protonephridial ribs (external ribs or rods, 62 in number) are developed from the outer rim of the funnel and lie outside and alternate with the flame-cell leptotriches along their exposed lengths. Adjacent ribs are interconnected by a "membrane" of apparent extracellular matrix. Pores (Plate Ⅰ:2) found open at the attachment points of protonephridial ribs to the flame cell.

2.3 Protonephridial ducts

Protonephridial ducts include the primary collecting-ducts, longitudinal ducts and transverse ducts in *M. expansa*. Primary collecting-ducts (Plate Ⅱ: 5,7) which have an intercellular lumen connect the capillaries with transverse or longitudinal ducts. Four longitudinal ducts are arranged as dorsal and ventral ducts on each side of the worm and lie just inside the margin of the medullary parenchyma. Posterior to the neck region, the dorsal duct is of a smaller diameter and carries fluid toward the scolex. Ventral ducts are positioned somewhat ventrally in the parenchyma and usually have a wide lumen that serves to carry fluid in a direction away from the scolex. The dorsal and ventral ducts of each side are made confluent in the scolex by a network of vessels each at least as wide in diameter as the longitudinal canals. At the posterior end of the worm all four ducts open separately to the exterior. The situation in gravid proglottids was not observed since these were already shed from the strobila of the worms recovered. In the posterior border of each proglottis the ventral longitudinal excretory duct of each side of the worm is connected by a transverse duct. In contrast, no transverse ducts connect the dorsal longitudinal ducts. No structural differences other than size and thickness of the basal lamina have been found among these ducts at electron-microscope level (Plate Ⅱ:4 - 7). The lumen is intercellular and the duct wall is supported by a well-developed basal lamina. The inner surface of these ducts is enhanced by numerous bead-like microvilli.

3 Discussion

3.1 Modification of protonephridium concept

Some 50 years ago, Hyman (1951) defined two main types of nephridia in invertebrates: (1) protonephridia with closed inner ends, and (2) metanephridia with open inner ends. Since then the concept of "protonephridia with closed inner ends" has been used in most text books, other more specialised books and research literature, although in some invertebrates a flame-bulb weir consisting of membrane-covered slits, clefts and pores perforate the cytoplasm lining the lumen of the cell (or called cylinder) have been found (Howells, 1969; Rohde and Watson, 1993; Bartolomaeus and Ax, 1992; Bartolomaeus, 1998). Howells studied the nephridial system of *M. expansa* and found that pores which connect the nephridial lumen to the intercellular space of the connective tissue exist at the junction of the flame cell and nephridial duct. He considered these pores were nephrostomes and believed the excretory and/or osmoregulatory system of *M. expansa* was not a protonephridial system. From the present study and the knowledge of the literature the concept of protonephridial system is improper, and it is proposed should be modified as follows: a protonephridium is a flame cell system consisting of flame cells, canal cells and nephropore cells in general, and that the flame cells (flame bulbs) function as a filtration apparatus with or without slits (pores) opening to the intercellular space of connective tissue (parenchyma).

3.2 Phylogenetic significance of protonephridia

To date, three main types of flame bulbs are distinguished in the Platyhelminthes by Rohde (2001): type 1 has two (or three) cilia arising from a terminal cell and rootlets extending along the weir; type 2 has many cilia arising from a terminal cell and the proximal canal cell closely aligned with it; and type 3 has a non-terminal perikaryon forming many flame bulbs, each with many cilia and a single row of longitudinal ribs. Each main type of flame bulb appears in various structural forms. Type 1 is found in Catenulida: *Catenula*, *Suomina*, *Stenostomum* and *Retronectes* (Rohde and Watson, 1993, 1994a; Rohde, 2001); type 2 in most Rhabditophora, except Lecithoepitheliata and Rhabdocoela: *Microstomum*, *Macrostomum*, Gotte's larva (Polycladida), Proseriata (*Monocelis*), *Urastoma*, Prolecithophora (*Archimonotresis*), Tricladida, Monogenea (Poly- and Monopisthocotylea) and Trematoda (Aspidogastrea and Digenea), Cestoda (Gyrocotylidea, Amphilinidea and Eucestoda) (Rohde et al., 1990; Rohde, 1993, 2001); and type 3 in Lecithoepitheliata and Rhabdocoela. The most likely evolutionary sequence is that type 3 is derived from type 2 and perhaps that type 2 is derived from type 1. Except for the above three main types, there are still other types such as the type found in the haplopharyngid *Haplopharynx rostratus*. No weir was found in this species, there being neither ribs nor 'membranes', but large numbers of exocytotic vesicles are aggregated around the capillaries of the three terminal cells which form a terminal complex. In this species, excretion and/or osmoregulation occurs apparently by exocytosis (Rohde, 2001). In addition, different species might have different numbers of cilia, ribs and leptotriches. Thus, in *M. expansa* there are 89 cilia, 62 internal ribs, 62 extrernal ribs, numerous internal leptotriches and few external leptotriches; in *Hypoderaeum conoideum*, there are 115 cilia (Chen and Chen, 1996); in *Echinosotoma miyagawai*, there are 125 cilia (Chen and Chen, 1996); in *Baltoplana magna*, there are 24 cilia (Rohde and Watson, 1994b).

It seems that the number of cilia increases as the animal develops and comes to attain a certain number in fully developed protonephridia. One pair of protonephridia which is the characteristic of most larvae of invertebrates (acoelomates-platyhelminths, some pseudcoelomates and some coelomates) is considered as the plesiomorphic condition. A serial arrangement of several protonephridia represents the apomorphic condition. All protonephridia are composed of three different sections with an interspecific varying number of cells in general: the terminal region, the duct and the nephropore region. Protonephridia consisting of only three cells: terminal, duct and nephropore cell are assumed to present the plesiomorphic protonephridial design. As is well known, the excretory and/or osmoregulatory system of animals plays a central role in animal homeostasis. Much work has been done to make clear the phylogenetic lineages and development of kidneys in vertebrates. In contrast, very little has been achieved in this regard in invertebrates, especially in the developmental area. There are only a few documented accounts of how the protonepheridia develop (Rohde and Watson, 1988; Bartolomaeus, 1989a, 1997, 1998), or what is the relationship between protonephridia and metanephridia (Bartolomaeus, 1989b; Bartolomaeus and Ax, 1992), or how they evolve (Xylander and Bartolomaeus, 1995). Clearly, much work is still needed to be done if we are to achieve the right phylogenetic conclusion.

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Explanations of Plates

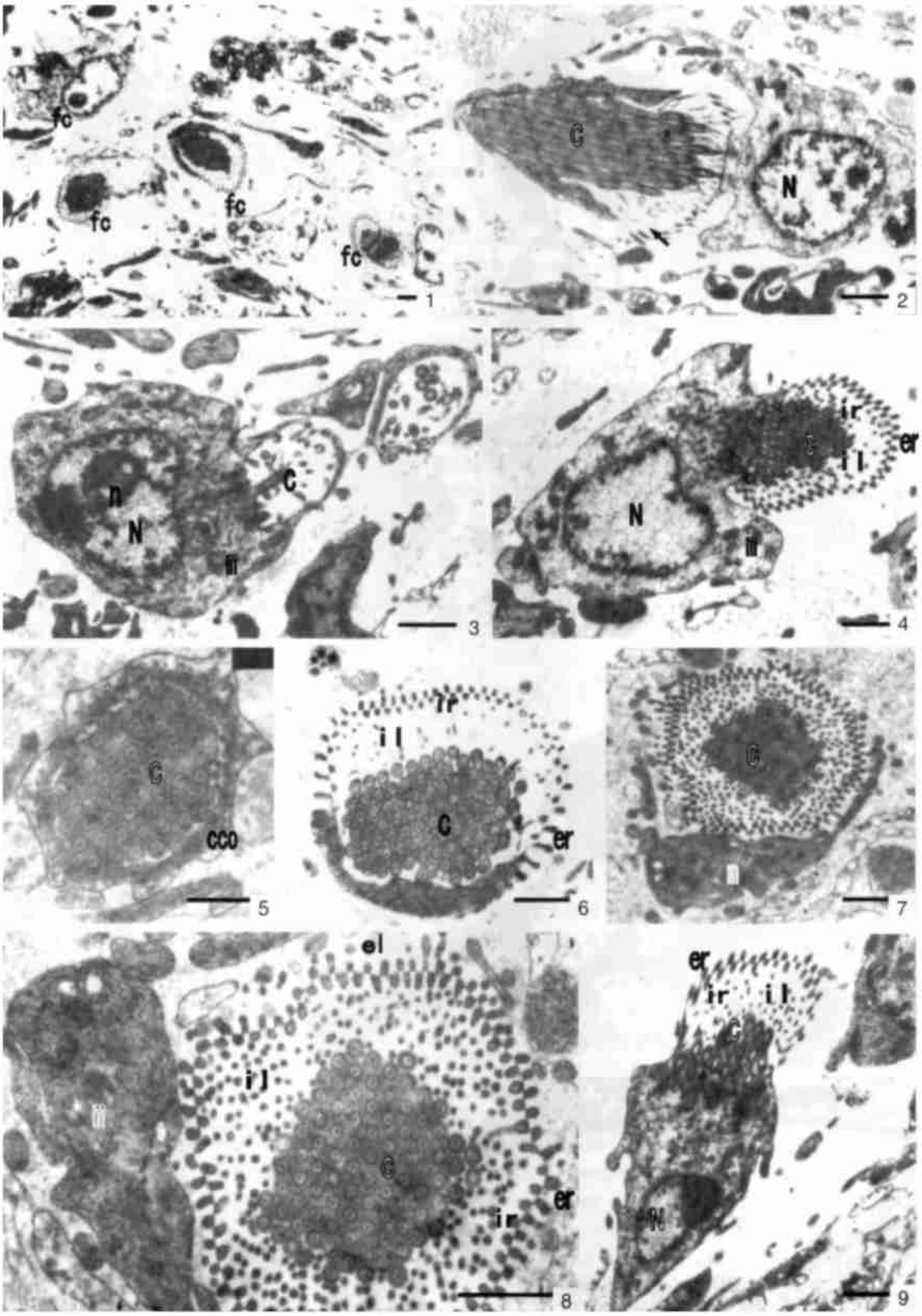
Plate Ⅰ

1. Section cut from scolex, at least four clear flame cells at the figure ×4 255.
2. Longitudinal section through flame bulb and terminal cell. Note pores connecting protonephridial lumen to the intercellular space of the connective tissue (arrow) ×8 405.
3. Oblique longitudinal section through a terminal cell. Nucleus and nucleolus can be seen ×9 075.
4. Oblique longitudinal section through a terminal cell. Nucleus and mitochondria are in the cell body, fused cilia in bulb lumen, internal and external ribs form the weir (filtration apparatus), with many internal leptotriches but few external leptotriches ×7 974.
5. Cross section through flame bulb near protonephridial capillary, do not contain a septate junction between cytoplasmic cords ×12 747.
6. Cross section through middle of flame bulb, no septate junction between cytoplasm of terminal cell and proximal canal cell ×9 333.
7. Cross section through a flame bulb, showing a complete cross face of the weir which composed of 62 internal and 62 external ribs ×8 077.
8. Amplified Figure 7 to show 89 cilia, which are "9 + 2" structure, formed the flame. Internal and external ribs are formed by bundles of a few microtubules. Inside each internal leptotriches there is at least one microtubule to support it ×16 048.
9. Oblique longitudinal section through terminal cell and flame bulb showing heterochromatin inside the nucleus ×8 486.

Plate Ⅱ

1. Oblique longitudinal section from a flame bulb, mitochondria can be seen near the rootlets of the cilia ×9 158.
2. Oblique longitudinal section near the base of rootlets, the structure of base body resembles that of centriole, there is no sheath outside it ×8 300.
3. Longitudinal section showing cilia and their branched rootlets ×9 763.
4. Proximal canal cell and a part of protonephridial capillary ×7 200.
5. Primary collecting-ducts with bead-like microvilli lining the lumens ×13 468.
6. Protonephridial duct with numerous microvilli to expand the surface for excretion and/or osmoregulation ×4 722.
7. Primary collecting-ducts drain to protonephridial duct ×16 025.

b: Base body. bmv: Bead-like microvilli. c: Cilia. cap: Capillary. cco: Cytoplasmic cords. el: External leptotrich. er: External rib. fc: Flame cell. il: Internal leptotrich. ir: Internal rib. N: Nucleus. n: Nucleolus. pcd: Primary collecting-duct. pcc: Proximal canal cell. ro: Rootlet of cilium. Scale bars = 1 μm.



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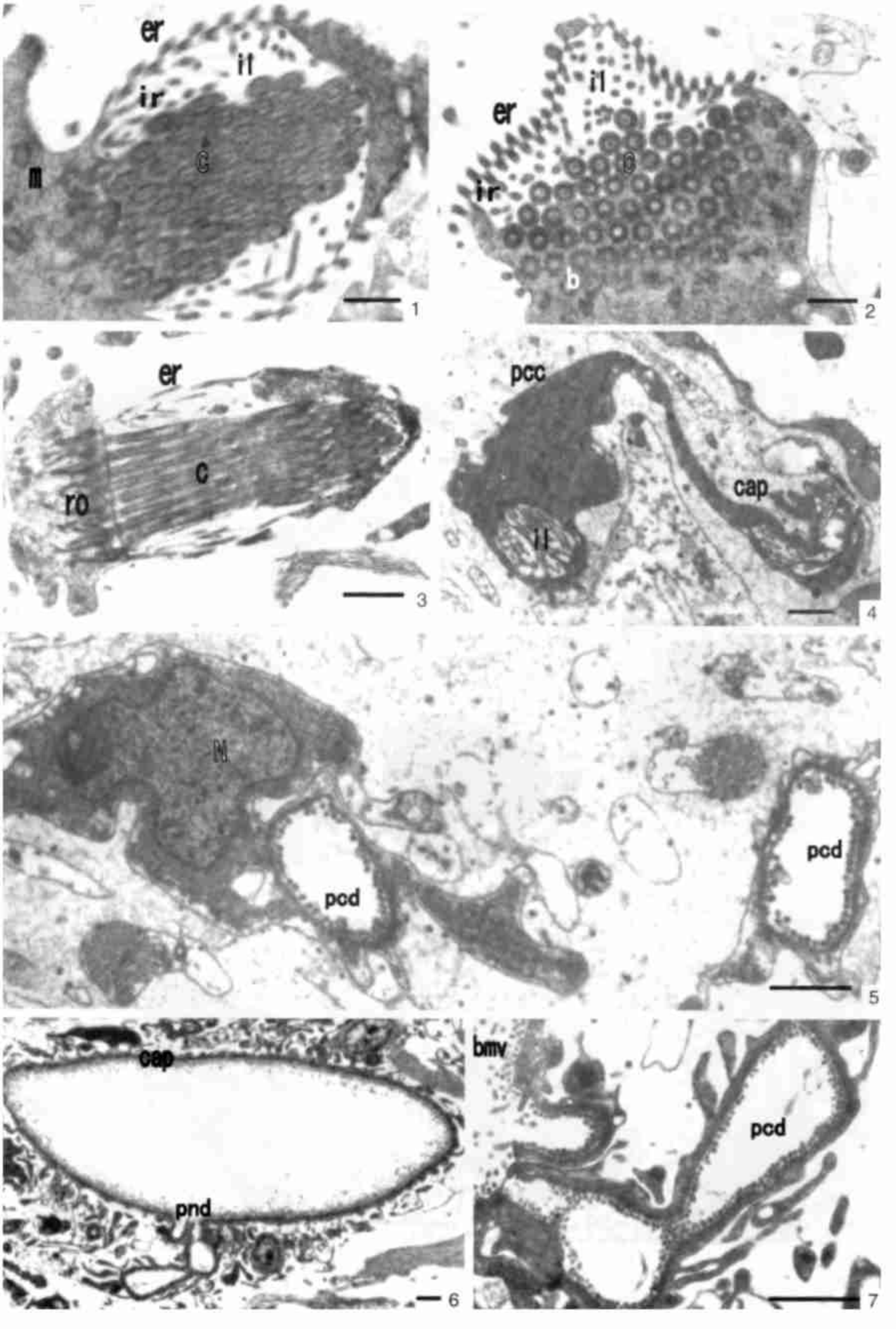

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